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CHARACTERIZATION OF *DESMA* (*NOVELLA PENTADESMA*) SEED AND OIL

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ABSTRACT

In this study, the characterization of desma seed and oil was carried out to determine the biochemical properties of the seed, physco-chemical and nutritional qualities of the oil using standard procedure. The results showed that the fat, protein, carbohydrate, crude fibre, ash and oxalic acid contents of the seed were 53.3, 25.0, 9.5, 4.1, 5.4 and 2.7 %, respectively. The fat comprised 0.03-0.13 % lecithin, 52:46 cephalin ratio and neutral triglycerides of phosphatides. The analysis of the oil revealed specific gravity at 25^0C, refractive index, acid value, saponification value and iodine value as 0.920, 1.464 n_D^{50}, 1.008 mg NaOH/g of oil, 186 mg KOH/g of oil and 112 gI_2/100g of oil, respectively. The oil is practically free of toxic components and contains more unsaturated fatty acids than many other vegetable oils with Food and Agricultural organization reference values. The high proportion of unsaturated fatty acid (1.0 % oleic) makes it an important source of essential fatty acids in the diet. The results obtained were within the American Standard for Testing of Material standard specifications.

Keyword- Desma seed and oil, cosmetic characterization, bio-chemical, physic-chemical properties

1.0 INTRODUCTION

Novella pentadesma is among the most important group of edible oil seeds that is found abundantly in South-South Nigeria. The seed has been reported to contain more oil than its close associate, *Novella allanblankia* seed. The oil is used in the food industry for the manufacture of cake, chocolate, butter and liquor of bean wine; and energy industry for the production of biodiesel. The seed is a good source of minerals and vitamins particularly calcium, phospohorus, potassium and iron (Baraski *et al.*, 2006).

The oil is non-chlesterone, polyunsaturated and semidrying. The seed contains fats, proteins, carbohydrates, crude fibre, ash and oxalic acid, thus emphasizing its potential uniqueness in many industrial applications. The oil contains neural triglycerides; oleic and linoleic acids which are the major fatty acids and are present in approximately equal amounts. The un-saponifiable matter in desma oil includes sterols, triterpenes and triterpene alcohols which include at least six compounds: copherols, desmamin and desmamolin which are not found in other edible vegetable oils. It is practically free of toxic components (Altisent, 2006).

Many researchers have studied the physicochemical, nutritional and biochemical properties of oils from various seeds. Hills and Murphi (1988) have characterized lipases from the lipid bodies and microsomal membranes of erucic acid-free oilseed-rape and found out that microsomal lipase was greatly inhibited at higher pH values, whereas the lipid-body lipase was much less affected. Akpan *et al.* (2012) have characterized castor oilseed and reported that the tested parameters, which include specific gravity, refractive index, acid value, saponification value and iodine value to fall within the ASTM

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specifications. However, studies on the characterization of desma seed and oil were not undertaken in the previous studies. Hence the objective of this research is to characterize desma seed and oil with a view to enhancing its economic value in the food and energy industries.
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2.0 MATERIALS AND METHODS

2.1.1 Sample Preparation

Desma seeds were obtained from the plantation communities at Ovia North-east and South-west local governments of Edo State, respectively. Damaged seeds were discarded, then seeds in good condition were cleaned, shelled and dried at 105°C for 30 min. Seeds were grounded using a grinder prior to oil extraction. All chemicals used in the study were of analytical grade and used without further purification. The extraction of desma seed oil was carried out by soxhlet extraction. The desma seed powder was extracted using hexane as a solvent for 6 hours.

2.1.1 Analysis of Desma Seed Oil

The weight of the oil extracted from 500g of seed powder was determined to calculate the lipid content. The result was expressed as the percentage of lipids in the composition of the dry seed powder. The acid value of desma seed oil was determined according to AOAC (1989) standard procedure. Percentage free fatty acids (FFAs) were calculated using the constant for oleic acid as factor. Iodine value was determined according to British standard BS 684: section 2.13:1976. The saponification value was determined according to British standard BS 684 2.6:1977. The unsaponifiable matter was determined according to AOCS Ca 6a-40 (1989). The Cielab coordinates (L*, a*, b*) were directly read using the spectrophotometer (Minolta chromometer CR 100). The levels of Triacyl glycerides in desma seed oil was determined according to the equation suggested by Akpan *et al.* (2012):

TAG (%) = 100 – [(free fatty acid, %) + (unsaponifiable matter, %)]

Fatty acid composition of seed oil was determined using gas chromatography (GC). About 0.1 ml oil was converted to methyl ester using 1 ml NaOMe (1M) in 1 ml hexane before being injected into the gas chromatograph. A GC analysis was performed on a Shimadzu gas chromatograph equipped with flame ionization detector and capillary column (30 m × 0.25 mm × 0.25 µm films). The detector temperature was programmed for 280°C with flow rate of 0.3 ml/min. The injector temperature was set at 250°C. Nitrogen was used as the carrier gas. Identification of the peaks was performed by comparing retention times with those of genuine standards analyzed under the same conditions.

The TAG profile of Desma seed oil was determined by high-performance liquid chromatography (HPLC), Waters model 1515 equipped with refractive index detector. The TAG of the oil were separated using a commercially packed spherisorb C18 column (150mm × 4.8µm ×3mm) from Waters. The mobile phase was a mixture of acetone:acetonitrile (63.5:36.5) set at a flow rate of 1ml/min. Sample preparation involved sample dilution with acetone:acetonitrile (63.5:36.5) before injection of 20 µL of the sample into the HPLC with total running time of 50 min. To determine the functional groups in Desma seed oil, FTIR and NMR spectroscopy methods were employed. FTIR of the products was recorded on a Perkin Elmer Spectrum GX spectrophotometer in the range 400-4000 cm^{-1}.

FTIR was used to measure functional groups of desma seed oil. A very thin film of desma seed oil was applied to NaCl cells (25 mm diameter × 4 mm thickness) for analysis. ^{1}H and ^{13}C NMR analysis was performed with NMR spectroscopy model Joel FCP 400 MHz with the solvent $CDCl_3$. ^{1}H and 13C NMR of desma seed oil was recorded on a Bruker 300 NMR spectrophotometer. For this analysis, 20 mg of the MRSO sample was dissolved in 1ml of $CDCl_3$ and introduced into the NMR tube.

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3.0 RESULTS AND DISCUSSIONS

3.1 Bio-chemical Properties of Desma Seed

The proximate contents of desma seed were shown in Table 1. In general, from the three replicates (extracts from samples) the seeds contain about 53.3% fat, 25 % protein, 9.5 % carbohydrates, 4.1 % crude fibre, 5.4 % ash and 2.7 % oxalic acid.

Table 1: Proximate composition of desma seed (%, moisture free basis)

Parameter	% Composition
Fat	53.3
Protein	25
Carbohydrate	9.5
Crude fibre	4.1
Ash	5.4
Oxalic Acid	2.7

3.1.1 Protein Composition and Solubility

Desma seed contains 25% protein. The proteins in the seed are mostly located in the outer layers of the seed. Based on their solubility, desma proteins have been classified as albumin (8.6%), globulins (67.3%), prolamin (1.3%) and glutelin (6.9%) fractions.

Globulin is the predominant protein fraction in the desma seed. It is composed of two components, α – Globulin, the major fraction accounts for about 60-70% of the total seed globulin, while β - Globulin is a minor component contributing 25% to the globulin fraction. α – Globulin is a high molecular weight protein (250,000 – 360,000 MW) and has a sedimentation co-efficient of 11-13 S. It is an oligomeric protein composed of six diametric units of molecular weight of about 50,000 – 60,000. The diameric unit is of the A-B type linked by a disulfide bond. β - Globulin is a minor component of desma seed globulins. It has a molecular weight of 15,000, and is rich in acidic and hydrophobic amino acids.

An understanding of the solubility characteristics of desma protein is essential for developing methods of preparing protein concentrates and isolates as well as for assessing their applications as functional ingredients in various food systems (Akpan *et al.*, 2012).

The major proteins of desma flour, being globulins, are salt soluble. About 84% of the total protein can be solubilized by extraction with successive water and salt solutions. The ionic strength of the solution affects the solubility of desma protein by causing dissociation of electrostatically associated subunits.

Maximum extraction of desma protein is obtained above pH 9 at solvent water/powder ratio of 15:1. Very little protein is extracted between pH 2 and 8. However, desma protein can be maximally extracted at pH 6 if the ionic strength of the solution is increased to 1M with sodium or calcium chloride.

Isolated desma protein is insoluble in water between pH 6 and 7. It becomes progressively more soluble as the pH is made more acidic or basic. The minimum solubility in water around pH 6-7 reflects the lowest net charge or the isoelectric point. The solubility of desma α – Globulin is markedly affected by ionic strength. Thus, increasing ionic strength from 0 to 1M at pH

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7 progressively increases solubility to almost 90%. The pH of minimum solubility decreases as the ionic strength is increased, reflecting increase cation binding and neutralization of the anionic protein.

3.1.3 Carbohydrate content

Desma seed contains 10% carbohydrates comprised of glucose (3.2%), fructose (2.6%), sucrose (0.2%), raffinose (0.2%), stachyose (0.2%), planteose (0.6%), and several other minor oligosaccharides. In addition, it also contains 4% crude fibre present mostly in the seed coat. Hemicellulose A was found to contain galacturonic acid and glucose in the ratio of 1:12.9, while hemicellulose B contained galacturonic acid, glucose, arabinose, and xylose in the ratio of 1:3.8:3.8:3.1.

3.1.4 Mineral and Vitamin content

Desma seed is a good source of minerals, particularly calcium, phosphorus, potassium, and iron. Desma seeds are an important source of certain vitamins, especially niacin, folic acid, and vitamin E (Table 2). The vitamin A content of the seed is, however, very low. Desma oil is rich in tocopherols. However, the proportion of γ and δ tocopherols is more than that of α tocopherol. The latter has the highest vitamin E activity. Hence, the vitamin E activity of desma oil is less than that of soybean oil.

Table 2 Mineral and Vitamin Content of Desma Seed

Composition (mg/100g)	Content
Calcium	1020
Phosphorus	620
Iron	34
Sodium	72
Potassium	767
Vitamin A (IU)	Trace 60
Thiamin	0.17
Riboflavin	0.028
Niacin	6.78
Pantothenic acid	0.6
Ascobic Acid	0.5
Folic Acid	107
Vitamin E	
Total (α – tocopherol equv.)	36.30
α – Tocopherol	1.1
β- Tocopherol	0.4
γ – Tocopherol	34.6
δ – Tocopherol	0.2

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3.1.5 Antinutritional Factors

Desma seed contains antinutritional factors hence suitable for human consumption after processing. However, it contains negligible amounts of oxalate and phytate, which adversely affect mineral bioavailability in human nutrition. Oxalic acid is mostly present in the seed coat, which impact a slightly bitter taste to the whole seed because of chelation of calcium. Oxalic acid can be removed by treating the seed with hydrogen peroxide at pH 9.5.

Desma seeds contain a substantial amount of phosphorus, most of which is makes up phytic acid or as phytin (a calcium and magnesium salt of inositol hexaphosphate).

3.1.6 Lipid Content

The lipid of desma seeds are mostly comprised of neutral triglycerides with small quantities of phosphatides (0.03 to 0.13% with lecithin: caphalin ratio of 52:46). The phosphatides also contain about 7% of a fraction soluble in hot alcohol but in soluble when cold. Desma oil, however, has a relatively high percentage (1.2%) of unsaponifiable matter.

The glycerides are mixed types, principally oleo-dilinoleo, linoleo-dioleotriglycerides and triglycerides with one radical of a saturated fatty acid combined with one radical each of oleic and linoleic acids. The glycerides of desma oil, therefore, are mostly triunsaturated (58mol %) and diunsaturated (36 mol %) with small quantities (6 mol %) of monounsaturated glycerides. Trisaturated glycerides are practically absent in desma oil.

The unsaponifiable matter in desma oil includes sterols (principally comprised of β-sitosterol, campesterol, and stigmasterol), triterpenes and triterpene alcohols which include at least six compounds of which three were identified (viz; cycloartanol, 24-methylenecycloartanol, and α – amyrin), to copherols, and desmamin and desmamolin which are not found in any other edible vegetable oils (Akpan *et al.*, 2012). Among the pigments spectrophotometrically identified, pheophytin A (λ max 665-670 nm) was found to markedly predominate over pheophytin b (λmax 655nm). The pleasant aroma and taste principles contain C_5 – C_9 straight – chain aldehydes and acetylpyrazine.

3.2 Desma Seed Oil

3.2.1 Fatty Acid Composition

Table 3 shows the fatty acid composition of desma seed oil. The oil was classified as a polyunsaturated, semidrying oil; it contains more than 60% unsaturated fatty acids. Oleic and linoleic are the major fatty acids and are present in approximately equal amounts:

Table 3 Fatty acid composition of desma oil

Fatty Acid	Values (%)
Palmitic (C 16:0)	8.2
Stearic (C 18: 0)	4.1
Arachidic (C20: 0)	0.5
Oleic (C 18:1)	47.2
Linoleic (18:2)	40.1

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The saturated fatty acids, principally comprised of palmitic and stearic acids, accounting for less than 20% of the total fatty acids. Arachidic and Linolenic acids are present in very small quantities.

3.2.2 Endogenous Antioxidants

Desma oil is resistant to oxidative rancidity. It also exhibits noticeably greater resistance to autoxidation than would be expected from its content of tocopherols (vitamin E). This unusual stability to oxidation is often attributed to the presence of a large proportion of unsaponifiable matter (approximately 1.0 – 1.2%) in desma oil compared with those in other vegetable oils. Moreover, the unsaponifiable matter itself includes substances, such as desmamol and phytosterol, which are normally not found in other oils (Akpan *et al.*, 2012).

The remarkable stability of unrefined desma oil is attributed to the presence of endogenous phenolic antioxidants, viz; desmamin, desmamolin, and desmamol (or desmaminol) (Figure 1).

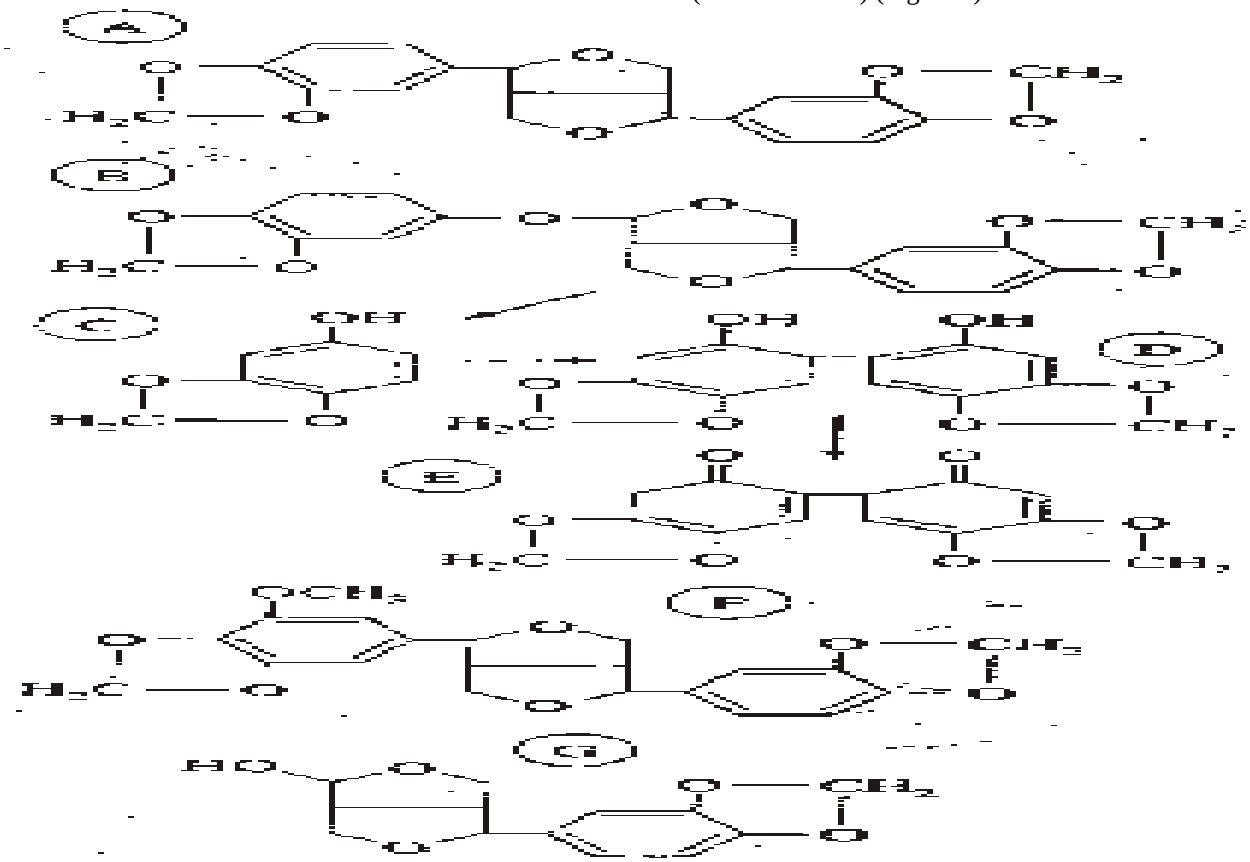

Figure 1: Structures of natural antioxidants found in desma oil. A. Desmamin B, Desmamolin; C, Desmamol; D, Desmamol dimer; E, Desmamol dimer quinone; F, Desmangolin; G, Desmamin.

Table 4 shows The concentrations of desma oil and their absorption characteristics.

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Table 4: Concentrations of desmamin, desmamolin, and desmamol in desma oil and their absorption characteristics

Parameter	Desmain	Desmamolin	Desmamol
Concentration (mg/100g oil)	295 – 880	125 – 458	Trace – 5.6
λ max	287	288.5	298
ε max	23.0	21.8	29.5
λ max	236	236	234
ε max	27.0	24.8	21.4

Desmamolin differs from desmamin in having an oxygen atom connecting one of its Methylene dioxyphenyl groups to the central tetrahydrofurfuro uv absorption with two maxima, at 287.5 and 23.5nm. This absorption of desma oil is, most likely primarily if not exclusively, due to its content of desmamin, desmamolin and desmamol.

3.3 Physico – Chemical Properties of Desma seed Oil

A number of parmeters are used to characterize vegetable oils. Data on some important characteristics of desma oil are summarized in Table 5. Desma oil is dextrorotatory, which is unusual for an oil devoid of optically active fatty acid glycerides. The unsaponifiable fraction of the oil, however, does contain optically active minor constituents, which are responsible for the optical rotation of the oil.

Table 5 Physico-chemical Properties of Desma Oil

Parameter	Unit of Measurement
pH	7.11
Colour	Not darker than 2-3 amber
Smoke point (^{0}c)	165
Flash point	319
Solidifying point (^{0}c)	-3 to-4
Specific gravity ($28^0/28^0$c)	0.920
Refractive index (n_D^{50})	1.464
Viscosity at 28^0C (standard)	9.425
Titer (^{0}c)	22
Acid value (mg Na0H/g of Oil)	1.008

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Saponification value (mg KOH/g of Oil)	186
Iodine value (gI_2/100g of Oil)	112
Free fatty acid (as % oleic)	1.00
Unsaponifiable matter (%)	2.30
Reichert – Meissel value	0.51
Polenske value	0.27
Hydroxyl number	3.30
Thiocyanogen value	74
Hehner value	9.57

3.4 Nutritional Quality of Desma Seed and Oil

Desma proteins are rich in sulphur-containing amino acids and low in lysine, which is unusual for oilseed proteins. Table 6 shows that among other essential amino acids, desma protein is borderline deficient for threonine, isoleucine, and valine contents compared with FAO (2007) reference values.

Table 6: Essential amino acid composition of Desma seed

		Protein Isolate			
Amino Acid	Flour	Water Extraction	Alkali Extraction	Salt Extraction	FAO/WHO Reference Protein
Tryptophan	2	1.8	-	-	1
Threonine	3.3	3.2	3.6	3.7	4
Valine	4.6	4.5	4.9	5.2	5
Methionine + crystine	5.9	3.7	3.2	2.1	3.5
Isoleucine	3.8	3.5	4	4.1	4.7
Leucine	6.6	6.6	6.7	6.6	7
Phenylalanine + tyrosine	8.1	7.9	8.7	8.1	6
Lysine	2.6	2.1	2.4	2.2	5.5

Data expressed as g/16g nitrogen

During the preparation of a protein isolate (>90% protein), there is some loss of methionine, cysteine, and tryptophan. This may reflect the selective recovery or elimination or certain proteins by the isolation methods employed (Akpan *et al.*, 2012).

The protein efficiency ratio (PER) of desma seed, flour and isolated protein are 1.86, 1.35, and 1.2, respectively. Supplementation of desma seed protein with lysine can increase its PER to 2.9. The biological value of desma seed protein

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is 62, which is lower than that of soybean (Akpan *et al.*, 2012).

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The amino acid composition of desma complements that of most other oilseed proteins. Tryptophan, which is limiting in many oilseed proteins, is adequate in desma. The availability of amino acids from desma seed protein can be affected by the method of processing. The high level of sulphur-containing amino acids in desma seed protein is unique. It suggests that desma protein should be more widely used as a supplement for methionine and tryptophan, and should be an excellent protein source for baby and weaning foods. The use of desma seed protein would eliminate the problems encountered when foods are supplemented with free methonine, which is unstable (Akpan *et al.*, 2012)..

Desma oil is practically free of toxic components. The oil contains more unsaturated fatty acids than many other vegetable oils with FAO reference values. The high proportion of unsaturated fatty acids renders it an important source of essential fatty acids in the diet. Linoleic acid is required for cell memberance structure, cholesterol transportation in the blood, and for prolonged blood clotting properties. Desma oil is rich in vitamin E but is deficient in vitamin A. The crude oil contains a relatively low amount of free fatty acids. The minor constituents, desmamin and desmamolin, present in desma oil protect the oil from oxidative rancidity.

4.0 CONCLUSIONS

The characterization of desma seed and oil lead to the following conclusions:

1. The results showed that the fat, protein, carbohydrate, crude fiber, ash and oxalic acid contents of the seed were 53.3, 25.0, 9.5, 4.1, 5.4 and 2.7 %, respectively.

2. The fat comprised 0.03 to 0.13 % lecithin, 52:46 caphalin ratio and neutral triglycerides of phosphatides.

3. The characterization analysis of the oil revealed that specific gravity at 25^0C, refractive index, acid value, saponafication value and iodine value were 0.920, 1.464 n_D^{50}, 1.008 mg NaOH/g of oil, 186 mg KOH/g of oil and 112 gI_2/100g of oil, respectively.

4. The oil is practically free of toxic components and contains more unsaturated fatty acids than many other vegetable oils with FAO reference values.

5. The high proportion of unsaturated fatty acid (1.0 % oleic) makes it an important source of essential fatty acids in the diet. The results obtained are within the ASTM standard specifications.

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