Proximate analysis, extraction, and characterization of oil from Terminalia catappa fruit

Abstract

Context

Terminalia catappa, also known as tropical almond, is a well-known plant recognized for its edible parts, including fruit, bark, leaves, and roots. It is also noted for its medicinal usefulness and numerous pharmacological actions.

Aim

This study aims to analyze the proximate composition of the seeds of tropical almonds and extract and characterize the oil from Terminalia catappa seeds and mesocarp. The goal is to determine the fruit's nutritional content, evaluate the oil's properties, and identify potential applications of the extracted oil.

Materials and Methods

Standard methods are used to assess physicochemical parameters such as saponification, acid, peroxide, iodine, and specific gravity. The seed's proximate composition is also analyzed, revealing moisture, ash, crude fiber, fat, protein, and carbohydrate content.

Results

The results indicate that the saponification value (mg KOH/g), acid value (mg KOH/g), iodine value (mg iodine/mg), peroxide value (mg/peroxide/kg), and specific gravity of the oil are 162, 1.68, 89, 1.40, and 0.95 respectively. The proximate composition reveals that the seed contains 23.24% moisture, 5.50% ash, 12.30% crude fiber, 16.51% fat, 21.22% protein, and 39.99% carbohydrate. These findings suggest that tropical almond seed is a good source of protein, carbohydrates, and oil and contains minerals that can contribute valuable amounts of essential nutrients to the human diet. The low acid value suggests that the oil is edible, while the high saponification value indicates its potential in industrial applications such as cosmetics. The low iodine value reveals that it is a non-drying oil unsuitable for the paint industry. Additionally, the low peroxide value of the oil indicates low susceptibility to oxidative rancidity and deterioration, confirming the presence of antioxidants in the seed oil.

Conclusion

Terminalia catappa seeds exhibit a high level of most chemical components, making them a promising raw material for various industries. Their high protein value and low level of anti-nutrients indicate their potential usefulness in animal and poultry feed supplements. They also serve as beneficial dietary supplements and should be encouraged in diets.

Keywords

Terminalia catappa, tropical almond, proximate composition, seed oil, mesocarp oil, saponification value, acid value, peroxide value, iodine value, specific gravity,

nutritional content, protein, carbohydrate, dietary supplements, antioxidants, industrial applications.

1.0 *INTRODUCTION*

Seeds are one of the most important food sources, providing humans and animals with essential nutrients. These nutrients include carbohydrates, lipids, proteins, vitamins, and minerals (Aguirre et al., 2018). Seeds contain proteins and bioactive peptides classified as nutraceuticals. Proteins and peptides are essential in the human diet because they provide the raw materials needed for protein biosynthesis and are a good energy source. Incorporating seeds into the human diet provides nutritional and functional health benefits, reducing the risk of contracting some chronic diseases (Oso & Ashafa, 2021).

Oilseed crops have been grown around the globe under various agroclimatic situations and are considered essential crops due to their commercial value. The rising demand is also one of the main factors that have led the producers to increase their production of oilseeds. Besides the upsurge in the production of oilseeds, the world is still facing a significant supply shortage (Mustafa & Iqbal, 2021). Fatty acids are generally incorporated into a triglyceride structure in plant oils, which indicates that plant oils are a source of fatty acids (Rahman et al., 2022). Oils are liquid, while fats are solid at room temperature.

Nuts have been part of the human diet since prehistoric times. They are nutrient-rich foods and an excellent means of delivering health-promoting bioactive compounds. As such, they serve as important healthful snack items and are part of many traditional and new gastronomy recipes worldwide. Frequent consumption of nuts is highly recommended to obtain the full benefit of the nutrients, bioactive, and antioxidants they contain, together with their desirable flavor (Alasalvar et al., 2020).

1.1 Taxonomy of Terminalia catappa

Plant scientists have classified *Terminalia catappa* as follows:

Kingdom: *Plantae*
Division: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Myrtales*
Family: *Combretaceae*
Genus: *Terminalia L.*
Specie: *Terminalia catappa*

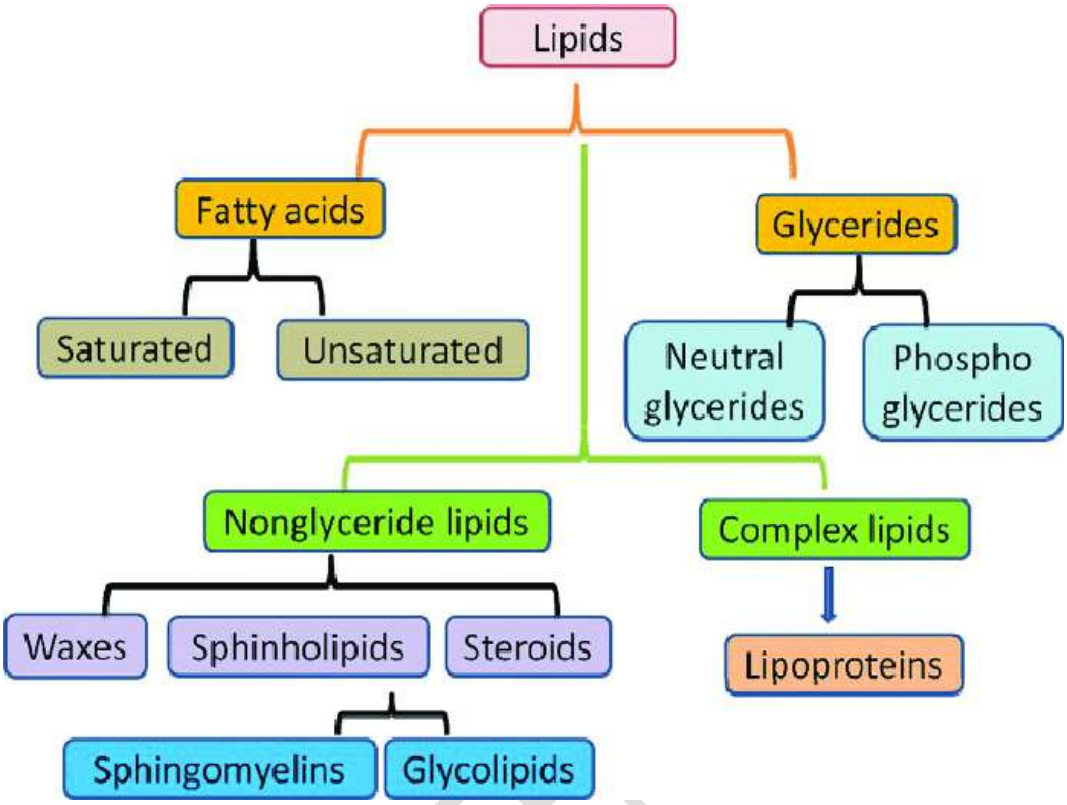


Figure 1: Terminalia catappa plant

Terminalia catappa, also known as the tropical almond, is the second-largest genus of the family Combretaceae (Fahmy et al., 2015). It is a well-known plant recognized for its edible parts, including fruit, bark, leaves, and roots. T. catappa has been acknowledged for its medicinally essential phytoconstituents, such as phenols, flavonoids, and carotenoids. Numerous pharmacological investigations have confirmed this plant's ability to exhibit antimicrobial, anti-inflammatory, anti-diabetic, antioxidant, anthelmintic, hepatoprotective, anti-tumor, hematological, and anticancer activities, all of which support its traditional uses (Saharan et al., 2022; Anand et al., 2015).

The tropical almond tree (Terminalia catappa) grows mainly in tropical areas of Asia. The tree grows from 10 m to 25 m high and has horizontal whorls of branches with shiny, ovate leaves 10-25 cm long, tapering below a narrow, heart-shaped base with an expanded, rounded apex. Its fruit is smooth and ellipsoid, 3-6 cm long, and prominently bi-ridged or keeled down to the sides, with fibrous and fleshy pericarp and hard endocarp. Studies have indicated that the leaves of Terminalia catappa are rich in tannins and a host of organic compounds that help condition the culture water, resulting in improved survival, growth, and health of cultured aquatic species (Dianala, 2019). It turns from green to purplish-yellow on ripening, containing a hard shell or nut covering the delicate edible seed. The ripe mesocarp of the fruit is mainly consumed by

children who neglect the seed. It is usually grown in full sun on well-drained soil. The branches are arranged in obvious tiers, forming canopy layers, giving the tree a pagoda-like shape.

Despite being classified mainly as a nut, an almond is a seed from the fruit of an almond tree. It is commonly known as a fruit in Nigeria—the almond flowers from March to May. Terminalia catappa oil is considered a high-quality edible because of its high percentage of linoleic and oleic fatty acids. Its high oil content also qualifies it for industrial use, especially biodiesel production. The world's production of Terminalia catappa fruit is estimated at 700,000 tons annually (Menkiti et al., 2017).

Almond oil, a rich source of macronutrients and micronutrients, is extracted for food flavorings and cosmetics (Ouzir et al., 2021). It is used as edible oil, mainly as a salad dressing and in vegetable dips. It is also used in the cosmetic industry, especially in hair, dry skin creams, anti-wrinkle, and anti-aging products. It is also used in aromatherapy and massage therapy since it suits any skin type. Bitter almond oil limits its uses to external applications.

In contrast, sweet almond oil is safe to ingest internally. It contains many vitamins, including E and K, that help skin regeneration and maintain elasticity, which is why the oil is used in many cosmetic products (Čolić et al., 2019). Almond oil is transparent, light yellow, fragrant, and can resist temperatures under 200°C.

Figure 2 Morphology of the palnt

1.2 Traditional Medicinal Uses of Almond Oil

Almond oil is regularly mentioned in the writings of famous herbalists throughout the ages. Historically, almond oil has been used for its numerous health and beauty benefits in ancient Chinese, Ayurvedic, and Greco-Persian

schools of medicine (Čolić et al., 2019). John Gerard (the eclectic herbalist Gerard) indicates that almond oil is a huge source of relief from pain on the outside through massage.

Dioscorides (author of Materia Medica) mentioned several recipes for ointments made using almond oil. Culpeper mentions the request for almond oil to massage the temples, believed to improve brain function and relieve stress. Almond has been used traditionally for curing wounds, anemia, insomnia, headache, sore throat, brain infections, kidney disorders, urinary infections, arthralgia, pityriasis, and hysteria (Mushtaq et al., 2015).

1.3 Therapeutic Properties of Almond Oil

- Anti-Inflammatory- It reduces inflammation when ingested.
- Antioxidant- Almond oil possesses mild antioxidant ability.
- Immune Booster -The topical as well as internal function of sweet almond oil boosts immunity and provides robust protection from various diseases
- Anti-hepatotoxic—Almond oil is recognized to aid the liver in eliminating toxins, which is performed by castor oil.
- Emollient- The excellent moisturizing property. It is used to remove excess dry skin.
- Sclerosant- Used to treat vascular issues like spider veins, hemorrhoids, and varicose veins.
- Laxative- Promotes defecation and relieves constipation. This laxative action is mild compared to more potent laxatives like castor oil.
- Analgesic-almond oil is a soft pain reliever.
- Muscle Relaxation- Massage with almond oil soothes stressed and sore muscles.
- Cicatrizant helps wounds heal faster.
- Anti-dandruff- It dissolves, leaving dandruff on the scalp.

(Karimi et al., 2021; Faghihi et al., 2022)

1.4 Importance of Terminalia catappa

Almonds may be small food, but they pack an enormous nutritional punch. They contain several vital vitamins and minerals, as well as healthy fats. Some of their excellent health benefits include lowering blood pressure, controlling blood sugar and cholesterol levels, and alleviating constipation, respiratory disorders, and anemia. Almonds are also great for hair, skin (psoriasis), and dental care. Almonds and almond oil have anti-inflammatory, immunity-boosting, and anti-hepatotoxic effects (Anand et al., 2015).

Further associations between almond oil and improved bowel transit have been made, reducing irritable bowel syndrome symptoms. Further, some studies show a reduced incidence of colonic cancer. Moreover, cardiovascular benefits have also been identified with almond oil elevating the levels of so-called "good cholesterol," high-density lipoproteins (HDL). At the same time, it reduces

low-density lipoproteins (LDL) (Williams et al., 2019). No research has been reported on oil extraction from Terminalia catappa's edible flesh and its applications. Therefore, the mesocarp and seed oil extraction and characterization from Terminalia catappa will be conducted.

1.5 Chemistry of Oils and Fats

Like most organic materials, Oils and fats are made up of three elements:
i. Carbon
ii. Oxygen
iii. Hydrogen
These elements combine to form chains known as fatty acids. Three of these chains join together to form a molecule known as a triglyceride. The triglyceride molecule is the basis of all oils and fats. Oils and fats vary in both their appearance and functionality due to differences in the types of fatty acid chains that join together to form the triglyceride molecule.

1.5.1 Lipids

Lipids are a large and diverse group of naturally occurring compounds. They are the biologically essential compounds in the building blocks of the structure and function of living cells. Chemically, lipids are the esters of glycerol and fatty acids or the triglycerides of fatty acids. They are generally insoluble in aqueous solution and easily soluble in non-polar solvents. Lipids are classified into simple, complex, and derived lipids based on their structure. Fats, oils, and waxes are simple lipids (Asokapandian et al., 2021).

1.5.2 Edible Fats and Vegetable Oils

The Codex Alimentarius, published in 1981, defines edible fats and oils as foodstuffs composed of glycerides of fatty acids. They may be of vegetable, animal, or marine origin and may contain small amounts of other lipids, such as phosphatides, unsaponifiable constituents, and free fatty acids naturally present in the fat or oil.

Fats from a vegetable origin show a predominance of unsaturated fatty acids, which make them liquefy at 25 °C and are specifically called oils. They are generally rich in oleic and linoleic acid, and saturated fatty acids are reduced to less than 20%. On the other hand, fats with around 30-80% saturated fatty acids are solid and are animal fats.

The main classes of fatty acids are saturated, monounsaturated, and polyunsaturated. Saturated (butyric acid, caprylic acid, palmitic acid, etc.) and monounsaturated (oleic acid) fatty acids can be Synthesized in the human body. However, the two more simple polyunsaturated fatty acids (PUFAs), α-linolenic acid (ALA), and omega-6 fatty acids, such as linoleic acid (LA), cannot be synthesized in the human body and are recognized as essential fatty acids. While some long-chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be

synthesized from the parent omega-3 fatty acids (ALA), this is done at a meager conversion rate; hence it must be taken through diet to fulfill the daily intake requirement (Patel et al., 2020).

Oils from seeds and fruits can be divided among those with a predominant presence of monounsaturated fatty acids, as is the case of oleic acid (olive, rapeseed, palm, etc.), and those with a predominance of linoleic acid (sunflower, hemp, soybean, peanut, maize, etc.).

In the industry, fats are often converted into fatty acids hydrolyzed without soap formation. The commonest soaps are the fatty acid salts of sodium and potassium. Hard soaps are sodium salts, while soft soaps are potassium salts.

The formulae of these acids are as follows:

Acid	**Formula**
Lauric	$CH_3(CH_2)_{10}COOH$
Myristic	$CH_3(CH_2)_{12}COOH$
Palmitic	$CH_3(CH_2)_{14}COOH$
Stearic	$CH_3(CH_2)_{16}COOH$
Oleic	$CH_3(CH_2)_7 CH{=}CH(CH_2)_7COOH$
Linoleic	$CH_3C_2(CH_2)_{14}COOH$
Linolenic	$CH_3(CH_2)_4CH{=}CH\text{-}CH\text{-}CH\text{-}CH\text{-}(CH_2)_7\text{-}COOH$

Table 1: Formula of Fatty Acids

1.5.3 Classification of Fat

Fatty acids are either saturated or unsaturated.

a. Saturated Fats

A fatty acid is said to be saturated when the fatty acid molecule contains the maximum number of hydrogen possible. Certain fatty acids are already naturally saturated, so they cannot be made more complex than they are in nature.
Saturated fat is a type of dietary fat. It is one of the unhealthy fats, along with trans fat. These fats are most often solid at room temperature. Foods like butter, many baked foods, palm and coconut oils, cheese, and red meat have high amounts of saturated fat. However, Government recommendations advise consumers to limit their intake of saturated fats as Saturated fats raise your LDL

(bad) cholesterol. High LDL cholesterol increases your risk for heart disease and stroke, weight gain, diabetes, and other health problems (Arnett et al., 2019)

b. Unsaturated Fats

There are three types of unsaturated fatty acids: -

i. **Monounsaturated Fatty Acids**

Fatty acids in this category have what is known as one double bond in their chemical makeup. They are relatively stable to oxidation and the development of rancidity and are now considered the best type of fat to eat in nutritional terms. The most common sources of monounsaturated oil are olive oil and rapeseed oil.

ii. **Polyunsaturated Fatty Acids**

Polyunsaturated fatty acids contain two or more double bonds in their chemical makeup. They are the least stable fatty acids to oxidation and, as such, are best used in cold applications. Sunflower seed oil is the most common source of polyunsaturated fatty acids (Reka et al., 2021). Polyunsaturated fatty acids resolve inflammatory processes (Marion-Letellier et al., 2015).

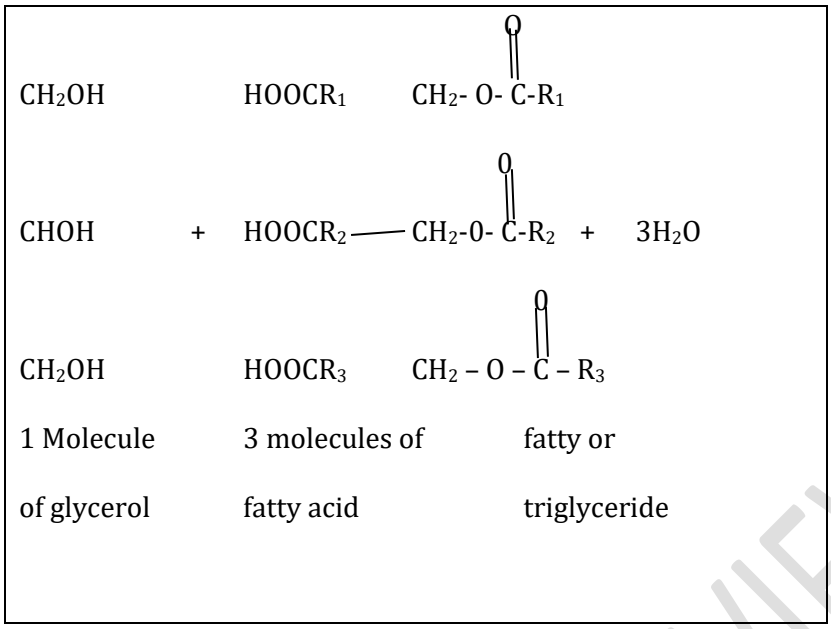
iii. **Trans Fatty Acids**

Trans fatty acids typically come from hydrogenated vegetable oils and animal fats. Recent scientific research suggests trans fats, although consumed in relatively small proportions, should be avoided due to their negative effect on blood cholesterol levels.

1.5.4 Triglycerides

Triglycerides are the most common type of fat found in nature. They form the virtual total weight of oils and margarine, both of which are made from triglycerides.

In ripe almonds, fatty acids appear mainly in the form of triglycerides. Almond oil is the conventional nut oil with the highest triglyceride content, about 98%, which results in a low acidity index. The triglycerides of all oils may be differentiated by the types of fatty acids they contain.



1.5.5 Oils

Oil is a triglyceride formed from unsaturated fatty acids and propan—1, 2, 3—triol. Oils are mainly from plants. They have double bonds between the carbons in the hydrocarbon tail, causing bends or kinks in the shape of the molecules. Because some of the carbons share double bonds, they are not bonded to as many hydrogen atoms as they could be if they weren't double-bonded to each other.
Generally speaking, oils containing more unsaturated fatty acids are liquid at room temperature, whereas those with more saturated fatty acids will be solid.

2.0 Almond Oil Obtaining Process

The almond oil-obtaining process is very similar to obtaining other nut oils. The nut is harvested before the autumn rains (August- September). After harvesting, the next step is de-hulling, which consists of removing the mesocarp that appears to have adhered to the nut and has not been lost by falling from the tree. After de-hulling, the nuts are typically exposed to the sun for two or three days (drying), as a general rule, or they are subjected to hot air ventilation to finish their drying. The humidity content is considerably reduced by up to 5-8% by drying. After that, cracking occurs, which consists of separating the shell and the seed. Finally, oil extraction takes place, generating a solid edible by-product. Some extraction systems will require previous grinding of the seeds. The most critical operations in almond oil extraction, which would need to be optimized to obtain a better quality final product, are drying and extraction.

2.1 Almond drying

Almond drying is a fundamental operation from a commercial point of view (deficient drying reduces the operational profitability and the shelf life of nuts susceptible to rancidity) and a sanitary point of view (adequate drying prevents

the growth and spread of fungus). Almond drying can be done in different ways: direct sun exposure, in a hot air oven, using a fan, in a hot air dryer, etc.

2.2 Almond oil Extraction

Different extraction methods can be used for almond oil extraction, although, as with other seeds, solvent extraction will provide the highest industrial yield. Traditional equipment uses high temperatures and chemical products, reducing the quality of the oil due to the appearance of undesirable flavors and the inactivation of vitamins and active substances that appear in the raw material, forcing the posterior necessity of refining the oils so they could not be defined as virgin oils. Matos and Acua 2010 evaluated three main influence parameters: extraction temperature, size of the almond particle, and solid/ solvent proportion regarding yield, and defined the optimal conditions as 90 C, 0.5 mm, and 1:3 proportion, respectively, reaching an oil yield of 44.59%. This yield can be improved if samples are irradiated with ultrasounds of 42 kHz.

In recent years, supercritical fluid extraction (CO) has improved its conception of alternatives to conventional solvent extraction methods. Femenia et al., in 2001, used pressures of 330 bar and temperatures of 50 °C to extract the oil contained in raw almonds, raw peeled almonds, and roasted almonds, obtaining oil percentages of 15-16%, 27- 33%, 49-64%, respectively. Leo et al., 2005 also extracted almond oil using this system, but using pressures of 350 to 550 bar, temperatures of 35 to 50 °C and solvent rates of 10 to 30 kg-1, and observed that the increase in extraction pressure and temperature caused an increase in oil yield. It was also observed that equal flow and pressure rate and the temperature increase caused an increase in yield of almost four times higher. An explanation for this phenomenon is that it increased oil solubility in CO2.
Experimental results were used to deduct that oil production, the initial stage of extraction, increased with an increase in the CO, flow rate of 10 to 30 kg-1, constant pressure, and temperature. Thus, oil production increased with pressure, temperature, and flow rate increments. Later on, Ma et al., in 2007, studied the factors that influence bitter almond oil extraction, finding optimal extraction conditions: extraction pressure (35 MPa), extraction temperature (50 °C), CO, flow rate (24 h), almond particle size (0.6 mm) and extraction time (2 hours). The factor sequence that affects extraction is almond particle size > extraction time > extraction pressure > CO, flow rate > extraction temperature. Under these conditions, almond oil yield reaches 53%.
An alternative to solvent use is the use of pressing with both hydraulic and Screw presses.

2.3 Characterization of Extracted Oil

The fatty glycerides of plant origin form an essential class of organic compounds: out of these, those that are solids at ordinary temperature are called fats, and those that are liquids are known as oils. Both of these categories are referred to as saponifiable oils. These are used in foods, the manufacture of soap and medicine, etc. Most fats and oils comprise glycerides of fatty acids containing 16

to 18 carbon atoms, such as oleic, stearic, and palmitic acid. The oil sample is filtered to remove suspended matter.

2.3.1 Qualitative Analysis of Fat Components

The qualitative examination aims to identify a sample as oil and provide information about its type and origin. Moreover, qualitative assessment of the actual condition of the oil sample and the detection of impurities are essential guides for the analytical methods to be applied.

2.3.2 Identification and Assessment of Type and Source

Besides smell and taste, which are often characteristic important clues to the identification of an oil sample, that is obtained from its superficial appearance (oily, lard-or tallow-like consistency), its solubility, especially in organic solvents, and its behavior on heating and saponification. Colour and transparency provide further proof of non-fatty constituents. The solubility of fat depends on the nature of the main constituents: the triglycerides. Whereas pure fats do not emulsify by mere shaking with water, stable emulsions indicate the presence of surface-active materials such as soaps, monoglycerides, and mucilaginous materials.

2.3.3 Acidimetric Chemical Constants

The main acidimetric constants are the acid, saponification, and ester values. These can be used to calculate the free fatty acid, total fatty acid, and fatty acids combined with esters and their molecular weights.

2.4 Proximate Composition

Proximate Analysis

Proximate analysis, also known as Weende analysis, was developed in 1866 by Hennberg and Stohmann; it is a chemical method of assessing and expressing the nutritional value of a feed, which reports the moisture content, ash content (minerals), crude fiber, crude fat, and crude protein present in a food as a percentage of dry weight. The proximate analysis gives the overall nutritional composition of a sample. According to an industry standard, the proximate analysis consists of five constituents: ash, moisture, proteins, fat, and carbohydrates. Proximate plant analysis gives valuable information and helps assess the sample's quality.
These methods evolved from thorough studies of the inherent properties of the component of interest and exploration of the unique advantage such properties have over others, thus allowing the component to be isolated or eliminated.

3.0 MATERIALS AND METHOD

3.1 Materials

Chemicals/Reagents

i. Carbon tetrachloride

ii. Chloroform

iii. Ethanol

iv. Hydrochloric acid

v. Kjedahl catalyst

vi. Petroleum ether

vii. Petroleum ether (40-60°C)

viii. Potassium hydroxide

ix. Potassium iodate

x. Potassium iodide

xi. Sodium hydroxide

xii. Sodium thiosulphate

xiii. Tetraoxosulphate (VI) acid

Apparatus/Equipment

i. Crucible tong

ii. Heating mantle

iii. Kjedahl flask

iv. Muffle furnace

v. Oven

vi. Soxhlet apparatus

vii. Specific gravity bottle

viii. Thermometer

ix. Water bath

x. Weighing balance

3.2 Methods

3.2.1 Sample Collection, Identification, and Preparation

Freshly matured *Terminalia catappa* fruits were plucked from the Faculty of Social Science at Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The fruits were identified using online sources, including the Missouri Botanical Garden (St. Louis, U.S.A.) and the E-Flora of Gandhinagar (Gujarat Forestry Foundation, Gujarat, India).

The fruits were transported to the laboratory in a polyethylene bag. Prior to analysis, the fleshes of the fruits were removed using a sharp knife, air-dried, milled into fine powder, sieved, and stored in an airtight polyethylene bag. The seeds were oven-dried, cracked open, milled into fine powder, and also stored in an airtight polyethylene bag.

3.2.2 Proximate Analysis

Determination of Moisture Content (AOAC, 2016)

Method:

A crucible was washed thoroughly and dried in an oven. It was then placed inside a desiccator to cool, after which it was weighed to get the Weight of the empty dish. Two grams of the sample were weighed into the crucible to get the combined Weight of the crucible and the sample. The sample was dried in an oven at a temperature of about 70-80°C for 2 hours and then at 105°C for another 4 hours until the Weight remained constant. After 4 hours, the crucible was placed in a desiccator to cool, and the final Weight of the crucible plus the sample was recorded.

Calculation:

$$\text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad \text{---------- 1}$$

Where:

- $W1$ = Initial Weight of empty crucible
- $W2$ = Weight of crucible + sample before drying
- $W3$ = Final weight of crucible + sample after drying

Determination of Ash Content (AOAC, 2016)

Method:

Two grams of the sample were weighed and transferred into a crucible. The sample was charred on a heater inside a flame cupboard to drive off most of the smoke. The sample was then transferred into a pre-heated muffle furnace at 550°C and left for 2 hours. After 2 hours, the sample, now grey in color, was removed from the muffle furnace, cooled in a desiccator, and reweighed.
The ash content was calculated using the method described below

Calculation:

Ash content (%) = $\frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$

$\frac{W_3 - W_1}{W_2 - W_1} \times 100$ ---------- 2

Where:

- $W1$ = Weight of empty crucible
- $W2$ = Weight of crucible + food before ashing
- $W3$ = Weight of crucible + ash

Determination of Crude Fibre (AOAC, 2016)

Method:

Two grams of the sample were defatted with petroleum ether and boiled under reflux for 30 minutes with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100 ml. The solution was filtered and washed with boiling water until the washings were no longer acidic. The residue was transferred to a beaker and boiled for 30 minutes with 200 ml of a solution containing 1.25 g of carbonate-free NaOH per 100 ml. The solution was heated for 30 minutes, filtered, washed with boiling water, and oven-dried. The oven-dried residue was ignited in a furnace at 550°C.

The fibre content was calculated as described below

Calculation:

Crude fiber (%) = loss in weight after ignition x 100

Determination of Lipid Content (AOAC, 2016)

Method:

Using a Soxhlet extractor

A clean boiling flask was dried in an oven at 105-110°C for about 30 minutes, 1U was transferred to a desiccator, and allowed to cool. 2g of the sample were weighed into a filter paper and transferred into the Soxhlet apparatus. 300ml of petroleum ether were weighed into the boiling flask of known Weight and placed on the heating mantle. The Soxhlet extractor was connected and allowed to reflux for about 4 hours. After a clear colorless solution was obtained, the petroleum ether was collected. The flask containing the extracted oil was dried in the oven at 110°C for an hour, transferred into a desiccator to cool, and then weighed.

Calculation:

% Fat Weight of fat x 100 ----------3

Weight of sample

Determination of Protein Content (AOAC, 2016)

Method:

Two grams of the sample were weighed into a digestion flask. 5g of Kjedahl catalyst was added, 8.0 g of K,SO, and 1g CuSO,(catalyst) were added, followed by 25 ml of concentrated sulphuric acid and five glass beads to prevent bumping during heating. The flask was heated until frothing ceased and the solution cleared. It was cooled, and black particles that appeared at the mouth and neck of the flask were washed with distilled water. The flask was reheated and allowed to cool again. The digest was then transferred with several washings into a 250 ml volumetric flask containing 30 ml boric acid and indicators (0.1% bromocresol green solution and 0.1% methyl red solution). It was made up to the mark with distilled water. The solution was distilled until 150 ml of distillate was obtained. The boric acid-receiving solution was titrated with 0.1 M HCl to a purplish-pink color.

Calculation:

Protein (%) = % Nitrogen x F

Nitrogen (%) = V_a, -V_b x M× 14.01 ----------4

Where:

- *Vs* = Volume of acid used to titrate the sample
- *Vb* = Volume of acid used to titrate the blank
- *M* = Molarity of HCl used
- *W* = Weight of sample
- 14.01 = Atomic Weight of nitrogen
- *F* = Protein-nitrogen conversion factor (6.25)

Determination of Carbohydrate Content

Calculation:

Carbohydrate (%) = 100 - (% protein + % fat + % moisture + % ash + % crude fibre)

3.3 Extraction of Oil

3.3.1 Extraction by Cold Maceration

The crushed almond flesh and nuts were weighed and soaked in 1000 ml of N-hexane. After 48 hours, the mixture was filtered and evaporated to dryness. The oils were then characterized accordingly.

3.3.2 Determination of the Percentage of Oil from *T. catappa* Seed

Petroleum ether (300 ml) was poured into a round bottom flask. Ten grams of the sample were placed in a thimble and then inserted in the center of the extractor. The Soxhlet apparatus was heated to 40-60°C. As the solvent boiled, the vapor rose through the vertical tube into the condenser at the top. The liquid condensate dripped into the filter paper thimble containing the solid sample to be extracted. The extract seeped through the pores of the thimble, filled the siphon tube, and flowed back down into the round bottom flask. This process continued until the extraction was completed, as indicated by the decolorization of the oil-solvent mixture in the extractor. The thimble was then removed from the tube, dried in an oven, cooled in a desiccator, and weighed to determine the amount of oil extracted.

3.3.3 Determination of the Percentage of Oil from *T. catappa* Mesocarp

30g of the sample were placed in the thimble and inserted in the center of the extractor. Approximately 150 ml of petroleum ether was poured into the round bottom flask. The apparatus was heated to 40-60°C and allowed to run for 3 hours of continuous extraction using the Soxhlet apparatus. Finally, the solvent was distilled, and the percentage of oil extracted was determined.

The percentage yield of the oils was calculated as follows:

$$\% \text{ yield} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100 \quad \text{---------- 5}$$

3.4 Characterization of the Extracted Oil

3.4.1 Physical Properties

The analysis was conducted using AOAC (2016) methods.

1. **Color Determination**

The oil sample in a glass tube was viewed and matched against a standard color and recorded.

2. **Specific Gravity Test**

A clean and dried gravity bottle was weighed, and its weight was recorded. The bottle was then filled with oil, weighed, and its weight recorded. The weight of the bottle when filled with distilled water was also recorded.

$$\text{Specific gravity} = \frac{\text{weight of oil}}{\text{Weight of equal volume of water}} \quad \text{---------- 6}$$

3.4.2 Chemical Properties

1. **Determination of Acid Value**

25ml of diethyl ether and 25 ml of ethanol were mixed in a 250 ml beaker. The resulting mixture was added to 10 g of oil in a 25 ml conical flask, and a few drops of phenolphthalein were added. The mixture was titrated with 0.1 M NaOH with consistent shaking until a dark pink color was observed. The volume of 0.1 M NaOH used was noted.

Free fatty acid = Titre value x M x 5.61 ---------- 7

Weight of sample

Where M = Normality of KOH

Acid value = 2 x free fatty acid

2. **Determination of Saponification Value**

2g of the sample were weighed into a conical flask, and 25 ml of 0.1 M potassium hydroxide was added. The content, which was constantly stirred, was allowed to boil gently for 60 minutes. A reflux condenser was placed on the flask containing the mixture, and a few drops of phenolphthalein indicator were added to the warm solution. The mixture was then titrated with 0.5 M HCl to the endpoint until the pink color of the indicator disappeared. The same procedure was used for other samples and a blank.

The expression for saponification value (SV) is given by:

SV= 56.1 x N x (Vo-Vi) ---------- 8

Where:

- *Vo* = the volume of the solution used for the blank test
- *Vi* = the volume of the solution used for the determination
- *N* = normality of HCl used (0.5)
- *m* = mass of the sample

3. **Determination of Iodine Value**

0.4g of the sample was weighed into a conical flask, and 20 ml of carbon tetrachloride was added to dissolve the oil. 25ml of Dam reagent were added to the flask using a safety pipette in a fume chamber. The flask was vigorously swirled and placed in the dark for 2 hours and 30 minutes. After this period, 20 ml of 10% aqueous potassium iodide and 125 ml of water were added using a measuring cylinder. The content was titrated with 0.1 M sodium thiosulphate solution until the yellow color almost disappeared. A few drops of 1% starch indicator were added, and titration continued by adding thiosulphate drop-wise until the blue coloration disappeared after vigorous shaking. The same procedure was used for the blank test and other samples.

The iodine value ([V) is given by the expression:

Iodine Value = $\frac{12.69 \times (V_1 - V_2) \times N}{M}$ ---------- 9

Where:

- N = molarity of sodium thiosulphate used
- $V1$ = volume of sodium thiosulphate used for the blank
- $V2$ = volume of sodium thiosulphate used for determination
- m = mass of the sample

4. **Determination of Peroxide Value**

$$PV = \frac{S \times M \times 1000}{W} \quad \text{---------- 10}$$

Where:

- S = volume of sodium thiosulphate used
- M = molarity of sodium thiosulphate used
- W = weight of the sample

4.0 RESULTS AND DISCUSSION

Proximate Composition

Table 2: Proximate Composition of T. catappa seed

Parameters	Composition (%)
Moisture	23.24
Ash	5.50
Fat	16.51
Crude Protein	21.22
Crude Fibre	12.30
Carbohydrate	39.99

The moisture content of the almond seed was found to be 23.24%. This result indicates that the almond seed has a high moisture content, making it unsuitable for long-term preservation. This value is significantly higher compared to 5.50% for cashew nuts (Barreca et al., 2020) and 5.10% for African oil beans (Akinlabu et al., 2019).

The proximate analysis results also indicate that the nut is a rich source of carbohydrates. Carbohydrates are easily digested and provide the necessary calories in most people's diets. Therefore, the results confirm that almonds are an energy-rich food source.

The ash content was determined to be 5.59%, indicating the presence of various minerals.

The protein content was found to be 21.229%, suggesting that *Terminalia catappa* seed can support growth and maintain a positive nitrogen balance, thus indicating a high dietary protein quality. In human diets, protein quality and quantity are significant concerns. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend a daily intake of 0.88 g of protein per kg body weight for children aged 1-10 years. As many people in developing nations rely almost exclusively on plant proteins, an adequate serving of almond nuts should be encouraged in their diets.

The results also show that *T. catappa* contains fiber. Fiber improves appetite satisfaction, facilitates the movement of food through the digestive system, and prevents constipation (Chikezie, 2017; Alka et al., 2018). Fiber is advantageous in human nutrition, as soluble fiber appears to reduce the blood's low-density lipoprotein (cholesterol) level (King et al., 2015).

The high seed oil content indicates that the almond seed is a cost-effective source of edible oils that can be used as an alternative to conventional oil seeds such as palm oil, groundnut oil, and soybean oil (Andersen, 2016)).

Table 3: Physicochemical properties of *T. catappa* seed Oil

Properties	Composition
Saponification (mg KOH/g)	162
Acid value (mg KOH/g)	1.68
Iodine value	89
Specific gravity	0.95
% yield	46
Colour	Yellow
Peroxide value (mg Iodine/g)	1.40

The acid value of *Terminalia catappa* seed oil was determined to be 1.68. This value is low compared to 2.15 for melon seed oil (Ng et al., 2015) and 4.30 for camphor seed oil (Miller & Yeung, 2022). Low acid values indicate the edibility of *T. catappa* seed oil.

The iodine value of almond seed oil was found to be 89. The iodine value measures fats and oils' unsaturation and indicates double bonds' presence in their molecular structure. According to Petkova and Antova (2015), iodine values below 100 classify the oil as non-drying. Hence, *T. catappa* oil is non-drying because its iodine value is lower than 100. Therefore, *T. catappa* seed oil is not suitable for paint making but is appropriate for use in the cosmetic industry.

The low peroxide values of the oil indicate low susceptibility to oxidative rancidity and deterioration, confirming the presence of antioxidants in the seed oil.

T. catappa's specific gravity was observed to be 0.95, indicating less dense than water.

5.0 CONCLUSION AND RECOMMENDATION

The study's results conclude that *Terminalia catappa* seeds exhibit a high level of most chemical components, making them a promising raw material for various industries. They also serve as useful dietary supplements. Their high protein value and low level of anti-nutrients indicate their potential usefulness in animal and poultry feed supplements.

The chemical properties of *T. catappa* seed oil showed a high saponification value and a low iodine value. As a non-drying oil, *T. catappa* seed oil is not suitable for paint making but is recommended for use in cosmetics.

Based on these findings, we recommend that *T. catappa* be used in food supplements due to its high nutritional value and qualify for industrial use due to its high oil content.

Consent:
Informed consent was obtained from all participants involved in the study.

Ethical Approval:
The Institutional Ethics Committee approved the study protocol.

References

Aguirre, M., Kiegle, E., Leo, G., & Ezquer, I. (2018). Carbohydrate reserves and seed development: An overview. *Plant reproduction*, *31*, 263-290. DOI:10.1007/s00497-018-0336-3

Akinlabu, D. K., Owoeye, T. F., Owolabi, F. E., Audu, O. Y., Ajanaku, C. O., Falope, F.Ajani, O. O. (2019, December). Phytochemical and proximate analysis of African oil bean (Pentaclethra macrophylla Benth) seed. In *Journal of Physics: Conference Series* (Vol. 1378, No. 3, p. 032057). IOP Publishing. DOI:10.1088/1742-6596/1378/3/032057

Alasalvar, C., Salvadó, J. S., & Ros, E. (2020). Bioactives and health benefits of nuts and dried fruits. *Food chemistry*, *314*, 126192. DOI: 10.1016/j.foodchem.2020.126192

Alka, G., Anamika, S., & Ranu, P. (2018). A review on watermelon (Citrullus lanatus) medicinal seeds. *Journal of Pharmacognosy and Phytochemistry*, *7*(3), 2222-2225.

Anand, A., Divya, N., & Kotti, P. (2015). An updated review of Terminalia catappa. *Pharmacognosy reviews*, *9*(18), 93.DOI:10.4103/0973-7847.162103

Andersen, A. J. C. (2016). *Refining of oils and fats for edible purposes*. Elsevier.

AOAC International. (2016). *Official Methods of Analysis of AOAC International* (20th ed.). Rockville, MD: AOAC International.

Arnett, D. K., Blumenthal, R. S., Albert, M. A., Buroker, A. B., Goldberger, Z. D., Hahn, E. J., & Ziaeian, B. (2019). 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*, *140*(11), e563-e595. https://doi.org/10.1161/CIR.0000000000000677

Asokapandian, S., Sreelakshmi, S., & Rajamanickam, G. (2021). Lipids and oils: an overview. *Food biopolymers: Structural, functional and nutraceutical properties*, 389-411. DOI https://doi.org/10.1007/978-3-030-27061-2_16

Barreca, D., Nabavi, S. M., Sureda, A., Rasekhian, M., Raciti, R., Silva, A. S., ... & Mandalari, G. (2020). Almonds (Prunus dulcis Mill. DA webb): A source of nutrients and health-promoting compounds. *Nutrients*, *12*(3), 672. DOI:10.3390/nu12030672

Chikezie, U. N. (2017). Phytochemical and proximate composition of Tetracarpidium conophorum (African Walnut) seeds. *International Journal of Research Studies in Biosciences*, *5*(10), 25-31.DOI:10.24297/jbt.v7i1.6411

Čolić, S., Zec, G., Natić, M., & Fotirić-Akšić, M. (2019). Almond (Prunus dulcis) oil. *Fruit oils: chemistry and functionality*, 149-180. DOI:10.1007/978-3-030-12473-1_6

Dianala, R. D. B. (2019). Utilization of the tropical almond tree leaves in aquaculture. *Fish for the People*, *17*(3), 41-43. http://hdl.handle.net/20.500.12066/5794

Faghihi, A., Zohalinezhad, M. E., & Najafi Kalyani, M. (2022). Comparison of the effects of abdominal massage and oral administration of sweet almond oil on constipation and quality of life among elderly individuals: a single-blind clinical trial. *BioMed Research International*, *2022*. https://doi.org/10.1155/2022/9661939

Fahmy, N. M., Al-Sayed, E., & Singab, A. N. (2015). Genus Terminalia: A phytochemical and biological review. *Montin.) species. Med Aromat Plants*, *4*(5), 1-22. http://dx.doi.org/10.4172/2167-0412.1000218

Karimi, Z., Firouzi, M., Dadmehr, M., Javad- Mousavi, S. A., Bagheriani, N., & Sadeghpour, O. (2021). Almond as a nutraceutical and therapeutic agent in Persian medicine and modern phytotherapy: A narrative review. *Phytotherapy research*, *35*(6), 2997-3012. https://doi.org/10.1002/ptr.7006

King, F. S., Burgess, A., Quinn, V. J., & Osei, A. K. (Eds.). (2015). *Nutrition for developing countries*. Oxford University Press.

Marion- Letellier, R., Savoye, G., & Ghosh, S. (2015). Polyunsaturated fatty acids and inflammation. *IUBMB life*, *67*(9), 659-667. https://doi.org/10.1002/iub.1428

Menkiti, M. C., Agu, C. M., Ejikeme, P. M., & Onyelucheya, O. E. (2017). Chemically improved Terminalia catappa L. oil: A possible renewable substitute for conventional mineral transformer oil. *Journal of Environmental Chemical Engineering*, *5*(1), 1107-1118. https://doi.org/10.1016/j.jece.2017.01.037

Miller, D. D., & Yeung, C. K. (2022). *Food chemistry: A laboratory manual*. John Wiley & Sons.

Mushtaq, A., Khaliq, M., Saeed, A., Azeem, M. W., & Ghania, J. B. (2015). Almond (Purunus amygdalus L.): A review on health benefits, nutritional value and therapeutic applications. *International Journal of Chemical and Biochemical Sciences*, *8*, 103-106.

Mustafa, G., & Iqbal, A. (2021). Economics of Oil Plants: Demand, Supply, and International Trade. *Oil Crop Genomics*, 393-413. DOI:10.1007/978-3-030-70420-9_19

Ng, S., Lasekan, O., Muhammad, K. S., Hussain, N., & Sulaiman, R. (2015). Physicochemical properties of Malaysian-grown tropical almond nuts (Terminalia catappa). *Journal of food science and technology*, *52*, 6623-6630. DOI:10.1007/s13197-015-1737-z

Oso, A. A., & Ashafa, A. O. (2021). Nutritional composition of grain and seed proteins. *Grain and seed proteins functionality*, 31-47. DOI:10.5772/intechopen.97878

Ouzir, M., Bernoussi, S. E., Tabyaoui, M., & Taghzouti, K. (2021). Almond oil: A comprehensive review of chemical composition, extraction methods, preservation conditions, potential health benefits, and safety. *Comprehensive reviews in food science and food safety*, *20*(4), 3344-3387. https://doi.org/10.1111/1541-4337.12752

Patel, A., Rova, U., Christakopoulos, P., & Matsakas, L. (2020). Introduction to essential fatty acids. *Nutraceutical Fatty Acids from Oleaginous Microalgae: A Human Health Perspective*, 1-22. DOI:10.1002/9781119631729.ch1

Petkova, Z., & Antova, G. (2015). Proximate composition of seeds and seed oils from melon (Cucumis melo L.) cultivated in Bulgaria. *Cogent Food & Agriculture*, *1*(1), 1018779. DOI:10.1080/23311932.2015.1018779

Rahman, H., Sitompul, J. P., & Tjokrodiningrat, S. (2022). The composition of fatty acids in several vegetable oils from Indonesia. *Biodiversitas Journal of Biological Diversity*, *23*(4). DOI:10.13057/biodiv/d230452

Reka, B., Sindhu, D., & Asha, S. (2021). Bio-Active Compounds and its ImmunomodulatoryProperties.DOI: http://dx.doi.org/10.5958/2322-0996.2021.00010.

Saharan, A., Dureja, H., & Dhiman, A. (2022). TERMINALIA CATAPPA LINN.: A TREASURY OF PHARMACOLOGICAL BENEFITS. *NeuroQuantology*, *20*(22), 829. DOI:10.48047/nq.2022.20.22.NQ10064

Spector, A. A., & Kim, H. Y. (2015). Discovery of essential fatty acids. *Journal of lipid research*, *56*(1), 11-21. https://doi.org/10.1194/jlr.R055095

Williams, P. T., Bergeron, N., Chiu, S., & Krauss, R. M. (2019). A randomized, controlled trial on the effects of almonds on lipoprotein response to a higher carbohydrate, lower fat diet in men and women with abdominal adiposity. *Lipids in health and disease*, *18*, 1-9. DOI:10.1186/s12944-019-1025-4