IMPACT OF DEGUMMING ON THE PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES OF *CITRULLUS LANATUS* (WATERMELON) SEED OIL

Victor O. Apeh[1]*,Obi U. Njoku[1], Okwesili F.C. Nwodo[1],
Kenchukwu V. Iloabuchi[2], Kingsley O. Omeje[1], Gabriel E. Eze[3]
[1]Department of Biochemistry, University of Nigeria, Nsukka.
[2]Konstanz Research School of Chemical Biology, Department of Biology, University of Konstanz 78457
[3]Department of Science Laboratory Technology, University of Nigeria, Nsukka
*E-mail: victorapeh@yahoo.com

Abstract
Due to increased consumption of vegetable oil, degumming has become imperative towards ehnancing quality and stability of the oil for human consumption. The present study was to determine the influence of degumming on the nutritional composition of the crude and degummed Citrullus lanatus seed oil (CLSO). The mineral and vitamin as well as physicochemical compositions of crude and degummed oil were carried out using standard methods while amino acid profile of the oils were evaluated using UHPLC. LD_{50} of the oils revealed that the oils are safe at 5000 mg/kg body weight. Analysis of mineral contents of crude and degummed Citrullus lanatus seed oils shows markedly increase ($p<0.05$) in Fe, Ca, Se, Zn and Mn, post degumming although Zn and Se contents were unchanged ($p>0.05$).Seventeen amino acids were detected in oils, eight were hydrophobic and nine were hydrophilic. The GC-MS shows that the crude oil has twelve compounds: four saturated fatty acids, five unsaturated fatty acid and three non-fatty acids. Degummed oil has ten compounds: three saturated fatty acids, three unsaturated fatty acids and four non-fatty acids of the oil. The nutritional and physicochemical properties of the oil revealed that degummed oil enhanced the qualities of the oil.

Keywords: amino acid profiles, *Citrullus lanatus*, degumming, fatty acid profile, linoleic acid

Received: 11.04.2020 Received in revised form: 20.05.2020 Accepted: 22.06.2020

1. INTRODUCTION

Increasing awareness on the health benefits of natural dietary constituents has led to the development of range of functional foods through research. Currently, the food industry is tasked with developing new food products, including seed oils with special health benefits. Interests in oil seeds as nutritional supplements for preparation of animal feed and human consumption has increased in recent years (Adewuyi*et al*., 2009). Oil seeds are important sources of nutrient and can serve as high quality dietary sources to meet nutritional requirements (Escudero*et al*., 2006). Among seed oils with beneficial properties, *Citrullus lanatus* have been well studied (Madhavi*et al*., 2012; Nwankwo*et al*., 2014). *C. lanatus* fruit is edible and belongs to the family *Cucurbitaceae*, which are rich in vitamins, minerals, fatty acids, and phytochemicals. The seed have been used to make emulsions for the treatment of catarrhal, disorders of the bowels, urinary passage and fever (Nwankwo*et al*., 2014). While the seed have also been reported to possess antimicrobial and laxative activities (Sharma *et al*., 2011), the roots and leaves are known to possess analgesic and anti-inflammatory properties (Madhavi*et al*., 2012). Although several literatures have reported the health benefits of crude *C. lanatus* seed oil, there is currently no documented evidence on the nutritional potentials of the degummed oil.
The importance of degumming in oil refining process cannot be over-emphasised as it removes phosphatide (gum) along with some other unwanted minor compounds without destroying the beneficial ones (Adewuyi*et al*., 2009). Although refining losses are experienced during degumming, foaming, settling and discoloration of oil in processing and storage (Eickhoff, 2000), it also has the potential to improve nutritional quality, palatability, and may enhance availability of micro and macronutrients. One of the least expensive ways of increasing protein levels in the diets of low income families is by encouraging the consumption of local

indigenous edible seeds, especially seed oils and legumes which have been found to be rich in protein (Singh *et al*., 1993). Seed oils are important sources of nutrient and can serve as high quality dietary sources to meet nutritional requirements (Perumal*et al*., 2001; Escudero*et al*., 2006). Polypeptides similar to those found in olive oils have been detected in other vegetable oils (Hidalgo *et al*., 2002), which opens the way to use polypeptides and amino acids to classify the oils.

In this study, we compared the nutritional potentials of degummed *C. lanatus* seed oil with the crude oil in order to establish the beneficial outcome of the degumming process on *C. lanatus* seed oil and, ultimately, maximize its nutritional benefits and potentials in the food industry.

2. MATERIALS AND METHODS

2.1 Collection and preparation of seed

The plant material used in this study was the fresh *Citrullus lanatus* seeds. The seeds of *Citrullus lanatus* (water melon) was procured from the dealers within Zuba in Bwari area council of FCT Abuja, the identity of the plant was confirmed and Voucher specimens (UNN/PSB/2721-03) were deposited at the herbarium at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The seeds were sun-dried for 72 h and then ground using commercial grinder (TSK-949, Westpoint, France), put in air tight container and stored in a desiccators for further analysis. Similar procedure has been reported by Ekpa (1989).

2.2 Extraction of oil

The pulverized samples were extracted for 6 h using *n*-haxane and soxhlet apparatus equipped with thimble. The extracted oil was then concentrated using rotary evaporator (EYELA, N-NSeries; Rikakikai Co Ltd., Tokyo, Japan) to obtain n-hexane-free crude *C. lanatus* seed oil. After concentration, crude *C. lanatus* seed oil was immediately placed in a hot air oven maintained at 60°C for 24 h to ensure complete removal of solvents. The concentrated oil sample was then stored in a bottle in a cool dry place before degumming.

2.3 Degumming procedure

A sample of oil (200 ml) was taken and heated to 70°C for 1 h in oil test bath followed by addition of 5 % v/v water. The mixture was then stirred for 10 min using a magnetic stirrer. During this process, a colloidal suspension was observed beneath the oil layer (perhaps a mixture of the fat-soluble impurities - phospholipid lecithin complexed with metals contained in the starting *C. lanatus*). Consequently, the mixture was again heated at 80°C for 15 min and then allowed to cool to room temperature. The sample was then centrifuged at 4000 rpm for 15 min to remove the colloidal suspension and was put in air tight container and stored in a dark cupboard to avoid oxidation due to light for further analysis.

2.4 Median Lethal Dose/Lethality (LD_{50}) Test

Determination of the acute toxicity was carried out using a modified method described by Lorke (1983). Three groups of adult albino mice consisting of three animals per group were used in the first phase. The crude or degummed oil was given orally in doses of 10,100 and 1000 mg/kg body weight (b.w) to animals in group 1, 2 and 3, respectively. The result of this first test was used as a basis for selecting the subsequent oral doses, 1500, 2000, 2500, 3000, 3500 for second phase and 4000, 4500 and 5000mg/kg b.w for third phase. Animals were fed with the normal rat feed and water and were observed for behavior and death.

Animal handlings wasas stipulated in the guidelines of the Ethical Committee on the Use and Care of Experimental Animals of UNN.

The LD50 was calculated using the expression of Lorke (1983) as follows:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality
D_{100} = Lowest dose that produced mortality

2.5 Chemical analyses

2.5.1 Determination of mineral elements

Mineral content, zinc (Zn), iron (Fe), selenium (Se), manganese (Mn) and calcium (Ca) of each oil sample (crudeand degummed oil) was determined using the atomic absorption spectrophotometer (AAS; Mode 303 Perkin Elimer Norwalk USA). According to the method of Ekpete *et al*. (2013) two grams of each oil sample was digested in a muffle furnace at 550°C. Digested oil samples (moistened with nitric acid/perchloric acid) were analyzed for mineral contents using the AAS. Validation was carried out with standard solutions of each mineral before and during AAS analysis of oil samples. For Ca determination, 1.0 mL lithium oxide solution was added to the original solution to unmask Ca from Mg. The rest of the minerals were determined after digestion. The concentration of minerals in ppm with dilution factor and dividing by 1000, as follows:

Mineral element =

$$\frac{\text{absorbency (ppm) x dry weight x Df}}{\text{Wt of sample x 1000}}$$

Dilution factor for all the minerals analyzed were 100.

2.5.2 Determination of vitamins

The vitamins in the dried samples were determined using the methods of association of vitamin chemists (AOVC 1966) vitamin A, D and E were determined using the spectrophotometer method described by Kirk and Sawyer, (1991) at 325nm.

2.5.3 Quality analyses of oils

Free fatty acid, density, refractive index, viscosity, moisture, saponification, specific gravity, acid value, iodine and peroxide values were determined according to the method of AOAC (2000).

2.5.4 Determination of fatty acid profile

The chromatographic analysis was carried out in a capillary fused silica column VF-5 MS (30 m × 0.25 mm I.D., 0.25 mm film thickness) from Varian (Middleburg, The Netherlands). A quantityof 2 µl of oil sample were injected into the gas chromatograph inlet where it is vaporized and was swept onto a chromatographic column by the carrier gas (usually helium). The sample flows through the column and the compounds comprising the mixture of interest are separated by virtue of their relative interaction with the coating of the column (stationary phase) and the carrier gas (mobile phase). The latter part of the column passes through a heated transfer line and ends at the entrance to ion source where compounds eluting from the column are converted to ions. The temperatureof the injectormaintained at 80 ºC for 0.1 min during injection and then programmed at 200 ºC min^{-1} to 300 ºC which was maintained for 13 min. The injector liner (3.4 mm i.d.) was filled with a Carbofrit plug (Restek, Bellefonte, USA). The GC oven temperature program was 80 ºC for 1.0 min, followed by a 25 ºC min^{-1} ramp to 180 ºC and a final ramp of 5 ºC min^{-1} to 280 ºC (held for 5 min). Carrier gas was helium (99.9999% purity; Air Products, Allentown, USA) at a constant flow-rate of 1.3 mL min^{-1}.

The database of National Institute Standard and technology (NIST) was used in interpreting themass spectrum of GC-MShaving more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The GC retention time was the basis for the identification and characterization of chemical compounds in various oil extracts. The mass spectra were computer matched with those of standards available in mass spectrum libraries.The Name, Molecular weight and structure of the components of the test materials were ascertained.

2.5.5. Determination of amino acid profile

The amino acid analysis was performed at the Department of Biochemistry, University of Nigeria, Nsukka, using the Ultra High Performance Liquid Chromatography (UHPLC) equipped with an FLD-3000 Fluorescence Detector (Thermo Electron SAS,

Waltham, MA, USA). The amino acids (AAs) were analysed using method described by Habran *et al*. (2016) with minor modifications. After derivation with 6-aminoquinolyl-N-hydroxy-succinimidyl-carbamate (AccQ-Tag derivatization reagent, Waters, Milford, MA, USA) according to Hilbert et al. (2003), free amino acids were measured according to Habran *et al*. (2016). Briefly, amino acids were analysed using an UltiMate 3000 UHPLC system. Separation was performed on a AccQ•Tag Ultra column, 2.1 x 100 mm, 1.7 µm (Waters, Milford, MA, USA) at 37°C with elution at 0.5 ml min-1 (eluent A, sodium acetate buffer, 140mM at pH 5.7; eluent B, acetonitrile ; eluent C, water) according to the gradient described by Habran *et al*. (2016). Chromatograms corresponding to excitation at 250 nm and emission at 395 nm were recorded. A control was performed before each run of 2 oil samples, to ensure consistency in retention time and a stable baseline. Chromeleon software, version 7.1 (Thermo Electron SAS, Waltham, MA USA) was used to calculate peak area. A standard of 20 amino acids (Alanine, Arginine, Aspartic acid, Asparagine, Cysteine, GABA, Glycine, Glutamic acid, Glutamine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine, Valine) purchased from Sigma (St Louis, Missouri, USA) was used after the control and in the middle of each run to calibrate amino acid quantification. Seventeen amino acids were identified and quantified as described by Pereira et al. (2006).

2.6 Data Analysis

The results were analyzed using the SPSS (Version 21). Data were expressed as mean ± standard deviation (mean ± SD). Student's t-test was used for comparison between two sets of data. Where the variables of three or more are to be compared, one-way analysis of variance (ANOVA) was used for comparison. Duncan test was used for post-hoc, where $p \leq 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Acute toxicity test of *C. lanatus* seed oil

The results in Table 1 shows that no death was recorded in phases 1, 2 and 3 of LD 50 test both in the crude and degummed oil at the concentration of 5000mg/kg body weight which was the highest concentration. Acute toxicity test was carried out with the extracted crude and degummed oil using albino mice.

The assessment of the median lethal dose (LD_{50}) (the dose that kills 50% of test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general toxicity screening of chemical and pharmacological agents (Chinedu*et al*., 2013).

Table 1. Median lethal dose (LD 50) of crude and degummed *Citrullus lanatus* oil on albino mice

Dose in mg/Kg b.w	Number of deaths recorded in C. Oil	Number of deaths recorded in D. Oil
Phase 1		
10	0/3	0/3
100	0/3	0/3
1000	0/3	0/3
Phase 2		
1500	0/3	0/3
2000	0/3	0/3
2500	0/3	0/3
3000	0/3	0/3
3500	0/3	0/3
Phase 3		
4000	0/3	0/3
4500	0/3	0/3
5000	0/3	0/3

The result revealed that *Citrullus lanatus* (watermelon) seed oil was safe up to a dose of 5000 and 5000 mg/kg body weight for crude and degummed *Citrullus lanatus* oil respectively. This report does not agree with the lethal dose value (2500mg/kg b.w) earlier obtained for crude *Citrullus lanatus* seed oil by Madhavi*et al*. (2012).

The difference in LD_{50} might be attributed to influence of geographical region and climatic condition on the seed as well as oil quality. Soils are crucial and therefore are linked to the atmospheric/climate system through the carbon, nitrogen, and hydrologic cycles, according to Eric (2013).

3.2 Effect of degumming on vitamins and minerals in C*itrullus lanatus* seed oil

Tables 2 and 3 depicts increased values in Ca, Mn, Zn, Se and Fe contents post degumming. Also, we observed significant higher (p<0.05) values in the vitamin A and E contents of degummed *Citrullus lanatus* when compared to crude *Citrullus lanatus*.

The degumming procedure must be capable of releasing the bound forms of the vitamin/minerals through the breaking of bonds linking the vitamin to protein or to carbohydrate for subsequent quantification. These may have accounted for increases in vitamins/mineral we reported in our study. Both vitamin E and selenium independently enhance the immune responses and an additive effect in increasing hemagglutinin titers (Arthur *et al*., 2003).

Arthur *et al*. (2003) have reported that vitamin E supplements act to improve the immune response by reducing the production of PGE2, which in turn moderates cyclooxygenase activity. Therefore the availability of these minerals and vitamin underscores the indispensability of this oil in our diet for our overall well-being.

3.3 Physicochemical properties of crude and degummed oil.

Result in Table 4a show that a significant reduction ($p < 0.05$) in moisture, and specific gravity of the dgummed oil compared with the crude oil of *Cirullus lanatus*. While density and moisture content of oil reduced after degumming there were no significant reduction in refractive and index.

Physical properties depicted in Table 4 showed that only specific gravity and moisture reduced after degumming while others were not significantly reduced post degumming.

Table 2. Vitamin Constituents of crude and degummed *Citrullus lanatus* seed oil

Vitamins	Crude (mg/100g)	Degummed (mg/100g)
Vitamin A	0.89 ± 0.01^{a}	1.13 ± 0.13^{b}
Vitamin D	0.57 ± 0.03^{b}	0.59 ± 0.03^{b}
Vitamin E	1.01 ± 0.02^{a}	1.17 ± 0.19^{b}

Values are expressed as mean ± SD. (n=3).
Values across the row with different superscripts differ significantly (p<0.05).

Table 3. Mineral constituents of crude and degummed *Citrullus lanatus* seed oil

Minerals	Crude (mg/100g)	Degummed (mg/100g)
Calcium (mg/kg)	71.55 ± 0.80^{a}	86.80 ± 0.31^{b}
Manganese	0.32 ± 0.01^{a}	0.42 ± 0.01^{b}
Zinc	3.77 ± 0.12^{a}	3.92 ± 0.06^{b}
Selenium	3.46 ± 0.45^{a}	3.83 ± 0.09^{b}
Iron	4.55 ± 0.09^{a}	6.50 ± 0.27^{b}

Values are expressed as mean ± SD. (n=3).
Values across the row with different superscripts differ significantly (p<0.05).

The reduction in physical properties except viscosity could be attributed to the removal of some fat-soluble impurities (phospholipid lecithin complexed with trace metals) that might have contaminated the oil. A low value of these parameters, suggests that degumming improved the quality of the oil. According to Aremu*et al*. (2015), the amount of impurities contained in the oil affects the degree of reflection caused by a ray of light during refractive index determination of the oil.

It is possible that those impurities in the crude oil hindered non-polar interactions and thus increased fluidity of the oil giving rise to increased viscosity after degumming. It is also possible that partial removal of minor components like free fatty acid, phospholipids and other impurities increased the surface tension of degummed oil thereby increased the viscosity of the oil. Likewise, Njoku *et al*. (2010) reported that free fatty acids, mono-and diacylglycerols decreased the surface tension of edible oil. The extraction of 2480 g of dry *C. lanatus* seed yielded of 654.84 g of oil which represented percentage yield of 26.40 %. This is comparable to yield obtained earlier by Tarun*et al.* (2013),Madhavi*et al*. (2012) and Orasenaya (2000). However, the present oil yield was lower than that reported by Oyedeji and Oderinde (2006) for Nigerian watermelon seeds (oil contents 45%). Such variation in oil yield from different regions might be due to the varied agroclimatic conditions employed for the propagation of such fruits. The moderately high yield of the oil suggests that the oil from watermelon seed would be sufficient for some industrial and pharmaceutical applications

3.4 Chemical properties of *Citrullus lanatus* seed oil

After degumming the oil, the free fatty acid, acid value, peroxide value, saponification value and iodine value of the oil were significantly lower ($p < 0.05$) when compared with the crude oil. The chemical properties of extracted degummed oil were significantly decreased ($p<0.05$) when compared to the extracted crude oil. These decreases in the values of the degummed oil samples were in line with other refining processes (Gordon, 1993; Aremu*et al*., 2015). The free fatty acid content of crude oil was significantly ($p < 0.05$) higher in comparison with the degummed oil. It has been observed that high free fatty acid level of crude oil is mainly due to high activity of the lipase; whereas in degummed oil the lipase activity might have been reduced which resulted in decrease of free fatty acid content in the oil (Njoku*et al*., 2010).

Table 4 a. Effect of degumming on the physical properties of crude and degumed oil

Characteristics	Crude	Degummed
Specific gravity at 20ºc	0.86±0.01[a]	0.81±0.01[b]
Refractive index at 40º C	1.34±0.06[a]	1.24±0.04[a]
Viscosity (Poise)	0.55 ±0.04[a]	0.59 ±0.02[a]
Density (mg/ml)	0.92±0.02[a]	0.89±0.01[a]
Moisture (%)	0.81±0.01[a]	0.20±0.01[b]

Table 4 b. Effect of degumming on the chemical properties of crude and degummed oil

Characteristics	Crude	Degummed
Free fatty acid (%)	1.87±0.03[a]	1.79±0.01[b]
Acid value (mgKOH/g)	3.77±0.06[a]	3.59±0.01[b]
Peroxide value (meq/kg)	3.27±0.07[a]	2.11±0.01[b]
Saponification value (mgKOH/g)	140.33±0.26[a]	112.28±0.43[b]
Iodine value (g I/100g of oil)	132.78±0.43[a]	110.51±0.50[b]
Phospholipid content (mg/dl)	0.028± 0.01[a]	0.017± 0.02[b]

Values are expressed as mean ± SD. (n=3).
Values across the row with different superscripts differ significantly (p<0.05).

Autoxidation of oil is aided by the presence of free fatty acids and other oxidized compounds (Aremu*et al*., 2015). The crude oil value did not compared favorably with the result by Edidiong*et al*. (2013) that stated 4.5% for the crude *Citrullus lanatus* seed oil. The free fatty acid which have hydrophilic hydroxy groups and hydrophobic hydrocarbons (Eunok and David, 2006), decrease the surface tension of edible oil and increase the diffusion rate of oxygen from the headspace to the oil to accelerate the oxidation of oil. This also explains increased viscosity as free fatty acid decreases. High quality oils are low in free fatty acids (Njoku*et al*., 2010).

Acid value gives an indication of the quality of fatty acids in oil (Njoku *et al*., 2010). After degumming (Table 4b), acid values decreased significantly (p<0.05). These values however accounted for the presence of free fatty acids in the oils as an indicator of the presence and limited lipase hydrolysis of the oil after degumming (Gordon, 1993). Low acid value in oil indicates that the oil could be stable over a long period of time and protect against rancidity and peroxidation.

Peroxide value (PV) is the most common indicator of lipid oxidation (Supatcha *et al*., 2015). In the present study, peroxide value after degumming of watermelon seedoil got reduced by 35.47%. This might be due to removal of hydratable compounds and other degrading impurities. Peroxides (R-OOH) are primary reaction products formed in the initial stages of oxidation, and therefore give an indication of the progress of lipid oxidation (Supatcha *et al*., 2015). The lipid alkyl radical reacts with atmospheric oxygen to form peroxy radicals.

Decrease in peroxide value of the oil after refining, improves its suitability for the long term storage because of its low level of oxidative and lipolytic activities. The unrefined vegetable oils are characterised by greater PV, compared to refined oils (Aremu *et al*., 2015; Omeje*et al*., 2019). WHO/FAO (1994) stipulated a permitted maximum PV of not more than 10 meq of oxygen/kg of the oils; therefore, the oils may be suitable for consumption. The iodine value is used in quantifying the amount of double bond contained in the oil which reflects the susceptibility of the oil to oxidation. Decrease in iodine value after degumming represent the decrease in unsaturation of oil which gives the oil a better stability in terms of oxidation. The decrease in iodine value denotes decrease in the degree of unsaturation of the oil (Kirk and Sawyer, 1991).

3.5 Fatty acid profile of crude and degummed oil

The gas chromatography-mass spectra of the crude and degummed oils showed that the crude oil comprised of four saturated fatty acids (hydroxyl methyl palmitin, palmitic acid, arachidic acid and methyl palmitin) covering 37.4% of the total concentration of the oil, five unsaturated fatty acids (methyl linoleate, linoleic acid, Ricinoleic acid {oleic acid}, ethyl linoleate and linoleic acid chloride) comprising 61.98%. The degummed oil comprised of three saturated fatty acids (hydroxyl methyl palmitin, hydroxyl methyl stearin acid and palmitic acid) accounting 32.15% of the total concentration of the oil and three unsaturated fatty acids (methyl linoleate, linoleic acid and linoleic acid chloride) in amount of 66.72%. Degummed oil has higher concentration of unsaturated fatty acid than the crude oil, in the same vein degummed oil has higher concentration of linoleic acid than CO.

The result in Table 5b shows that percentage of unsaturated fatty acid increased by 0.51%. Linoleic acid was the predominant unsaturated fatty acid and their methyl esters.

While palmitic acid and its methyl ester derivatives were the predominant saturated fatty acid contained in the oil. Degummed oil has higher concentration of unsaturated fatty acid than the crude oil (CO), in the same vein degummed oil has higher concentration of linoleic acid than CO. The higher percentage of unsaturated fatty acid might be due unclear reaction mechanism which converts some saturated fatty acid to unsaturated fatty acid and this report is in line with the report of Wani*et al*. (2013).

Table 5 a. Fatty acids found in crude *Cirullus lanatus* seed oil

No	RT	Common name	Molecular formula	Mol.wt.	Percentage area
1	8.22	Citral	$C_{10}H_{16}O$	152	0.12
2	8.55	Citral	$C_{10}H_{16}O$	152	0.17
3	16.75	Methyl Palmitin	$C_{17}H_{34}O_2$	270	0.20
4	18.37	Palmitic acid	$C_{16}H_{32}O_2$	256	32.06
5	19.83	Methyl linoleate	$C_{19}H_{34}O_2$	266	1.49
6	21.75	Linoleic acid	$C_{18}H_{32}O_2$	280	46.66
7	23.06	Ricinoleic acid	$C_{18}H_{34}O_3$	298	1.61
8	23.35	Arachidic acid	$C_{20}H_{40}O_2$	312	1.68
9	24.30	Ethyl linoleate	$C_{20}H_{36}O_2$	280	0.84
10	24.65	Hydroxy methyl palmitin	$C_{19}H_{38}O_4$	330	3.46
11	26.45	Linoleic acid chloride	$C_{18}H_{31}ClO$	298	11.38
12	27.76	Squalene	$C_{30}H_{50}$	410	0.33
		Total saturated fatty acids			37.20
		Total unsaturated fatty acids			61.98

Table 5 b. Fatty acids found in Degummed water melon (*Cirullus lanatus*) seed oil

No	RT	Name of compound	Mol formula	Mol. Weight	Percent. area
1	7.33	Citral	$C_{10}H_{16}O$	152	0.29
2	8.28	2, 4-Dodecadienal	$C_{12}H_{20}O$	180	0.11
3	8.60	Citral	$C_{10}H_{16}O$	152	0.18
4	17.94	Palmitic acid	$C_{16}H_{32}O_2$	256	16.81
5	19.57	Methyl linoleate	$C_{19}H_{34}O_2$	294	0.71
6	22.12	Linoleic acid	$C_{18}H_{32}O_2$	280	59.42
7	24.30	Linoleic acid chloride	$C_{18}H_{31}ClO$	298	6.59
8	24.75	Hydroxyl methyl palmitin	$C_{19}H_{38}O_4$	330	2.17
9	26.79	Hydroxy methyl Stearin	$C_{21}H_{42}O_4$	358	13.20
10	27.87	Squalene	$C_{30}H_{50}$	410	0.53
		Total saturated fatty acids			32.15
		Total unsaturated fatty acids			66.72

The presence of ricinoleic acid or epoxyoleic acid ($C_{18}H_{34}O_3$) which is an oxygenated fatty acid in the crude and its absence in degummed suggests an oxidation reaction in CO more than degummed. The most common oxygenated acid has a hydroxyl, epoxy, or furanoid unit (Hammam*et al*., 2001). Arachidic acid is also a product of hydrogenation of arachidonic acid. The contents of total saturated fatty acids for the crude *Citrullus lanatus* seed oil (37.2%) in the present study were higher than those reported (17.8%) by Ziyada and Elhaussien (2008) in *Citrullus lanatus* var. Colocynthoide, this also varied slightly from those (12.0-36.0%) investigated for different species of musk melon seed oils (Tilak*et al*., 2006). There is no documented report yet on saturated and unsaturated fatty acids of degummed *Citrullus lanatus* seed oil. We found the contents of total unsaturated fatty acids in the present study to be lower than those reported by Baboli and Kordi (2010) (81.6%). The total unsaturated fatty acids value in our report are comparable with those recorded (64.6-88.2%) for different species of muskmelon (Tilak*et al*., 2006). Linoleic acid is an essential fatty acid with many health benefits such as the inhibition of eicosanoid production, alteration in the production of several prostanoids, reduction of blood pressure, lowering of serum triacylglycerol (TAG) and cholesterol levels (Mabaleha*et al*., 2007).

3.6 Amino acid profile of crude and degummed *C. lanatus* seed oil

Amino acid analysis revealed that there are seventeen (17) amino acids: eight hydrophobic and nine hydrophilic. A total concentration of

190.20 and 242.56 mg/L of crude and degummed oil extracts respectively. The oil contains ten non-essential and seven essential amino acids. Hydrophobic group occupies 81.10% of crude and 88.81% of degummed oil, while hydrophilic group occupies 18.89% for crude and 11.20% for degummed oil. The non-essential amino acids occupy 47% for crude oil and 61.99% for degummed oil while essential amino acid occupy 52.93% and 38.01% for crude and degummed oil respectively.

Citrullus lanatus seed oil is a good source of essential amino acids, notably Alanine, Proline (non-essential), Tryptophan and Methionine (essential). The other amino acids are present in moderate amounts. The degumming with water was not always complete, because peptides and proteins present in *C. lanatus* oil were not too soluble in water, and explains the presence of small amounts hydrophilic amino acids in the oil after degumming was applied. The presence of different enzyme activities, which implies the presence of proteins, has been demonstrated in olive oils (Zamora et al., 2001) suggesting that some peptides are soluble in lipid matrixes, where they might be playing unknown functions however, only in few reports have proteins been recognized as components of olive oils (Hidalgo and Zamora, 2001; Hidalgo *et al*., 2002). Aspargine and glutamine were excluded from this study, since hydrolysis converts them into Asp and Glu (Gimeno-Adelantado*et al*., 2002).

Hydrolysis also leads to a partial conversion of Glu into pyroglutamic acid and to tryptophan (Gimeno-Adelantado*et al*., 2002). Proteins react with lipid oxidation products, yielding endogenous antioxidants in food systems (Hidalgo *et al*, 2000).

This suggest that removal of hydratable phosphatides enhanced the availability of amino acid composition of the degummed oil.

4. CONCLUSIONS

Degumming minimized the degree of auto-oxidation as evidenced in the physicochemical properties as well as improved the quality and nutritional content of the oil. Degumming equally enhanced the polyunsaturated fatty acid as well as linoleic acid component of the oil.

Table 6: Amino acid profile of degummed and crude oil of *Citrullus lanatus* seed

Amino acids	Crude (mg/L)	Degummed (mg/L)	Hydrophilic/ Hydrophobic	Essential/ Nonessential
Alanine	51.22±0.39a	67.57±0.57[a]	Hydrophobic	Non essential
Proline	12.00±0.04[a]	56.18±0.28[a]	Hydrophobic	Non essential
Hydroxyproline	6.86±0.13[a]	6.59±0.20[a]	Hydrophobic	Non essential
Isoleucine	0.00±0.00[a]	0.99±0.03$_b$	Hydrophobic	Essential
Methionine	11.77±0.16[a]	12.53±0.32[a]	Hydrophobic	Essential
Phenylalanine	0.37±0.02[a]	0.33±0.01[a]	Hydrophobic	Essential
Tryptophan	69.32±0.40[a]	71.86±0.23[a]	Hydrophobic	Essential
Valine	2.51±0.15[a]	5.90±0.21[a]	Hydrophobic	Essential
Cysteine	2.07±0.08[a]	1.99±0.04[a]	Hydrophilic	Non essential
Serine	4.96±0.05[a]	5.72±0.09[a]	Hydrophilic	Non essential
Aspartic acid	5.47±0.26[a]	5.39±0.27[a]	Hydrophilic (charged)	Non essential
Glutamic acid	2.91±0.17[a]	2.77±0.10[a]	Hydrophilic (charged)	Non essential
Tyrosine	2.29±0.14[a]	2.77±0.05[a]	Hydrophilic	Non essential
Threonine	0.67±0.03[a]	0.60±0.03[a]	Hydrophilic	Essential
Histidine	15.77±0.11[a]	0.32±0.02[a]	Hydrophilic	Essential
Norleucine	0.68±0.01[a]	1.05±0.14[b]	hydrophilic	Non essential
Glycine	1.44±0.11[a]	0.00±0.00[b]	hydrophilic	Non essential
Total	190.20	242.56		

5. ACKNOWLEDGMENTS

The authors wish to acknowledge the Mrs B.N. Okolo of Laboratory Unit of Bishop Shanahan Hospital, Nsukka for her technical and logistic input.

6. REFERENCES

[1] Adewuyi, A., Oderinde R. A and Ajayi I. A. (2009). The metal composition, proximate properties and the effect of refining on the physico-chemical characterization of Baphia nitida and Gliricidia sepium seed and seed oil. *J Food Technol.***7**(2), 43-49

[2] AOAC. 2000. Official methods of analysis of AOAC international 17th ed, Chaper 41: 26.

[3] Aremu, M.O, Ibrahim, H. andBamidele, T.O. (2015).Physicochemical Characteristics of the Oils Extracted from Some Nigerian Plant Foods – A Review. *Chem and Process Eng. Res* 32, 945-951.

[4] Arthur, J. R., McKenzie, R.C. and Beckett, G.J. (2003). Selenium in the immune system. *J. Nutrition* 133(5), 1457-1459.

[5] Association of Vitamin Chemists, Methods of vitamin assay (1966). Prepared by the Association of Vitamin Chemists, inc. Myer Freed, chairman, Methods Committee. Interscience Publishers, New York.

[6] Baboli, Z.M. and Kordi, A.A.S. (2010). Characteristics and Composition of Watermelon Seed Oil and Solvent Extraction Parameters Effects. *J. Am Oil ChemSoci*. 87,667-671.

[7] Chinedu, C.A., David, A. and Fidelis, S.A. (2013). A New Method for Determining Acute Toxicity in Animal Models. *Toxicology Intl* 20 (3), 224–226.

[8] Edidiong, A., Essien, A. andUbong, M.E. (2013). Chemical analysis of *Citrullus lanatus* seed oil obtained from Southern Nigeria. *Elixir Organic Chem*54, 12700-12703.

[9] Eickhoff, K.P. (2000). Degumming process in edible oil Industry' World Conference Proceedings on Edible Oils and Fat Processing - Basic Principles and Modern Practices, Edited by Singhal, S.C. (Ed) AOCS, Champaign, pp. 72-78.

[10] Ekpa, O. D. (1989). The effect of chemical preservation on the storage of some Nigerian crops and vegetables. *J. Chem Soc. Nig.* 14, 31 – 36.

[11] Ekpete, O.A., Edori, O.S. and Fubara, E.P. (2013). Proximate and mineral composition of some Nigerian fruits. *Br. J. Appl. Sci. Technol*.3, 1447–1454.

[12] Eric, C. B. (2013). The Potential Impact of Climate Change on Soil Properties and Processes and Corresponding Influence on Food Security. *Agriculture* 3, 398-417.

[13] Escudero N.L., Zirulnik, F., Gomez, N.N., S.I. Muciarelli, S.I. and Gimenez, M.S. (2006). Influence of a protein concretion from *Amaranthuscruentus* seeds on lipid metabolism. *Expert Biol and Med* 231, 50-59.

[14] Eunok, C. and David, B.M. (2006). Mechanism and factors for edible oil oxidation. *Comp.Rev. in Food Sci and Food Safety* 5, 169-184.

[15] Gimeno-Adelantado, J. V., Mateo-Castro, R., Dome´nech-Carbo, M.T., Bosch-Reig, F., Dome´nech-Carbo, A., De la Cruz-Canizares, J. and Casas-Catala, M.J. (2002). Analytical study of proteinaceous binding media in works of art by gas chromatography using alkyl chloroformates as derivatising agents. *Talanta*, 56, 71-77.

[16] Gordon, M. (1993). Fats, fatty foods, In: Ranken, M.D., Kill, R.C., (Eds), Food Industries Manual, 23rdedition. Blackie Academic and Professional, London, pp. 179 – 186.

[17] Habran, A., Commisso, M., Helwi, P., Hilbert, G., Negri, S., Ollat, N., Gomès, E., van Leeuwen, C., Guzzo, F. andDelrot, S. (2016).Roostocks/Scion/Nitrogen interactions affect secondary metabolism in the grape berry. *Frontiers in Plant Science*, 7, 1134.

[18] Hammami, I., Kamoun, N. and Rebai, A. (2001).*Biocontrol of Botrytis cinereawith essential oil and methanol extract of Viola odorata flowers*. ***Archives of ApplSci Res***3(5), 44-51**.**

[19] Hidalgo, F.J. and Zamora, R. (2000). The role of lipids innonenzimatic browning. *Grasas y Aceites* 51, 35-49.

[20] Hidalgo, F. J., Alaiz, M. and Zamora, R. (2001). Determination of Peptides and Proteins in Fats and Oils. *Anal. Chem*.73, 698-702.

[21] Hidalgo, F.J., Alaiz, M. and Zamora, R. (2002). Low Molecular Weight Polypeptides in Virgin and Refined Olive Oils, *J. Am. Oil Chem. Soc.79*, 685–689. *oc*. 8, 31-36.

[22] Hilbert, G., Temsamani, H., Bordenave, L., Pedrot, E., Chaher, N., Cluzet, S., Delaunay, J.C., Ollat, N., Delrot, S., Mérillon, J.M., Gomès, E. and Richard, T. (2015). Flavonol profiles in berries of wild *Vitis* accessions using liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance spectrometry. *Food Chemistry*, 169, 49-58.

[23] Kirk, R.S. and Sawyer, R. (1991). Pearson's Composition and Analysis of Foods.9th Ed. Longman Sci. Tech. Engl, pp. 607-617.

[24] Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of Toxicol53, 275-287.

[25] Mabaleha, M.B., Mitei, Y.C. and Yeboah, S.O. (2007). A comparative study of the properties ofselected melon seed oils as potential candidates for development into commercial edible vegetable oils. *J.Am. Oil Chem Soc*. 8, 31-36.

[26] Madhavi, P., Maruthi, R., Kamala, V., Habibur, R. andChinna, E.M. (2012).Evaluation of Anti-Inflammatory Activity of *Citrullus lanatus* Seed Oil by In-vivo and In-vitro Models. Intl Res J Pharmacy and Applied Sci2 (4), 104-108.

[27] Njoku, O.U., Boniface, J.A.E., Obitte, N.C., Odimegwu, D.C. and Ogbu, H.I. (2010). Some nutriceutical potential of beni seed oil. *Intl J. Applied Res. in Nat. Prod* 2(4), 11 – 19.

[28] Nwankwo, I.U., Onwuakor, C.E. and Nwosu, V.C. (2014). Phytochemical analysis and antibacterial activities of *Citrullus lanatus* seed against some pathogenic microorganisms. *Global J Med research*: *Microb and Pathology* 14 (4), 1101-1107.

[29] Omeje, K.O., Iroha, O.K., Edeke, A.A., Omeje, H.C. and Apeh VO. (2019). Characterization and fatty acid profile analysis of oil extracted from unexploited seed of African star apple (Udara). *Oilseeeds & Fats Crops and Lipids*, 26(10): 1-15.

[30] Orasenaya, M.O. (2000): Extraction before *Citrullus colocynthis*seed oil. *Nigerian J Nat Products and Med*4, 76-77.

[31] Oyedeji, F.O. andOderinde, R.A. (2006): Characterization of isopropanol extracted vegetable oil. *Journal of Applied Science*, 6,2510-2513.

[32] Pereira, G. E., Gaudillere, J.P., Pieri, P., Hilbert, G., Maucourt, M., Deborde, C., Moing, A. and Roin, D. (2006): Microclimate influence on mineral and metabolic profiles of grape berries. *J. Agric and Food Chem*, 54, 6765-6775.

[33] Perumal, S., Klaus, B. and Harinder, P.S.M. (2001): Chemical composition, protein fractionation, essential amino acid potential and antimetabolic constituents of an unconventional legume, Gila Bann seed kernel. *J. Sci. Food Agric.*, 82(20), 192-202.

[34] Sharma, S., Paliwal, S. andTilak, J.D.A. (2011): First report on laxative activity of *Citrullus lanatus. Pharmacologyonline*2: 790-797.

[35] Singh, U., Rao, V.P. and Subrahmanuam, N. (1993): Cooking, chemical composition and protein quality of newly developed genotypes of pigeon pea (*Cajanus cajan* L). *J. Sci. Food Agric*., 61: 395-400.

[36] Supatcha, J., Chi-Tang, H.O., Visith, H.O.C., Min-Hsiung, P.A.N., Sasivimon, C., Khanitta, R. and Monthana, W. (2015): Physicochemical properties of *Terminalia catappa* seed oil as a novel dietary lipid source. *J. Food Drug Anal* 23, 201-209.

[37] Tarun, K.G., Habibur, R., Dipankar, B. and Fulchan, A. (2013).*In-vitro* antibacterial study of *Aquilariaagallocha*heart wood oil and *Citrullus lanatus* seed oil. *Scholars J. Applied Med Sci*1 (1), 13-15.

[38] Tilak, R.M., Tukaram, A., Brij, M.L. and Valangaman, S.S. (2006). A study of seeds of musk melon (*Cucumismelo*L.), A lesser known source of edible oil. *Journal Science of Food and Agric*,10: 973-978.

[39] Wani, A.A., Sogi, D.S., Singh, P. andGo¨tz, A. (2013): Impacts of Refining and Antioxidants on the Physico-Chemical Characteristics and Oxidative Stability of Watermelon Seed Oil. J. Am. Oil Chem Society 90: 1423–1430.

[40] WHO/FAO, (1994): Fats and oil in Human Nutrition (Report of Joint Expert Consultation). FAO food and Nutrition Paper 57, Rome.

[41] Zamora**,** R, Alaiz, M. and Hidalgo, F.I. (2001).Influence of Cultivar and Fruit Ripening on Olive (*Olea europaea*) Fruit Protein Content, Composition, and Antioxidant Activity**.** *J. Agric. Food Chem.*49 (9):4267-4270.

[42] Ziyada, A.K. and Elhussien, S.A. (2008). Physical and chemical characteristics of *Citrullus lanatus* Var. Colocynthoide seed oil. J. Physiological Science 19, 69-75.