

Physico-Chemical Characteristics of Butter Extracted from Seed of *Tieghemella Heckelii* (Sapotaceae) Grown in the Rain Forest of West Ivory Coast

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Received Date: 27-Sep-2019

Accepted Date: 05-Oct-2019

Published Date: 16-Oct-2019

Abstract

The study examined the extraction of *Tieghemella heckelii* (Sapotaceae) seed butter and their physico-chemical properties in order to explore their potentials in food systems. After different steps of traditional extraction, the physico-chemical properties of *Tieghemella heckelii* almonds butter were studied. The analysis showed that this butter contains three vitamins, five fatty acids, and some polyphenols. Other properties such as the density, the unsaponifiable compounds, the color, the fat content, the peroxide index, the acidity and the pH were studied. The physico-chemical analyzing of *Tieghemella heckelii* (Sapotaceae) butter appeared beneficial for improving its nutritional statute. The fact that physico-chemical characteristics of *Tieghemella heckelii* (Sapotaceae) butter oil are comparable to other high value edible vegetable oils indicates its suitability as raw material for food, cosmetic and pharmaceutical products.

Key words: *Tieghemella heckelii* (Sapotaceae), butter, oil, physico-chemical characteristics

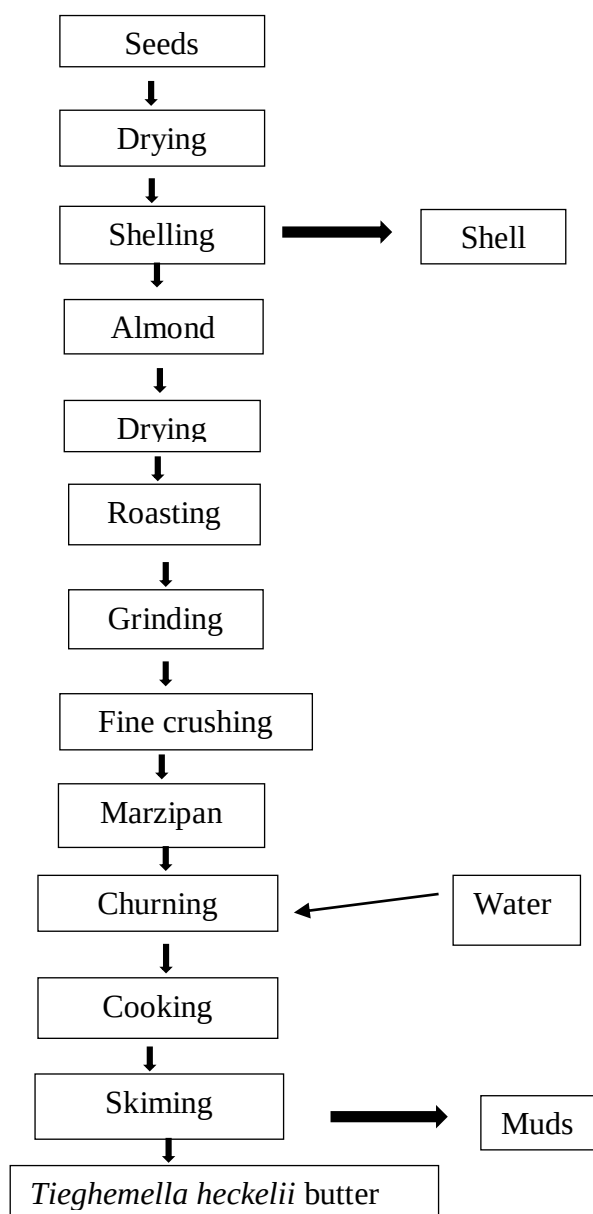
INTRODUCTION

In developing nations, numerous wild edible plants are exploited as food sources; hence they provide an adequate level of nutrition to the inhabitants (Aberoumand, 2009). Among these edible plants, appear the oleaginous plants. During these five last years, the consumption of the edible oils does not cease increasing; its production reached an average of 15 million ton/year. Indeed, this element essential with the nutrition can be of vegetable nature in the form of greasy substance (Bourachouche and Boudei, 2016). These greasy food substances are significant elements of our supply because they are rich in essential fatty acids, in vitamins and minerals (Trémoliers *et al.*, 1984). They can be extracted from seeds or the fruits of many vegetable species. The natural vegetation of Ivory Coast offers to the local populations many useful species of forms herbaceous, woody, as well as trees, which produce fruits, seeds, barks, sheets and wood (Aké *et al.*, 1992). Among these many arborescent and fruit-bearing forest species *Tieghemella heckelii* still called makoré or the 'wild mango' appears pertaining to the family of Sapotaceae. Traditionally in Ivory Coast, the seeds of *T. heckelii* are crushed and extracted with water, the supernatant is collected and water is drained and thrown. The butter obtained enriched in grease is used at various ends. It is used at food ends, medical for the treatment of the skin troubles (Tuani *et al.*, 1994), in cosmetic like pomade for the body and the hair and in soap factory. Butter extracts from almonds are consumed like oils crackling in the forest area of Ivory Coast. In spite of the importance and the role which plays this oilseed, few studies are devoted there. The few rare studies carried out related to the tree. Also to our knowledge few studies have been done on the butter extracted from *Tieghemella heckelii* almonds. This project aims to determine the physico-chemical properties of this butter in order to assess its nutritional benefits.

Materials and Methods

Plant Materials

Tieghemella heckelii fruits were collected from the western areas of the Ivory Coast. The fresh, firm and mature fruits were harvested and transported to the laboratory for butter processing before analyses.

Traditional process of *Tieghemella heckelii* butter extraction**Figure 1:** Different stages of *Tieghemella heckelii* butter production**ANALYSIS OF *Tieghemella heckelii* BUTTER****Yield of *Tieghemella heckelii* butter production by the traditional method**

The number of matters incoming and outgoing was measured using a precision balance. These measurements made it possible to calculate the yield making it possible to evaluate the losses and the output of the extraction method used. The output of extraction of the method used was calculated starting from the following formula:

$$R = \frac{m}{m_0} \times 100$$

Where: R = output of extraction

m_0 = matters incoming

m = outgoing

Determination of soap traces

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The procedure of the soap traces determination consists in putting 40ml acetone-water mixture (3%) in an Erlenmeyer, then add to it 0.5ml solution of the bromothymol blue as indicator, then to neutralize the acetone-water mixture by titration until the turn of the solution to the yellow with the hydrochloric acid with 0,01N. Add 10g of oil and mix well with the neutralized solution. Then titrate until the yellow turn with the hydrochloric acid with 0,01N. The soap oil content expressed in ppm is determined by the following expression:

$$\text{Soap oil content in ppm} = (V \times 3100) / P$$

Where: P = test sample (g)

V = hydrochloric acid volume

Moisture content

The moisture content of the samples was determined by drying oven (105°C for 24 h). It consists of weighing 5 g of oil in a crucible and stove at 103°C for 24h. Then crucible and its contents were withdrawn and let cool in the desiccator then to weigh. Remake the weighing process until constant weight. The content is expressed by the following expression: % moisture = $(P - P_1) \times 100 \div P_0$

Where: P = Weight of the empty crucible and its contents

P₁ = Loss of weight

P₀ = Test sample

Titratable acidity

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. The oil fat was mixing thoroughly before weighting. Weight accurately about 10 g of cooled oil sample in a 250 ml conical flask. Add 75 ml neutralized ethanol. Boil the mixture (in a water bath) for about 5 minutes. Then Add few drops of phenolphthalein as an indicator solution and titrate with NaOH (0,01N) drop by the drop and stirring the content till first definite change to pink colour. Note down the final burette reading. Acidity is expressed by:

$$\% \text{ Acidity value} = N \times V \times PM \times 100 / P \times 1000$$

Where: N = normality of NaOH solution

V = volume of NaOH solution

PM = molecular weight of oleic acid

P = weight of the sample

Peroxide compounds

The Peroxide compound informs us about the deterioration and oxidation step of the fat content. 30 ml chloroform acetic acid solution and 0.5 ml Potassium iodide were added to 5 grams of oil in an Erlenmeyer flask. After, a few drops of starch-based adhesive and also 100 ml distilled water were added to the mixture in Erlenmeyer. The mixture is agitated until appearance of purple color. Then, titration was done on that with thiosulfate solution (0.01 N) until purple color disappears and in parallel to carry out a dummy trial (without oil). The Peroxide compounds expressed in active oxygen milliéquivalents per kilogramme is calculated by the following relation.

$$\text{Peroxide compounds (méq g O}_2 \text{ / kg MG)} = (N \times (V_1 - V_0) \times 1000) / P$$

Where: N = normality of thiosulfate solution

V₁ = volume of thiosulfate solution

V₀ = volume of the sodium thiosulfate solution for the dummy trial

P = weight of sample

Determination of the pH

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It was carried out with three recoveries while plunging the pH-meter in oil to be analyzed and for approximately 15min until this one posts the constant value of the pH.

Relative Density

Using an analytical balance, successive weighing of an equal volume of oil and water to the same temperature (20°C) is carried out. The procedure of the density determination consists to clean the pycnometer carefully and to dry it then to determine the mass empty pycnometer. Then fill the pycnometer with distilled water to the mark and leave 30 min in a water bath at 20°C and determine the mass pycnometer filled with distilled water. Clean and dry the pycnometer, then fill it with oil to the mark and determine the mass of the pycnometer containing oil. The relative density is given by the formula below:

$$\text{Density} = (m_2 - m_0) / (m_1 - m_0)$$

Where: m_0 = mass of empty pycnometer

m_1 = mass of pycnometer filled with distilled water

m_2 = mass of pycnometer containing oil

Determination of the color

The color of oil was determined by the Spectrocolorimeter lovibond PFX 995. The principle is based on the measure by visual comparison of the sample colors and glass of reference. Measurement relates only to the red color, the yellow being fixed at 70. The color is expressed by the relation: Red X 70 yellow Y white Z blue

Where X, Y, and Z are positive relative decimal numbers.

Crude fat

The oil of *Tieghemella heckelii* almonds was extracted by the soxhelt method. Weigh 5g almond finely crushed. Then, introduce the sample into a beforehand damaged permeable cellulose cartridge with solvent and cover it with absorbent cotton packed well. Put the cartridge in the apparatus extractor of Soxhlet. This last is provided with a cooling agent by the top, a balloon of 250ml clean, dry, and tared and a heating balloon by bottom. Then pour hexane until its half. Lead the heating under conditions such as a reflux rate of 3 drops/s applies. The solvent will evaporate then cooled, and the liquid falls on the substance to exhaust in a way so that the cartridge is immersed. When the intermediate part is sufficiently filled with solvent, the siphon starts. And the solvent containing the substance turns over in the balloon charged in lipids. After the 3 hours necessary of extraction, recovers the cartridge, on the one hand, and solvent then the extract, on the other hand. The solution obtained passed in Rotavapor to drive out by distillation the major part of solvent, which makes it possible to recover the lipids alone. Finally eliminate the last traces from solvent by heating the balloon with the drying oven at 105°C for 3h. Cool in desiccator to room temperature and weigh.

$$\text{Calculation \% Crude fat: } = \frac{[(P_3 - P_2) \times 100]}{P_1}$$

Where: P_1 = weigh of sample

P_2 = weigh of empty balloon

P_3 = weigh of the balloon with extracted oil

Cruds protein content

The method of KJELDAHL was used to determine the crude protein content. This method is based on the quantification of the nitrogen content. The crude protein is calculated by multiplying the nitrogen rate N (%) by coefficient 6.25. This method includes/understands two great essential stages: Sulfuric mineralization (Digestion) and the distillation followed by titration with the hydrochloric acid (HCl). 1g of the sample was introduced into tubes of KJELDAHL. In these tubes, 12 ml of 98 % sulfuric acid were added. A pinch of copper sulfate pastilles (CuSO_4) as catalyst is added in order to increase the speed and efficiency of the digestion procedure and Potassium sulfate in order to increase the boiling point of sulfuric acid. The

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appearance of white smoke which shows that the evaporation of water is completed and that the liquor obtained is a colouring clear green. During the distillation step the ammonium ions (NH_4^+) are converted into ammonia (NH_3) by adding Sodium hydroxide (0.5 N). The released ammonia is pulled by the water vapor. This ammonia was titrated by hydrochloric acid (0.109 N) in the presence of red methyl as indicator. Titration is completed at the time of the turn of blue to the red. The result obtained, after titration, enables us to calculate the percentage of total nitrogen. The formula is as follows:

$$\text{Nitrogen rate N (\%)} = [V - V \times N \times 1.401] / P$$

$$\% \text{ Crude protein} = \text{Nitrogen rate N (\%)} \times 6.25$$

Where: V = volume of hydrochloric acid

N = normality of hydrochloric acid

P = weight of the sample

Unsaponifiables compound

The proportioning of unsaponifiables was carried out in accordance with standard AFNOR NF T 60-206. This method rests on the saponification of a test specimen of 5 g oil by 50 mL of a caustic potash solution (2N) hot, under backward flow for 20 min. After the addition of 50 mL of distilled water, the unsaponifiables are extracted by diethyl ether followed by washing with the distilled water until neutral reaction from washing. The organic phase is then filtered on anhydrous sodium sulfate and is evaporated vacuum by a rotary evaporator. The residue thus obtained is dried at 103°C then let cool in a desiccator and weight. The unsaponifiable compound is determined by the following relation

$$\% \text{ Unsaponifiable} = (m_1 / m_0) \times 100$$

Where: m_0 = weight of test specimen

m_1 = weight of dry residue

Total phenolic compounds

The protocol used is based on that described by Singleton *et al.* (1965) with some modifications. The contents of total phenolic compounds were measured using the Folin-Ciocalteu reagent based colorimetric essay. A volume of 200 μL of each extract was mixed with 1 mL of Folin-Ciocalteu reagent diluted 10 times and 800 μL of sodium carbonate (Na_2CO_3) 7.5 %. The absorbance was recorded using a spectrophotometer at 765 nm after 30 min. The amount of total phenolic compounds as gallic acid equivalent was calculated from the calibration curve equation obtained from the standard curve. The total phenolic content was expressed in mg per 100 g

Vitamin C or Ascorbic Acid

The proportioning is based on the reaction of oxidation of the vitamin C (ascorbic acid) by a solution of 2,6-dichloroindolphénol (DCPIP) in an acid medium (Hughes, 1983). The vitamin C is extracted starting from 0.5 g of the sample by 20ml from the oxalic acid 1%, with an agitation during 30min with the darkness. After centrifugation 18335 x g, a volume of 300 μL of supernatant is mixed with 2.7 mL DCPIP. The absorbance was recorded using spectrophotometer at 515 nm after 15s. The amount of ascorbic acid was calculated from the calibration curve equation obtained from the standard curve. The ascorbic acid content was expressed in mg per 100 g

Extraction fat soluble vitamin A and E

The method described by Jedlicka and Klimes (2005) was used to extract the vitamin A and E. 10 ml of 10% KOH in methanol-water solution in ratio 1:1 was added with 1g of oil extract. To avoid the process of

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oxidation during saponification, 0.025 g of ascorbic acid was added. The mixture is then carried to the backward flow in a bain-marie at 70 °C for 30 min. the mixture then is cooled and extracted with 15 ml of hexane. The hexanic phases were joined together and dried on anhydrous sodium sulfate for drying evaporation. The residue obtained (Approximately 0.3 g) is included in 10 ml of methanol for the analysis. The evaluation of vitamin A and E content was made by HPLC coupled to a fluorimetric detector. The analysis is made in isocratic mode on column Hypersil ODS RP18 (stationary phase). The mobile phase is a mixture Acetonitrile/methanol in ratio 80:20 with a flow of 1 mL/min. The standards were prepared by serial dilution (from 1/10 to 1/2). Retinol: 11.3 µg/100mL, Tocopherol: 21.07 µg/100mL. All calculations are made starting from the witness 100%. Fluorimetric detection: 455 nm.

Determination of fatty acid profile

Preparation of methyl esters of the fatty acid

The extraction and the methylation of the fatty acids were carried out directly on extracted butter. The various stages of esterification can be summarized as follows: Introduce 2 oil drops in a tube out of a glass of 5 ml. Add 1 ml of hexane and stirring for 2 seconds. With this mixture, 0.2 ml of methanolic soda ash (2N) was introduced and the whole solution is stirred for 10 seconds. Then, carry to the bain-marie at 50°C for 20 seconds and still shake for 10 seconds. Add 0.2 ml of hydrochloric acid methanolic (2N), stir and let elutriate. Take the surviving phase then inject the sample in the gas chromatography (GC).

Chromatography of methyl esters of the fatty acid

The methyl esters of the fatty acid was carried out by a GC of mark THERMO FINNIGAN provided with a detector with ionization of flame (FID) under the following operating conditions: Column capillary length: 30 m; Stationary phase: BPX70; Gas vector: nitrogenize; flow 30 mL/min; Volume injected: 1µl; Temperature of the column: 180°C; Temperature of injection: 240°C; Temperature of detector FID: 230°C. Calibrations were carried out for different acids purchased from Sigma-Aldrich.

RESULTS

Traditional process of *Tieghemella heckelii* butter extraction



Figure 2. *Tieghemella heckelii* almond oil
(After extraction)



Figure 3. *Tieghemella heckelii* butter
(at ambient temperature)

Chemical parameters

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The various methods used showed that butter does not have any soap trace. The water content and the pH are respectively 0.063% and 7.2. The peroxide value found in this study is about 0.83 méq O₂ / kg and its acidity is 0.2 (Table 1).

Table 1. Chemical parameters of *T. heckelii* almond butter

Chemical parameters	Content
Soap trace	0 ± 0
Moisture (%)	0.063±0.005
Acidity (%)	0.2±0.01
Peroxide (meq/kg)	0.83±0.035
pH	7.2±0.057

The obtained values are averages ± standard deviation of triplicate determinations

Physical parameters

The density is one of the purity criteria of oil. It informs about the group to which oil belongs. The density value found in this study is about 0.910. Concerning the color that is a subjective indicator used by food industry for the fast monitoring of oil quality is 3.76 (Table 2).

Table 2. Physical parameters of *T. heckelii* almond butter

Physical parameters	Value
Rendement (%)	30.11±0.24
Color	3.76±0.057
Density	0.91±0.005

The obtained values are averages ± standard deviation of triplicate determinations

Chemical constituents

Butter is extracted starting from the seeds of the almond of *Tieghemella heckelii* by the extraction method using hexane solvent but according to the literature; it remains the best suitable one. The result shows that taking 3 hours is sufficient for a good output. The fat content obtained shows that the *T. heckelii* almonds seeds are rich in fat content with an average of 58.25%. But no protein is found in the *T. heckelii* butter. The unsaponifiable content giving an account of characteristics of oil quality is 4.59 %. The butter also contains 0.256 mg/g of polyphenol. Its composition in vitamin A, C and E is respectively of 0.014mg/g, 0.49 mg/g and 0.036 mg/g (Table 3).

Table 3. Chemical constituents of *T. heckelii* almond butter

Chemical constituents	Content
Butter (%)	58.25±0.11
Protein (mg/g)	0±0
Unsaponifiables (g/100g)	4.59±0.09
Polyphenol (mg/g)	0.256±0.003
Vitamin C (mg/g)	0.49±0.0035
Vitamin A (mg/g)	0.014±0.003
vitamin E (mg/g)	0.036±0.005

The obtained values are averages ± standard deviation of triplicate determinations

Fatty acid profile

The butter of studied *T. heckelii* contains a relatively low number of different fatty acids in extremely different proportions (Table 4). GC analysis of the fatty acid showed that it contained five fatty acids. The

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most abundant are 59.54% oleic acid, 17.47% of stearic acid; the medium is 6.65 % of linolenic acid, 2.58% of palmitic acid and the minor fatty acids is 0.689% of linoleic acid.

Table 4. Analysis of Fatty Acids in the oil of *T. heckelii* almond butter

Fatty acids	Content (g/100g)
oleic acid	59.54±0.04
stearic acid	17.47±0.068
linolenic acid	6.65±0.037
palmitic acid	2.58 ± 0.028
linoleic acid	0.689±0.002

The obtained values are averages ± standard deviation of triplicate determinations

DISCUSSION

The moisture content was 0.063%, which is low and, therefore, beneficial for prolonging the shelf life *T. heckelii* almond butter. Chew *et al.* (2011) reported that reduced moisture content ensured the inhibition of microbial growth; hence, it is an important factor in food preservation. The reduction in moisture content as observed in this study may indicate good stability of *Tieghemella heckelii* butter. The soap trace was not detected in this oil.

Acidity is largely used by many manufacturers of foodstuffs like an indicator of the oil deterioration (presence of free fatty acid) (Tarmizi and Ismail, 2008). It is recommended for an edible oil to have a low rate of acidity (lower than 3.3% standard imposed by Codex Alimentarius) to support long conservation without deterioration. The low acidity value was a result of lower hydrolysis of triglycerides and signified that the oil could have a long shelf life, which allows it to be consumed as virgin edible oil.

The acidity of *T. heckelii* butter (0.2%) is comparable with that of vegetable oils such as the olive oil (0.5-1.5%), the sunflower oil (0.2 %), and the shea butter (0.39%) (Dahouenon *et al.*, 2012).

The peroxide value generally indicates the level of the rancidity of oil. It is a very useful criterion and a satisfactory sensitivity to appreciate the first stages of oxydative deterioration (Chimi, 2005). The peroxide value was 0.83 meq O₂/kg of oil, which is less than 10 meq O₂/kg of oil, allowed for crude oils by Codex Alimentarius Committee (Firestone, 1997).

This value obtained is lower than the shea butter (8.1-10.1) (Dahouenon *et al.*, 2012), cotton seed oil (< 10) (Bozdogan, 2017). It was shown that oils fraiches have a peroxide index lower than 10 meqO₂/kg and they become rancid when the peroxide value is on the beach from 20 to 40 meqO₂/kg (Onyeike *et al.*, 2002). The found peroxide is rather low, which enables us to conclude that the butter of *T. heckelii* will resist deterioration better.

Color is an important quality parameter of edible oil, both in the refining process and in the marketplace. It is also frequently monitored in the product line according to some commercial standard in order to maintain a consistent quality (Bayley, 1948). The color of *T. heckelii* oil is 4.5 which normalizes its color of edible oils. The lower the value is, the more colouring tends towards yellow gold appreciated by consumers (Soumanou *et al.*, 2009).

This work shows that the yield of *T. heckelii* butter extraction by the traditional method is higher (30.11%) than shea butter (20 to 42%) (lovett, 2015).

The Unsaponifiable is consisted of the whole of the extractable compounds by organic solvents (hexane, ethyl ether) directly after treatment of the greasy substance by alcoholic potash. It represents the nonglyceridic part of oil. It is very rich in secondary metabolites such as cholesterol, phytosterols, ergosterol, pigments, and fat soluble vitamins. The Unsaponifiable gives an account of oil quality characteristics (Mostefa, 2010). According to Bockisch (1993) and Elmadfa, (1995), Unsaponifiable makes up 5 to 6% of vegetable oils. Unsaponifiable of *T. heckelii* butter (4.59%) is similar to that author but largely exceeds cotton seed oil (1.5%), sunflower oil (0.4% to 1.4%), and olive oil (1.5 %) (Lambert, 2005).

This Unsaponifiable content of *T. heckelii* butter would confer to his oil significant therapeutic virtues (Charrouf, 2002).

The total phenolic compound is naturally present in the fruits, the vegetables, the seeds and the flowers (Ojeil *et al.*, 2010). Various studies show that phenolic compounds have positive effects on human health (Stark and Madar, (2002); Visioli and Galli, (2002); Carluccio *et al.*, (2002)). With their antioxidant properties, phenolic compounds have been discovered that can be important for their effects on human health. The amount of total phenols in *T. heckelii* butter (256 mg/kg) is, on average lower than olive oil (800 mg to 1g/kg) (Visioli and Galli, 1998).

Vitamins A and E are a fat-soluble chemical substance while vitamin C is water soluble. Vitamin A is necessary to ensure normal growth as well as healthy skin, eyes, teeth, gums and hair. Vitamin E is necessary for the formation and functioning of red blood corpuscles, muscles and other tissue. According to Rader *et al.* (1997), vegetable oils are the main dietary sources of vitamin E, which decreases the risk of cardiovascular diseases and cancer. Vitamin C strengthens the body immunity against infections, helps in collagen and thyroxin synthesis and enhances iron absorption. Total amount vitamin E (tocopherols) was 36mg/kg, which was lower than reported for peanut oil (398.60mg/kg); grapeseed oil (140.60mg/kg) and olive oil (216.80mg/kg) (Xu *et al.*, 2007). The quantity of vitamin E found in *T. heckelii* seed butter could contribute to its oxidative stability. The vitamins A and C composition of *T. heckelii* almond butter are 0.014 mg/g and 0.49 mg /g respectively. *T. heckelii* almond butter thus has a high level of vitamin C content and an appreciable amount of vitamin A content. Its vitamin C is higher than that of *P. africana* seeds ($0.92 \pm 0.02\text{mg}/100\text{g}$) groundnut ($9.8\text{mg}/100\text{g}$) and *D. edulis* ($25.76 \pm 1.51\text{mg}/100\text{g}$) seeds [20].]. Its Vitamin A content is also higher than those of *P. africana* ($0.89 \pm 0.01\text{ug}/100\text{g}$) and *D. edulis* ($1.13 \pm 0.04\text{mg}/100\text{g}$) (Ujowundu *et al.*, 2010).

The results obtained showed that *T. heckelii* almond butter (7.2) tends towards neutral. It is similar to those of Dura and Tenera Palm kernel oil (6.49 and 6.72, respectively) (Akpakpan *et al.*, 2018).

The fatty acid content of *T. heckelii* butter has two majority fatty acids that are oleic acid 59.54% and stearic 17.47%. This result is in agreement with those of Gwali *et al.* (2012), who reported that the dominant fatty acids of shea butter were oleic and stearic acids. The linolenic acid value (6.65 %) is similar to shea butter oil value (6 – 8%) (Okullo *et al.*, 2010) but lower than sunflower oil and soya bean oil linoleic acid values (74% and 53% respectively) (Maritz *et al.*, 2006). Linoleic acid is an essential fatty acid that is vital in nutrition because of its un-saturation. The linoleic acid value content of 6.65 % makes *T. heckelii* butter oil a moderate source of essential fatty acids in the human diet (Maritz *et al.*, 2006).

Conclusion

Based on the results of the physico chemical properties of *Tieghemella heckelii* (Sapotaceae) butter and oil, it could be concluded that this butter and oil could become valuable resources to produce high value of vegetable oil. Most of the values such as unsaponifiable, vitamin C, A and E value, polyphenol value, peroxide value, acidity value, fatty acid value, comply with the standard specifications. Acidity and peroxide value show that *Tieghemella heckelii* (Sapotaceae) butter and oil will resist deterioration better and have long conservation without deterioration. Unsaponifiable and total phenolic content can be important for their effects on human health. *Tieghemella heckelii* (Sapotaceae) butter is rich in oleic and stearic acid indicating that oil was stable and tolerant to rancidity. Such properties are very required on the international market for technological uses in cosmetics and food industry.

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