
Nutritional Potential of Co-products of Two Species of Benin Cucurbits

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Abstract: In the perspective of sustainable development, which involves the use of all biomass, two oilseeds have been selected according to their potential interests: *Citrullus lanatus* and *Lagenaria siceraria*. The physico-chemical characterization (oil and extraction cake) has been done. Thus, for extracted oils, unsaturated fatty acids are in the majority with the predominance of oleic and linoleic acids (21.31 to 44.36% and 16.20 to 70.35% respectively). The study of unsaponifiable fractions revealed that: *Citrullus lanatus* oil contains 134 mg/100g sterols (including 78.60% β -sitosterol) and 83.9mg/100g tocopherols (including 73.7% α -tocopherol) and *Lagenaria siceraria* oil contains 124mg/100 sterols (including 58.14% β -sitosterol) and 76.4mg/100g tocopherols (including 66.9% α -tocopherol). Phospholipids (0.39 and 0.40% respectively for *Lagenaria siceraria* and *Citrullus lanatus*) were quantified on the basis of phosphorus content. This study shows the nutritional value of oils through their compositions in essential fatty acids, their richness in phytosterols and tocopherols on the one hand and the ways in which co-products resulting from the extraction of selected seeds on the other hand are valued. Research showing the influence of stabilizing agents on the quality and stability of oils should be carried out to make their use easier. Tests of the use of these oils in the fields of cosmetics, pharmaceuticals, paints and polymer synthesis industries could be carried out.

Keywords: Biomass, *Citrullus Lanatus*, *Lagenaria Siceraria*, Chemical Characterization

1. Introduction

Our human societies are facing challenges that are recognized as vital [1]. These issues are those of the availability of resources, particularly water and energy, health, particularly in its relationship to food and the environment, and socio-cultural traditions. Addressing these challenges requires a strong mobilization of innovation capacities, with a determination to propose viable and sustainable responses [2]. Agriculture, and particularly the cereal professions, can and should play a role in determining the best options for addressing these issues and their treatment [3]. The valorization of biomass for chemistry and energy is motivated by the need to develop new sectors based on the identification of plant species adapted to these uses.

As a result, food seeds known and appreciated by African populations such as Cucurbitaceae species, commonly known as squash in the Republic of Congo, "pistachios" in Côte d'Ivoire or "Egusi" in Benin and Nigeria [4], are vegetable sources rich in protein and can thus constitute a solution to protein-caloric malnutrition. Cucurbits (Egusi) containing a large number of species are oil plants unconventional. Their seeds are widely used throughout the world in association with other foods to meet human protein and lipid needs. Previous studies on the ecobotanical characteristics [5] and fruit or seed yield [6] of species grown in West Africa have been widely published.

Very few studies on the nutritional aspects of the seeds of the different cucurbit species have revealed that the species *Citrullus lanatus* and *Lagenaria siceraria* have more

interesting nutritional potential [7-9].

The oils extracted from Cucurbits are rarely studied in the literature. To our knowledge, no studies on the nutritional properties of co-products of cucurbit species in particular: *Citrullus lanatus* and *Lagenaria siceraria* (species more appreciated in Benin) have been carried out as in the case of conventional oils and cakes (coconut, palm, palm kernel, soya and their cakes). Thus, it is appropriate that the oils and cakes of *Citrullus lanatus* and *Lagenaria siceraria* be characterized in order to enhance the value of the seeds of these species. This work entitled "Nutritional potential of co-products of two species of cucurbits in Benin" therefore aims to fill this gap.

2. Materials and Methods

2.1. Materials

2.1.1. Seeds

The collection of the seed lot analysed was carried out in the North (Savalou) of Benin (West Africa). They were properly dried in the sun until the seeds "sounded". The seeds were separated from the husks, properly sorted and cleared of all impurities, then shelled and finely ground. The almonds were finally packaged at 25°C in aluminium foil before the oil was extracted.

2.1.2. Oil

The non-conventional oils were obtained by extracting the seeds (finely ground) in Soxhlet for 6 hours at a temperature of 70°C. Traces of hexane were removed by rotavapor. The extracted oils were packaged in dark bottles and in an inert atmosphere of dinitrogen.

2.2. Methods

2.2.1. Physico-chemical Characterization of Oils

The water and volatile matter contents are determined according to standard NF T 60-201 and hexane extractives (oil content) by standard NF V03-924. The acidity, peroxide value and saponification value are determined respectively according to the protocol of standards NF T60-204, NF T60-220, NF ISO 3657.

2.2.2. Determination of the Mineral Elements of the Oils

The mineral elements (N, P, Ca, Mg, Mn, Zn, Cu, Na) were determined by ICP (Inductively Coupled Plasma) after mineralization of the sample. The principle of PCI is based on atomic emission and emission phenomena occurring in a plasma. The very high temperature of the plasma (7000 to 10000 K) compared to that of a flame (1000 K) allows a better dissociation of the chemical species. The samples are dissolved according to the dry digestion procedure. The Varian Vista spectrometer is equipped with a CCD (Coupled Charge Device) detector. The device (Jobin Yvon JY) was at the following wavelengths: $\lambda = 214.914$ nm for phosphorus and $\lambda = 589.592$ nm for sodium. The assays were carried out by performing a calibration that respects the conditions of the analyzed medium (matrix, acidity). The calculations were

performed by interpolation with respect to the calibration range. The validation of analytical results is based on the analysis of internal reference samples (controls), the mineral content of which is known.

2.2.3. Determination of Fatty Acid Composition by GPC

i. Preparation of Methyl Esters of Fatty Acids

The methyl esters have been prepared according to the NF T60-233 standard protocol.

ii. Mass Composition of Fatty Acids

To determine the fatty acid composition, 1 μ L of a hexane methyl ester solution was injected into an Agilent 6890 HP series GC (Agilent, USA) equipped with an INNOWAX column (Agilent, USA), 30m long, 0.32mm internal diameter and a 0.25 μ m film thickness. The injector was in split mode, ratio 1/80 at 250°C. The carrier gas was helium at a flow rate of 1.5 mL/min. The flame ionization detector was at 270°C. The furnace temperature was programmed as follows: 150°C for 3 minutes followed by an increase of 3°C/min to 220°C (26.3mm) which was kept constant until the end of the acquisition (35.3mm).

Peak identification was done by comparing the retention times of methyl esters of fatty acids of vegetable oils such as olive oil, sunflower oil and palm oil, injected under the same operating conditions. In order to verify the reproducibility of the results, each injection was repeated three times under the same operating conditions.

2.2.4. Determination of Total Oil Sterols

i. Determination of the unsaponifiable content

Unsaponifiable contents were determined using the IUPAC method [10].

ii. Preparation of the sterol fraction

To determine the total sterols, 0.5 g of oil, 1mL of cholesterol and 5mL of alcoholic KOH were introduced into a flask with 2 grains of pumice stone and refluxed for 15 min. 5ml of ethanol are then introduced into the flask from the top of the refrigerant. 10mL of this solution are introduced into a chromatography column filled with aluminum oxide ($0.063 < I < 0.2$ mm). Elutions were made successively with 5ml ethanol and 30 mL diethyl ether. The fraction obtained was after evaporation of the solvent, redissolved in 1mL of chloroform.

iii. Preparative CCM of the sterol fraction

20 μ L of a cholesterol standard solution and 400 μ L of the unsaponifiable oil fraction were successively deposited using a Linomat IV-Y CAMAG applicator (Merck, Ref. 022-786) on a silica plate 60 (Alltech, 20 \times 10 cm, 250 μ m thick). The elution was made by a chloroform/diethylether mixture (90/10, %v/v). The part containing the cholesterol deposit was revealed by nebulizing a $\text{Cu}^{++}/\text{H}_3\text{PO}_4$ mixture (1/1, %v/v) and baking at 180°C for 10 min.

The sterol band corresponding to the cholesterol spot was scraped off and the sterols were desorbed in chloroform (10mL/g silica) at room temperature under magnetic agitation for 5 min. Once the silica became transparent, a Millipore filter filtration (0.45 μ m, Ref. SLFH 013 NL) allowed the total sterols to be recovered without solid

contaminants.

iv. Sterol composition and content

1 μ L of this sterol fraction was injected with GPC to determine the sterol content of the oil. The sterol analysis was performed under isothermal conditions (285°C) in a GPC CG 8000 apparatus (Fisons Instruments) equipped with a SAC-5 column (Sigma-Aldrich, USA), (length 30m, 0.25mm internal diameter and 0.25 μ m film thickness). The temperature of the flame ionization detector is maintained at 300°C and that of the injector in split mode, ratio 1/100 at 300°C. The integration of the peaks was done using a Merck D2000 integrator. The carrier gas was helium (1.5 mL/min). To identify the peaks, we injected standards of cholesterol, β -sitosterol and stigmasterol (Sigma quality products, concentration of 1mg/mL). In order to verify the reproducibility of the results, each injection was repeated three times under the same operating conditions.

2.2.5. Determination of Tocopherol Composition and Content by HPLC/UV

The analysis of oil tocopherols was performed by HPLC in the normal phase. A solution of 20mg oil per mL hexane and isopropanol (99: 1) was filtered through a millipore filter with a diameter of 0.45 μ m. The device and its accessories (pump, injector, detector) are products of Agilent 1100 Series (France), comprising a quaternary pump, a manual injector with a 20 μ L injection loop and a DAD detector (with diode strips). The column was of type Luna Si 60, 5 μ m, 4.6 x 250mm (Phenomenex, France). The solvent mixture under isocratic conditions was composed of hexane and isopropanol for HPLC (99: 1, % v: v). The column flow rate was 1 mL/min and the pressure 33 bar with a DAD detector at 295nm wavelength. The peaks were identified by injecting tocopherol standards (Sigma aldrich products). The calibration curves were plotted using a dilution range of 0.3 to 8 μ L/mL.

3. Results and Discussion

3.1. Physical and Chemical Characteristics of the Extracted Oils

The physico-chemical characteristics of the extracted oils are presented in Table 1. They are compared to other oils commonly used in Benin for various applications. The oil contents (51.20 and 53% respectively for *Citrullus lanatus* and *Lagenaria siceraria*) are higher than those of cotton and groundnut oils [11]. These values are consistent with those obtained by [12]. Compared to the standards and some parameters of these oils (Table 1), it appears that the extracted oils meet all the quality criteria that would give them a good application under less expensive conditions [13-15].

3.1.1. The Refractive Index (IR) and Iodine Index (ID)

The refractive index shows a low dispersion of values for both oils. This table shows a difference between the iodine indices according to the oils. The two indices IR and ID are important criteria for identifying oils. According to Wolff

cited in [16], *there is a close relationship between the iodine index and the refractive index*. For a non-polymerized, unoxidized oil, the two indices vary in the same direction. This allows oils to be classified as non-drying oils (ID<100 and 1.467<IR<1.472), semi-siccative oils (100<ID<130 and 1.470<IR<1.478) and drying oils (ID>130 and 1.481<IR<1.482). The examination of Table 1 shows that this report is not always respected. But the results obtained show a clear distinction between *Lagenaria siceraria* oils (IR = 1.23; ID =100.54) and *Citrullus lanatus* oils (IR=1.36; ID = 112.8). The refraction indices obtained with the oils analysed comply with the standards of the codex alimentarius. These high iodine index values can be explained by low oxidation of unsaturated fatty acids.

3.1.2. The Peroxide Value and the Acid Value

When an oil is not stored properly, its quality can deteriorate in various ways, but most often by hydrolysis or oxidation. In this case, it becomes unfit for consumption. Thus, the peroxide value which makes it possible to assess the degree of oxidation of an oil and the acid value which measures the quantity of free fatty acids resulting from triglyceride hydrolysis reactions are two quality criteria for reporting on the condition of an oil. The values of the peroxide indices obtained for the different oils (Table 1) comply with the standards of the codex alimentarius, which sets the value below 10 mEq of peroxides/kg of oil. This means that the oils analysed are very poorly oxidised. This low oxidation would be due to the extraction conditions which can cause a low oxidation of unsaturated fatty acids leading to their increase. This would justify the high values of the iodine indices obtained. From an acidity point of view, the values of the acid indices obtained in Table 1 range from 1.04 \pm 0.05 (*Lagenaria siceraria*) to 1.20 \pm 0.03 (*Citrullus lanatus*). However, according to the codex alimentarius, an oil of good quality must have little or no acidity. However, no sample analysed has zero acidity, but the results obtained with the oils still comply with codex standards.

3.1.3. The Saponification Index

The saponification index indicates the fatty acid content (esterified and free) of an oil. These values are 197.29 \pm 0.32 mg KOH/g (*Lagenaria siceraria*) and 195 \pm 0.21 mg KOH/g (*Citrullus lanatus*) and therefore compatible with a predominance of C18 fatty acids. These values are similar to those obtained for vegetable oils such as soybean (189-195mgKOH/g-oil), peanut (187-196 mg KOH/g-oil) and cotton (189-195 mg KOH/g-oil [17]). These values are higher than those obtained by [18]. *The strong saponification indices observed would justify a possible use of these oils in soap making.*

3.1.4. Content of Unsaponifiable Substances

The unsaponifiable contents are close (1.58) for *Lagenaria siceraria* oil and (1.65) for *Citrullus lanatus* oil. The study of their unsaponifiable fractions has shown that they have, in accordance with their high unsaponifiable content, high

levels of sterols and especially tocopherols, which guarantee good resistance to oxidation. The exceptional phospholipid contents of the oils are comparable to that of rapeseed oil, one of the richest in phospholipids [19].

Consequently, the physical and chemical properties of the oils presented in Table 1 are generally in agreement with the bibliographic data for the results that can be compared.

Table 1. Chemical characteristics of the extracted oils.

Characteristics (g/100g-MS)	C. I	LS
Oil content (%-MS)	51.20±0.22	53±0.20
Acidity (%-oleic)	1.20±0.30	1.04±0.50
Acid value (mg KOH/g)	1.98±0.01	2.56±0.01
saponification index (mg de KOH/g-oil)	195.29±0.21	197.29±0.32
Refractive index	1.36±0.32	1.23±0.32
Iodine index (g iodine / 100g oil)	112.8±0.45	100.54±0.50
Peroxide value (meq d ^o O ₂ /Kg oil)	0.99±0.02	1.06±0.01
unsaponifiable (%)	1.65±0.00	1.58±0.00
Total phospholipids	0.53±0.15	0.35±0.25

C. I: Citrullus lanatus; LS: Lageraria siceraria

3.2. Fatty Acid Composition of Oils

The levels of capric, lauric, myristic, palmitic, stearic, oleic, linoleic and in the analyzed oils are grouped in Table 2. The results obtained show profiles that are often specific to each type of oil. Gas chromatographic analysis of the total methyl esters of the fatty substances of the investigated seeds reveals the presence of three main fatty acids: palmitic acid (C16: 0), oleic acid (C18: 1) and linoleic acid (C18: 2). These oils have comparable profiles, although sometimes the percentages vary. However, we can see that these oils are very nutritious in view of their content of essential fatty acids (oleic and linoleic acid). The profile shows on the one hand the predominance of oleic, linoleic, palmitic acid and on the other hand a small percentage of myristic acid. In general, these results support the standards set by FAO. From the composition of the extracted oils, it appears that unsaturated fatty acids are strongly represented compared to saturated fatty acids which are practically only represented by palmitic acid. This high richness in unsaturated fatty acids could make oils of unsaturated types. Like olive, soybean, cotton, cotton, sunflower and groundnut oils, the oils studied are characterized by a majority presence of fatty acids with 18 carbon atoms. They could be used in various fields of food, cosmetics and soap-making due to their fatty acid composition and their physico-chemical characteristics.

Table 2. Fatty acid composition of extracted oils.

Fatty acids (%)	C. I	L. s
Myristic acid (C14: 0)	2.53±0.38	3.76±0.33
Palmitic acid (C16: 0)	15.35±0.48	12.05±0.19
Stearic acid (C18: 0)	11.77±0.01	9.48±0.01
Oleic acid (C18: 1, n-9)	20.31±0.02	14.04±0.03
Linoleic acid (C18: 2, n-9, 12)	49.20±0.00	60.10±0.01
Linolenic acid (C18:3, n-9, 12, 15)	0.42±0.22	0.15±0.00
Arachidic acid (C20: 0)	0.14±0.01	0.39±0.01
Gadoleic acid (C20: 1, n-9)	0.28±0.02	0.03±0.00

3.3. Sterol and Tocopherol Profile of Oils

3.3.1. Sterol Content

This family of compounds, especially β -sitosterol, helps to fight cardiovascular disease by reducing intestinal cholesterol adsorption [20]. β -sitosterol is the majority sterol followed by stigmasterol (Table 3). The total sterol contents (124 to 134 mg/100g oil) are in the same order of magnitude as those of olive oil (119-268 mg/100g), groundnut and palm oil (127-171 and 123-140 mg/100g respectively) [21]. However, based on the results obtained, several ways of recovering the oils are possible:

1. In oncology: some sterols are reported to have anti-cancer activities and inhibit colon, rectal and lung cancers.
2. In the diet, a phytosterol supplement would have a cholesterol-lowering effect and thus limit the risk of cardiovascular disease.
3. And in cosmetology: sterols are used in many products with anti-inflammatory activities, or to repair skin damage and shampoos.

Table 3. Composition of the sterols fraction of extracted oils.

Constituents	C. I	L. s
Campesterol (%)	3.53±0.03	11.56±0.38
Lanosterol (%)	0.01±0.00	0.15±0.00
Δ^5 -avenasterol (%)	5.35±0.22	7.14±0.19
Stigmasterol (%)	12.51±0.04	23.01±0.02
β -sitosterol (%)	78.60±0.50	58.14±0.45
total Sterols (mg/100g)	134.00±0.32	124.00±0.45

3.3.2. Tocopherol Content

Tocopherols and tocotrienols (tocols) are aromatic compounds with a chromanol ring (the carbon atom 6 carries a hydroxyl group) substituted by a chain with 3 condensed isopentenyl units. Tocotrienols are rarely found in oils, except in the case of palm oil. Tocopherols, more present, although in minor fraction, are interesting because of their antioxidant and also vitaminic properties, mainly due to α tocopherol (vitamin E) [22]. This vitamin, whose biological properties are numerous, is used in several fields, particularly in cosmetology, and in several fields related to medicine: cardiology, anti-inflammatory drugs, oncology. The results obtained are summarized in Table 4. Tocopherols, mainly α -tocopherol are the main compounds. We can also note the presence of δ -tocotrienol, rather rare in vegetable oils. δ -tocopherol, the most effective antioxidant, is present in all the oils studied.

Table 4. Composition of the tocopherols and tocotrienols in extracted oils.

Constituents	C. I	L. s
δ -tocotrienol (%)	0.70±0.01	0.66±0.01
(β + γ)-tocotrienol (%)	1.44±0.00	1.34±0.00
α -tocotrienol (%)	1.01±0.02	1.00±0.01
δ -tocopherol (%)	0.83±0.11	1.05±0.10
(β + γ)-tocopherol (%)	22.33±0.10	29.05±0.10
α -tocopherol (%)	73.70±0.22	66.90±0.33
total Tocopherols (mg/100g)	83.90±0.30	76.4±0.38

3.4. Extraction Cakes

3.4.1. Mineral Element Content of Extraction Cakes

Table 5 presents the mineral element composition of the extraction cakes in the seeds studied. The main minerals are phosphorus, nitrogen and potassium. The relatively high levels of N, P, K in *Citrullus lanatus* oilcake may favour its use in fertilizer formulations. In general, the mineral element composition of our extraction cakes is low compared to that of delipidated soybean, rapeseed, cotton, groundnut and palm nut cakes [23]. The proportions of N, P, K of *Citrullus lanatus* are higher than those of *Lagenaria siceraria*. This difference suggests the use of *Citrullus lanatus* oilcake as a replacement for chemical fertilizers in agriculture. These results show that oilcake can be a potential source of major and minor mineral elements, and can therefore be used to partially fill food and/or feed.

Table 5. Minerals elements of oilcakes.

Constituents	C. l	L. s
totaux mineral	9.40±0.00	6.30±0.00
N (%)	6.18±0.01	3.36±0.02
P (%)	1.23±0.00	0.88±0.02
K (%)	1.28±0.00	0.85±0.01
Mg (%)	0.48±0.00	0.57±0.00
Ca (%)	0.13±0.01	0.41±0.01
Na (%)	0.03±0.00	0.12±0.00

3.4.2. Organic Matter Content of the Oilcake

The organic matter content of the extracted oilcake presented in Table 6 reflects their digestive character and testifies to their possible use as second-generation biofuels. From the analysis of this table, it appears that the two seeds studied have a significant variation in their respective chemical composition. The humidity levels are all below 9% which should encourage their storage. Generally speaking, we can see on this table that proteins constitute the main component of the two meals while the proportions of the other components are variable. The total protein contents of *Citrullus lanatus* and *Lagenaria siceraria* are comparable to the data in the literature [24]. These values are lower than those of soybean meal (36-53% MS meal). The protein proportions of *Citrullus lanatus* are comparable to those of rapeseed and sunflower oilcake (20-25%MS). If we consider each meal individually, *Lagenaria siceraria* has the highest content of crude fibre (15.50%MS meal) and lignin (8.90%MS meal). The high proportions of starch and total sugars are indicative of the digestive character of the cakes studied and their use in animal feed [25]. Oilcake can be used as an energy source for some animals as long as it is not subject to rancidity. These exceptional levels could be explained by high proportions of polar lipids and/or a particular position of these lipids in the plant matrix. It is likely that polar lipids, especially phospholipids, are not extractable with hexane and require a prior acid attack to destroy cell walls. Nutrient-rich cakes could be used as livestock feed. However, they would constitute a lignocellulosic feedstock for second-generation bioethanol [26].

Table 6. Organic matter content of cakes.

characteristics (g/100g-MS)	C. l	L. s
Total organic matter	90.16±0.33	98.96±0.32
Total proteins	39.64±0.16	37.15±0.13
Cellulose Wende	12.50±0.50	15.50±0.45
Lignins	4.90±0.00	8.90±0.01
Lignocellulose	10.00±0.22	16.24±0.22
Hemicellulose	19.80±0.01	20.13±0.02
Starch starch	62.90±0.36	70.45±0.50
Total sugars	35.00±0.10	33.45±0.00

3.5. Amino Acids from Extraction Cakes

Table 7 gives the amino acid composition of the extraction cakes. The levels of essential amino acids for humans (isoleucine, leucine, lysine, methionine, phenylalanine, treonine, tryptophan, valine and histidine), are in the range of 1.54 - 2.05% MS cakes for leucine. The contents for other essential amino acids are in the range of 0.90 - 1.72% MS-tourteau. However, the methionine contents are low (0.50 - 0.64% MS oilcake). The amino acid compositions of the seeds are comparable to that of groundnuts [27]. These data confirm the nutritional value of seeds and their nutritional uses.

Table 7. Amino acids composition of extraction cakes.

Amino acids (% MS - cakes)	C. l	L. s
Valine	1.11±0.00	2.33±0.01
Isoleucine	0.93±0.01	1.66±0.00
Leucine	1.54±0.00	2.05±0.03
Lysine	0.64±0.00	0.14±0.00
Tyrosine	0.72±0.02	1.02±0.03
Methionine	0.64±0.01	0.50±0.00
Phenylalanine	1.25±0.00	2.01±0.01
Arginine	2.20±0.15	1.75±0.01
Serine	0.99±0.00	nd
Histidine	0.49±0.01	1.40±0.03
Glycine	nd	3.04±0.00
Alanine	1.13±0.00	2.34±0.02
glutamic acid	3.73±0.02	1.16±0.01
Aspartic acid	1.37±0.00	2.33±0.03
Treonine	0.90±0.00	nd
Proline	1.33±0.01	nd

nd=not detected

4. Conclusion

This work is a contribution to the characterization of co-products of *Citrullus lanatus* and *Lagenaria siceraria* from Benin. Fat contents and chemical characteristics are reported. The data obtained are also in compliance with standards and show their good qualities and possible uses in several areas such as food, soil amendment... etc. These species are of particular nutritional interest, given their chemical composition and nutritional potential.

The predominance of unsaturated fatty acids such as oleic acid and linoleic acid is compatible with the values obtained for iodine and saponification indices and justifies the liquid aspect of oils at ordinary temperature. This study has updated the data in the literature and should support the agri-food and

socio-economic interest of the species studied, which would constitute an important source of organic substances (proteins and lipids) and mineral substances (phosphorus, calcium, potassium, sodium, magnesium).

The development of forest resources for industrial purposes can contribute to the protection of our threatened forests. Indeed, the preservation of standing species through diversification and greater valorisation of their by-products can promote their development.

It would be interesting to set up a complete seed fractionation process to produce certain functional ingredients. These ingredients must be tested in food formulations to assess their sensory, nutritional and economic added value. This idea of fractionation could also be applied to other seeds that are widely used and known for their nutritional potential.

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