Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.549

Assessment of the Non-Triglyceride Constituents of Njangsa (*Ricinodendron heudelotii)* Seed Oil

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ABSTRACT

Njangsa seeds are widely used in West Africa as a spice for seasoning different dishes. The oil extracted from njangsa seeds is highly valued and expensive because of its aroma, taste, and bright golden colour. This study is carried out with the aim of determining the content of non-triglyceride component of njangsa seed oil. Njangsa seed oil was extracted with n-hexane, and some of its quality parameters were assessed. Spectroscopic analysis of the oil was carried out using IR and GC-MS spectroscopy. The yield of the oil extraction was 45.9% and the result of quality evaluation gave iodine value; 165.8 gI_2/100g, acid value1.39 KOH/g, Peroxide value 7.2 Meq/kg. The IR spectrum shows absorption peaks that revealed the presence of –OH, C=O, -C-O, C-C, C=C, and C-H functional groups. The GC-MS results revealed the presence of 24 different compounds of different classes that include alkanes, alkenes alcohols, esters, carboxylic acids, aldehydes, oxirane, and sulfur containing compounds. Njangsa seed oil therefore contains different type of non-triglyceride compounds that are responsible for its flavor, colour and other taste attributes that makes njangsa seed oil more appealing to comsumers.

Keywords: Njangsa, Chromatogram, Spectrum, Non-triglyceride

INTRODUCTION

Njangsa seed oil is extracted from Njangsa (*Ricinodendron heudelotii*) seeds from an oil seed tree called njangsa (*Ricinodendron heudelotii*), found in tropical West Africa (Simon and Leaky 2006). Njangsa plant has been a wild plant growing in the tropical West African forest, but because of its highly priced edible oil seeds, it is now being domesticated by commercial farmers in West Africa (Simon and Leaky 2006). Njangsa seed kernels are ground and used as spice for seasoning 'jollof' rice and pepper soup because of its aroma, flavour and the golden shiny colour it gives these African dishes. Njangsa seeds has high oil content, and the n-hexane extracted oil has about 52 % oil yield (Fai et al., 2018). The oil and the cake of njangsa seeds are edible and there has been no report of any toxic compound in the seed or report of toxicity as the result of the consumption of the njangsa seed kernels. The njangsa seed oil has iodine value of about 165 gI_2/100g of oil and saponification value of about 189mg/g of KOH and a drying oil, and can be useful as raw materials in paint and soap making industries (Fai et al., 2018).

Njangsa seed oil, like any other fixed vegetable oil is made up of mostly triglycerides, but also contain a small proportion of non-triglyceride matter (Narasinga Rao 2001). The phytochemicals that makes njangsa seed a spice are mostly oil soluble and are usually extracted with the njangsa seed oil during oil extraction. These phytochemicals constitute part of the non-triglyceride component of the njangsa seed oil, and are very important because they add more nutritional values to the njangsa seed oil. Generally vegetable oil are known as being

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triglycerides esters of long chain saturated and unsaturated fatty acids (Karmakar et al., 2020), but vegetable oil is more than just being triglycerides because vegetable oils generally contain non triglyceride components ranging from about 2 % to 10 % of the oil (Narasinga-Rao 2001), depending on the seeds from which it is extracted, method of extraction as well as refine or non-refined.

The non-triglyceride components of the vegetable oil are oil soluble phytochemicals found in oil bearing seeds or nuts that are extracted with the vegetable oils during oil extraction (Narasinga Rao 2001). These non-triglyceride component of the vegetable oil are very important because they define the colour, taste, flavour, and aroma in vegetable oil. Non-triglyceride components of some vegetable oil also add nutritional and physiological functions to oils. The classification of vegetable oils as edible and non-edible oils depends mostly on the type and the amount of non-triglyceride components of the vegetable oil (Menezes et al., 2006, Sharma and Singh 2008). The knowledge of compounds that constitute the non-triglycerides components of vegetable oils is therefore important to consumers of vegetable oils so that they could select wisely from the many types of vegetable oils in the market.

This research is therefore aim at determine the constituent of the non-triglyceride component of the njangsa seed oil.

MATERIALS AND METHODS

Collection and Processing of Seeds

The njangsa seeds were bought from a market by an agriculturalist and was identified by a botanist at the botany department Gombe State University. The seeds were sorted, sun dried, and oven dried at the temperatures of 40 ^{o}C for 24 hours, crushed with a corn miller, and keep in a dry polythene bags for oil extraction.

Oil Extraction

The oil was extracted using soxhlet extraction method with n-hexane as the solvent in the chemistry laboratory of Gombe state university. A 50 g of the crushed njangsa seeds was weighed into the extraction thimble, put into the extractor and was inserted into a weighed 500 cm^3 round bottom flask which was half filled with n-hexane and was mounted on a heating mantle. The condenser was connected and the water was allowed to be running through the condenser as the power was switched on. The extraction was allowed for about 8hours when there was no sign of oil in the seed cake. The extractor was replaced with the Liebig condenser and the solvent distilled off and recovered from the oil. The flask containing the solvent free oil was reweighed and recorded.

Calculation

$$\% \text{ Oil yield} = \frac{(W2-W1)x100}{\text{Weight of Sample (cm3)}}$$

Where:

W_2 = weight of flask and oil

W_1 = weight of empty flask

Preparation of Reagents

0.1 M NaOH: 4 g of sodium hydroxide was weighed and dissolved in 1000 cm^3 of distilled water.

Phenolphthalein indicator: 0.1 g of Phenolphthalein was weighed and dissolved in 50 cm^3 ethanol and then 50ml of distilled water was added in a 1000 cm^3 conical flask. This was then made up to the mark with boiled distilled water.

Starch indicator: 1 g of soluble starch was weighed and dissolved in 50cm^3 distilled water in a 250 cm^3 conical flask. This was

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then transmitted into the mark with boiled distilled water.

10 % aqueous potassium iodine: 10 g of potassium iodine was weighed and dissolved into 20ml of distilled water in a 250 cm^3 beaker. It was then transferred in 1000 cm^3 volumetric flask and distilled water was added up to the mark.

Wij's reagent: 7.8 g of iodine trichloride and 8.5 g of iodine were dissolved in glacial acetic acid in separate flasks and warned. After cooling, it was then transferred both into a 1000 cm3 volumetric flask and make up to mark with glacial acetic acid.

0.5M hydrochloric acid: this was prepared using the formula:

$$C_1V_1 = C_2V_2$$

$$C_1 = \frac{SG \times \%\ PURITY \times 10}{MW}$$

$$= \frac{1.19 \times 37\ 10}{36.5}$$

$$= 12.06M$$

$$C_1V_1 = C_2V_2$$

Where: $V_1 = \frac{C_2V_2}{C_1}$

$$V_1 = \frac{0.5 \times 100}{M_1}$$

$$V_1 = \frac{0.5 \times 100}{12.06}$$

$$V_1 = 4.145\ cm^3$$

Where C = concentration of HCl = 0.5 M

MW = molecular weight of acid = 36.5 g

V = volume needed = 100 cm^3

SG = specific gravity = 1.19

% purity = percentage purity = 37 %

Therefore, 4.145 cm^3 of HCl was taken into 100 cm^3 volumetric flask and dilute to mark with distilled water.0.5 cm^3 sodium thiosulphate: A 4 g of sodium thiosulphate ($Na_2S_2O_3$) was weighed and dissolved in 1000 cm^3 of distilled water. It was then transferred into 1000 cm^3 volumetric flask.

0.5 M alcoholic KOH: A 28 g of KOH was weighed and dissolved in 20 cm^3 of distilled water in a 25 cm^3 conical flask. It was then transferred into 1000 cm^3 volumetric flask and make up to the mark with absolute alcohol (ethanol).

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Characterization of Vegetable Oil Samples

Determination of iodine value

Official Method of Analysis (A.O.A.C.) (2023. was employed with little modification. A 0.2 cm^3 of oil sample was weighed into a 250 cm^3 conical flask and dissolved with 15 cm^3 carbon tetrachloride, and 25 cm^3 of Wijs reagent was added to the mixture. The flask was then stoppered and gently shaken and placed in the dark for 30 minutes. The excess iodine was determined by adding 20 cm^3 10 % (W/V) potassium iodine solution and 150 cm^3 water and titrating this with 0.1 M sodium thiosulphate using starch as indicator. The titration was continued until blue colour just disappeared after a vigorous shaking. A blank determination was carried out and the iodine value (IV) was determined. The procedure was repeated.

Calculation

The iodine value of oil sample is determined using the equation below;

$$\text{Iodine Value (g } I_2 \text{ / 100 g oil)} = \frac{12.69 \times C \times (V1 - V2)}{\text{Weight of Sample (cm3)}}$$

Determination of acid value (A.O.A.C.)

A 5.0 cm^3 of oil sample was weighed into a 250 cm^3 conical flask, 50 cm^3 solvent mixtures (1:1) of 95 % ethanol and diethyl ether were added and the solution was titrated with 0.1M potassium hydroxide using 1cm^3 of 1 % (W/V) of phenolphthalein as indicator until pink coloration persisted. The acid value was computed from the expression;

$$\text{Acid Value (mg KOH/ g oil)} = \frac{56.1 \times C \times V}{\text{Weight of Sample (cm3)}}$$

Determination of Peroxide Value (A.O.A.C.)

A 0.5 g of oil was weighed into 250 cm^3 conical flask. 10 cm^3 of chloroform and 15 cm^3 of acetic acid were added and the mixture stirred after which 1 cm^3 of (10% W/V) potassium iodide was added. The flask was stopped and shaken for 1 minute. The flask was placed in the dark for 5 minutes and 75cm^3 of water was added. The iodine liberated was titrated with standard (0.1M) sodium thiosulphate until yellow colour was almost gone.

Blank determination was conducted. The peroxide value (PV) was computed from the relationship:

$$\text{Peroxide Value (mg } O_2\text{/g oil)} = \frac{(V0 - V) \times C}{\text{Weight of Sample (cm3)}}$$

Spectroscopic Analysis of Vegetable Oil

IR analysis

The IR analysis of the oil was carried out in the Biochemistry laboratory of the College of Medicine of the Gombe State University using IR machine type' Perkin Elmer Spectrum Version 10.03.09'

Gas chromatography-mass spectrometer (GC-MS) analysis

The GC-MS analysis of the njangsa seed oil was carried out in the American University of Nigeria (AUN). The gas chromatography-mass spectrometer model; 'GC-MS-7890A, Agilant Technology Inert MSD-597CM' was used with column of agilant-1 fused silica; capillary column (30 m x 250 µm x 0.25 µm, composed of 5 % Phenyl Methyl Silox). For GC-MS detection, an electron ionization system with ionizing energy of 74 eV was used. Helium gas was used as the carrier gas at constant flow rate of 3.8379 cm^3/min and an injection volume of 1µl was employed with split-less injection mode, injection temperature of 270 ^{0}C and ion source temperature 250 ^{0}C. The oven temperature was programed initially at 80 ^{0}C for 0 minute, decrease by 10 ^{0}C for 1 minute then increased to 300 ^{0}C for 5 minutes. The flow control

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mode was at an average velocity of 72.418 cm/sec, pressure 32.475 psi, the column flow was 3.8379 cm^3/minute, and the purge flow was 1 ml/minute. The total flow was 54.838 cm^3/minutes. Mass spectra were taken at 74 eV, a scan of 27 minutes and fragment from 50 g to550 g.

RESULTS AND DISCUSSION

Oil Quality Parameters

The oil quality parameters of njangsa, seed oil are shown on Table 1. The soxhlet extraction method yielded 44.44% njangsa oil. Although the oil yields is within a close range, the yield of 44.44% for njansa seed oil is almost the same with 44.17%, of Harold et al., (2017) and 43.3% of Yaboah et al., (2011) for the same njangsa oil. The yield is also within the range of 44.9% to 54.7% as reported by Kapesu et al., (1995). Although these differences in the oil yields look small, they are very important when extracting in industrial scale since the differences are multiplied million times of the quantity. The iodine value of vegetable oils indicate the level of unsaturation of the oil. The iodine values of 165.8 mgI_2 /100g for njangsa seed oil shows that njangsa seed oil is a drying oil. (Ulrich poth 2002).

The iodine value of 165.8 mgI_2 /100g for njangsa is in the lower range of the range of iodine values for njangsa oil (Fai et al., 2018). However the difference is not much and could be because of errors from instrument. The difference could also be due to geographical location and soil variability. Acid value indicates the amount of free fatty acid in the oil. A high acid value indicates the level of deterioration by hydrolysis and also rancidity. The acid values of the oils analyzed is 1.39 mgKOH/g of oil. The acid value of 1.39 mgKOH/g for njangsa oil is higher than the value of 0.39 mgKOH/g obtained by Fai et al., (2018) and 0.64 mgKOH/g obtained by Nwachukwu (2019). This could be due to the deterioration of the oil in the seed due to storage.

The peroxides values of njangsa oil is 7.2 meq/kg. The peroxide value indicates the level of active oxygen in the vegetable oil and the value is related to storage or exposure to the environmental oxygen. A high peroxide value means the oil was not well stored or preserved and also indicates rancidity of the oil. Njangsa seed oil is considered fresh since it has peroxide value below 10 meq/kg (Gordon 2001). When the peroxide values of the oils is less than ten milligram equivalent it implies that the oil is very good (not rancid) and when it is above ten milligram equivalent it means the oil is still good (Gordon 2001).

Table 1: Oil quality parameters of njangsa seed oil

Vegetable oil	Iodinevalue (gI_2/100g)	Acid value (mg KOH/g of oil)	Peroxide value (meq/kg)	Percentage yield (%)
Njangsa oil	165.8	1.39	7.20	44.44

IR Results of Njangsa Seed Oil

The results of IR analysis of njangsa seed oil are presented on Figure1 and table 2. The IR analysis shows the functional groups in the oil. The Spectrum shows absorption peaks at 3423.09 cm^{-1} (strong and broad), 1743.96 cm^{-1} (strong and sharp), 1163.66 cm^{-1} (strong and sharp), 2855.25 cm^{-1}, 2855.28 cm^{-1} and 3007.28 cm^{-1} (strong and broad) and medium peaks at 1655.09 cm^{-1}, 300.28 cm^{-1} . The implications of these absorption peaks are presented on Table 2.

Bima Journal of Science and Technology, Vol. 7 (4) Dec, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i4.549

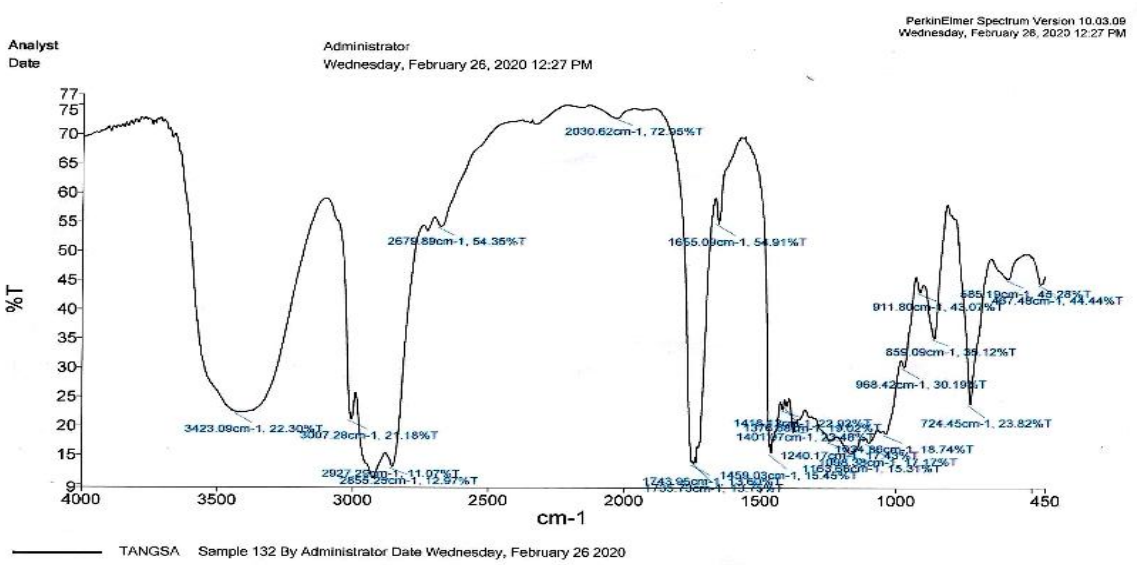

Figure 1: IR Spectrum of Njangsa seed oil

Table 2: Functional groups in constituents of Njangsa Oil

Vibrational frequency (cm^{-1})	**Functional group**	**Remark**	Probable compounds Azogu (2010)
Strong and broad peak at 3423.09 cm^{-1}	-OH group	Alchohol,	Hydroxy-compounds.
strong sharp peak at 1743.96 cm^{-1}	-C=O stretch	Carbonyl group	Carboxylic Acid
Strong and sharp peak at 1163.66 cm^{-1}	-C-O stretch	-C-O present	Aldehydes and ketones Ester
Strong peaks at 2927.29 cm^{-1}, 2855.25 cm^{-1} , 3007.28 cm^{-1}	CH_2-CH_2 , CH_2-CH_3 stretches	SP^3 C-H present	Alkanes
Medium peak at 1655.09 cm^{-1}	C=C stretch	C=C	
Medium peak at 3007.28 cm^{-1}	Vinyl C-H	Sp^2 C-H	Alkenes.

The implications of the absorption peaks shown on the Spectrum are presented on Table 2. The results on Table2 implies that the functional groups on the constituents of njangsa seed oils are; alcohols, carboxylic acid, esters, and alkenes. This confirms the result of the presence of free fatty acid as on the oil quality parameters (Table 1) and high unsaturation from high iodine value (table 1). The vegetable oil major constituents are triglycerides which are ester of fatty acid and glycerol which is confirmed by the presence of the C=O, stretch at 1735 cm^{-1} and C-O stretch at 1300 cm^{-1} to 1000 cm. The presence of hydroxy- compounds confirms the report of Narasinga (2001), that vegetable oil contains 2% to 8% non-glycerides component which includes hydrocarbons, tocols, sterols, and flavonoids (the reason why njangsa seed is used as a spice). Arrey et al., (2022) identified many flavour compounds in njangsa oil which included alcohols, aldehydes, esters, and hydrocarbons, and he concluded that the amount of the non-triglyceride matter in vegetable oils varies with the method of extraction.

GC-MS Analysis of Njangsa Seed Oil.

The GC-MS results of njangsa seed oil are presented on Figure 2, and Table 3. The GC

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chromatogram for njangsa oil (Figure 3) showed many peaks each of which was analyzed and interpreted on mass spectrum to reveal the presence of many compounds. However, the analysis of the fragmentation of the peaks shows that njangsa seed oil contains different types of compounds including free fatty acids (Table 3).

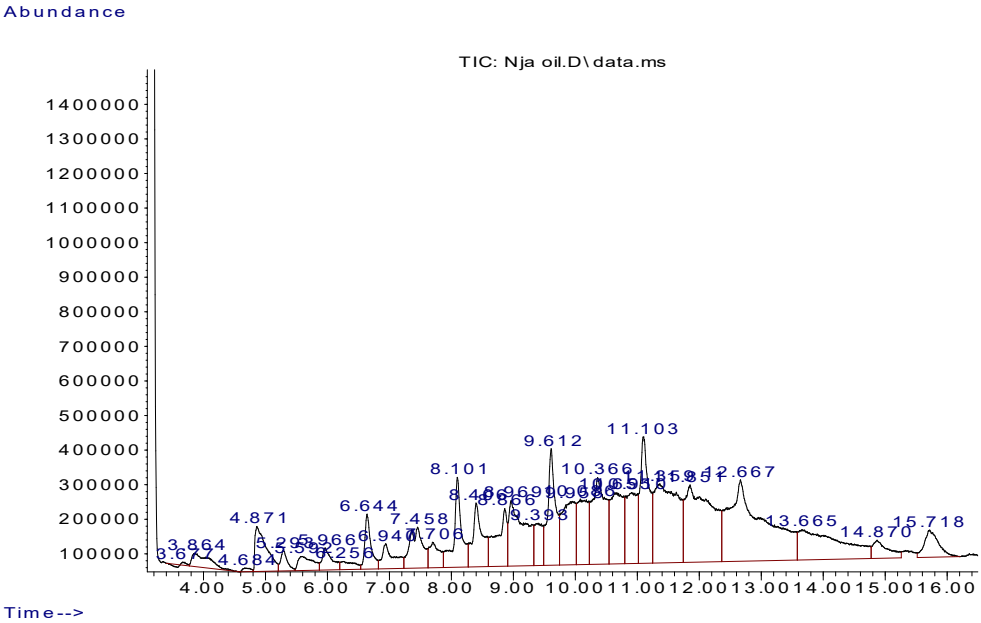

Figure2: GC-MS Chromatogram of Njangsa Oil

Table 3: List of compounds present in Njangsa oil

Compound	Formulae
-2,4-decadienal(EE)	$C_{10}H_{16}$
Oxalic acid cyclobutyl heptadecyl esrer	$C_{23}H_{42}O_4$
Decane,3,6-dimethyl	$C_{12}H_{26}$
1-octanol-2-butyl	$C_{12}H_{26}O$
Hexane, 2-methyl-4-methylene (isomers)	H_8H_{14}
Methoxy acetic acid, 2-tridecyl ester	$C_{16}H_{32}O_3$
Oxalic acid, cyclobutyl pentadecyl ester (isomers)	$C_{21}H_{38}O_4$
Tetracontane 3,5,24-trimethyl	$C_{48CH}H_{88}$
1-Trdecene	$C_{13}H_{26}$
Oxalic acid,cyclobutyl hexadecyl ester	$C_{12}H_{40}O_4$
Carbonic acid hexadecyl hexadecyl prp-1-en-2-yl ester	$C_{20}H_{38}O_3$
n-Hexadecanoic acid	$CH3(CH_2)_{14}COOH$
Tetracontane 3,5, 24 trimethyl	$C_{43}H_{88}$
Cyclohexadecane	$C_{16}H_{32}$
Oxalic acid, sobutyl hexadecyl ester.isomers	$C_{24}H_{46}O_4$
Carbonic acid, octadecyl prop-1-en-2-ylester(i)	$C_{22}H_{42}O_3$
17- pentatriacontene	$C_{35}H_{70}$
Oxirane, tetradecyl	$C_{16}H_{32}O$
1-decanol, 2-hexyl	$C_{16}H_{34}O$
Disulfide, di-tert-dodecyl	$C_{24}H_{50}S_2$
Cyclotetradecane	$C_{14}H_{28}$
Oxalic acid,cyclobutyl heptadecyl ester	$C_{23}H_{42}O_4$
Oxirane,[(dodecyloxy)methyl]-	$C_{15}H_{30}O_2$
squalene	$C_{30}H_{50}$

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The GC-MS analysis of the njangsa seed oil revealed the presences of 23 compounds, while Arrey et al., (2022) got 35 compound for Hexane extracted njangsa oil. This differences could be due to the influence in environmental conditions where the njangsa plants were grown. The number of compounds in the njangsa seed oil of this analysis is also higher than the number of compounds), enzyme assisted aqueous extraction which revealed only 13 compounds. This differences is due to the differences in the methods of oil extraction as concluded by Arrey et al., (2022) in his comparative study for hexane extracted njangsa oil and enzyme assisted aqueous extracted njangsa oil.

CONCLUSION

Njangsa seed oil was extracted from njangsa seeds and the yield of 44.44% was obtained. Quality evaluation of the njangsa seed oil show that the oil has iodine value above 150 $mgI_2/100g$ of oil implying that njangsa seed oil is a dryings oils. The acid value and peroxide value of njangsa oil are within the range of the good quality oil, implying that the oil had not undergone deterioration. The IR spectrum shows that the oil contain alcohols, esters, alkanes, alkynes, carbonyl and sulfides groups. The GC-MS results of the analysis revealed the presence of 24 non-triglyceride or volatile compounds in njangsa oil, that included hydrocarbons, carboxylic acids, esters, aldehydes, peroxides, and sulfides compounds many of which are polyfunctional compounds. These compounds are of different chemical classes and are responsible for taste, colour and flavour of the njangsa seed oil.

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