

Original Research Article

Evaluation of Chemical Composition and Physicochemical Properties of Giant Yellow Mulberry Fruit (*Myrianthus arboreus*)

ABSTRACT

Aims: This study aimed at evaluating the nutritional value of the pulp and seeds the giant mulberry (*Myrianthus arboreus*) fruit, and determine the physicochemical properties of its seed oil.

Study design: The experimental design was employed.

Place and Duration of Study: Ripe fruits of *Myrianthus arboreus* were harvested from a secondary forest in Fontem, South west region of Cameroon. The fruits were transported to the life science laboratory of the University of Buea for preparation and analyses.

Methodology: The methods used for the nutritional were carried out following standard by Association of Analytical Chemists. Oil was extracted using soxhlet method. UV-visible spectrophotometer and gas chromatography were used to analyse the physicochemical and fatty acid properties of the seed oil. Data were analysed using Statistical Package for Social Sciences version 21.

Results: Results on the proximate composition of the pulp and seeds revealed; carbohydrate; 5.03% and 28.06%, proteins; 17.22% and 24.40% and lipids 6.50% and 42.05% respectively. The seed had more energy (589.67Kcal/g) than pulp (143.95Kcal/g). The abundant minerals were K, Ca and P for pulp and Mg, K and P for the seeds and both samples contained appreciable amount of vitamins A and C. The analysis of antinutrients (phytate, tannin and oxalate) showed that the samples had moderately low content in these. For the oil quality indices, results revealed Free fatty acid, 2.82 mgKOH/g, saponification value, 196.62 mgKOH/g, Iodine value of 171.45 gI₂/100g, Peroxide value of 2.04 mEq/kg and P-anisidine value, 3.80. Fatty acid profiling showed that the oil was 45.06% saturated and unsaturated fatty acids accounts (54.94%) consisting of 46.01 monounsaturated and 8.93% Polyunsaturated (mainly oleic and linoleic).

Conclusion: A knowledge of the potentials this fruit could encourage its cultivation and wider consumption. Its consumption may positively impact the nutritional status and health of the consumer.

Keywords: Pulp, Seed, oil, fatty acid profile, *Myrianthus arboreus*, chemical composition, physicochemical properties, fatty acid profile.

1. INTRODUCTION

Food security still remains of utmost concern in sub-Saharan Africa. In Cameroon, the continuous high levels of insecurity in the Northwest and Southwest regions, the rise in conflicts over natural resources (water, pastures, etc.), and floods (in the Far North, North and West) continue to have a severe impact on food security. Update for the 2023 Global Report against Food Crises, reveals that 22% and 10% of the population are respectively in phase 2 and 3 of acute food insecurity in 2023. Consequently, there has been an increase in malnutrition and nutritional deficiencies with over thirty-seven percent (37%) of children under 5 years of age being stunted [1].

As food based coping strategy to the poor and borderline food consumption score in Cameroon, it is important to diversify foods and diets using agricultural biodiversity to improve nutrition and health.

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Comment [u2]: Nutritional Analyses were carried out by the standard methods described by Association of Official Analytical Chemists (AOAC). Which year of AOAC?

Comment [u3]: Results on the proximate composition of the pulp and seeds revealed that carbohydrate were 5.03% and 28.06%; proteins, 17.22% and 24.40% and lipids 6.50% and 42.05% respectively.

The sentence can be rewritten as: 'Results revealed that the proximate composition of the study were 5.03% and 28.06% of carbohydrates; 17.22% and 24.40% of proteins; 6.50% and 42.05% of lipids, respectively of pulp and seeds.'

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One of alternative responses to this is using indigenous edible fruits. They are plant species known by populations for their fruits eatable and they grow spontaneously in the wild. The majority of most regions of Cameroon is covered by equatorial forests full of many little-exploited forest fruits that could nevertheless contribute to improving the nutritional status of its populations. Indigenous fruit trees, growing spontaneously, has made enormous contribution to nutrition and security, health and income generation in sub-Saharan Africa in general and in Cameroon in particular [2-5].

Leaves are consumed as vegetables with highly nutritious values as well medicinal properties, their fruits are edible and sometimes contain seeds of high nutritional and economic importance. Fruits not only offer easily available energy, but also micronutrients necessary to sustain and support human growth and activity [6]. They are naturally rich in antioxidant compounds, their by-products (peels and seeds) also contain high levels of antioxidants and phytochemicals that can be used as functional ingredients to fortify foods. In addition to their high nutritional value, indigenous fruit trees bridge the "hunger gap" producing even when staple crops fail during times of food shortage. In the last two decades, research efforts have been channeled towards harnessing the nutrient potentials of both conventional and unconventional fruits as a way of enhancing food security. Most available edible indigenous fruits in Cameroon are at present not fully utilized. At their different levels of utilization, the fruits are eaten and their seeds are commonly cooked and consumed, either directly as snack or complimentary foods, or fermented and used as condiments in soups and sauces. Hence, the lesser-known or under-exploited fruits and their seeds have recently assumed a new status in developing countries, especially with the focus on sustainable agriculture and nutrient requirements [7].

Cameroon's flora and diversity, contains many indigenous fruit tree plants that could provide a balance for human food, health, and industrial utilization, however there is a limitation of consumer awareness regarding the nutritional and health benefits of regular fruit consumption. *Myrianthus arboreus* commonly called giant yellow mulberry or monkey fruit is one of such tree. It occurs in the forest zone of tropical Africa and widely distributed in the tropical regions of West Africa (Côte d'Ivoire, Guinea, and Nigeria), Central Africa (Angola, Cameroon) and East Africa (Ethiopia, Tanzania). This fruit is known under various vernacular appellations in various African countries, Ivorian call it "wougnan" and "oujoujou" (Ibo). In Cameroon, it is locally called "pernambuco" or bush pineapple and its fruit pulp is edible, its seeds are oilseeds and are eaten cooked or raw in some regions, yet the extraction of its oil is not widespread in Cameroon unlike in other African countries where the fruits are found. In previous studies conducted in and around Kahuzi-Biega National Park (KBNP) Eastern D.R. Congo, more than 40 oil producing plant species were identified, *Myrianthus arboreus* was one of plant species [8]. Moreover, in Côte d'Ivoire, Katou *et al.*, [9], was able to highlight this potential, characterizing the fatty acids and showed it's predominance of unsaturated fatty acids (95.58%). The study also showed that, the seeds of *M. arboreus* are rich in fat matter; making this plant a promising source of oil.

Despite the increase research and consumption of *Myrianthus arboreus* fruit in other African countries, its consumption and utilization in Cameroon is limited in that, the fruit trees are mostly known only in their immediate localities, also, there is a severe shortage of research data on their nutritional value and utilization. To fully appreciate this under-exploited fruit, its current status in terms of their nutrient potentials, current nutrition and health uses, concentrations of anti-nutritional and toxic factors and functionality in food systems and appropriate processing techniques will need to be examined. The results to such information about the fruit could justify the call for wider exploitation and full utilization in other food systems, which may encourage their wider distribution.

2. MATERIAL AND METHODS

2.1 Sample collection

Ripe fruits of *Myrianthus arboreus* were harvested from a secondary forest in Fontem, a locality found in the South west region of Cameroon. The fruits were transported in ice cold box to the life science laboratory of the University of Buea for preparation and analyses.

2.2 Sample preparation

Myrianthus arboreus fruits (Fig 1) were washed and their seeds (Fig 1) dehulled and dried in an oven at 50°C for 48 hrs. The pulps were removed and crushed using a mortar and pestle and the frozen for further analyses. The seeds on the other hand were crushed and ground into powder using an electric blender. The meal flour (Fig 1) obtained was divided into two portions, one portion used for oil extraction while the other was stored for further analyses.

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2.3 Oil extraction

The method employed was that of solvent extraction with the Soxhlet extractor apparatus as described by AOAC,[10]. A 250 ml Soxhlet extractor apparatus and hexane was used as the solvent. A mass of 30 g of yellow mulberry seeds powder was weighed into a muslin cloth which was placed in a Soxhlet apparatus thimble. A round bottom flask containing 250 ml of hexane was placed to the end of the apparatus and a condenser, tightly fixed at the bottom the extractor. The set up was heated up in a water bath at a temperature of 60 °C. The excess solvent in the oil was recycled by heating in a heating mantle at a temperature of 60 °C after the extraction. Quantity of oil extracted was determined gravimetrically. The oil yield was then evaluated as the ratio of the weight of the extracted seed oil to the weight of seed powder sample. Oil yield was calculated as;

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$$\% \text{ Oil yield} = \frac{\text{Weight in gram of extracted oil}}{\text{Weight in gram of extracted seed powder sample}}$$

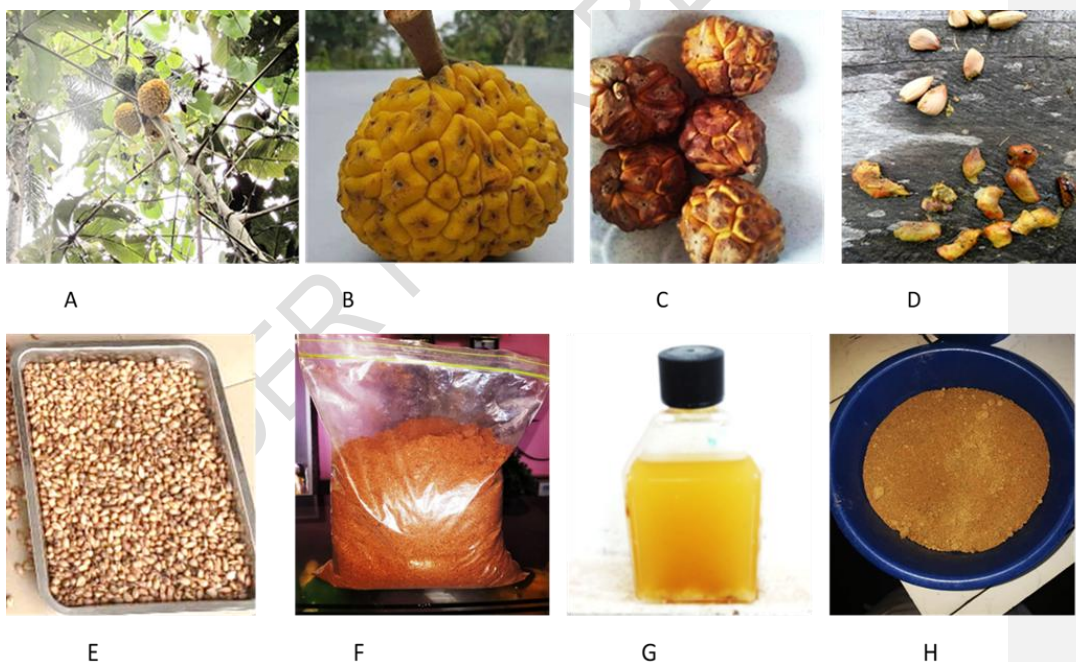


Figure 1: Photos showing sample preparation of *Myrianthus arboreus* pulp, seeds and oils
A = a typical giant yellow mulberry tree with fruits, **B** = fresh giant yellow mulberry fruit, **C** = ripe fruits with seeds, **D** = fresh decorticated seeds, **E** = oven dried seeds, **F** = powdered seeds prior to oil extraction, **G** = Extracted oil, **H** = defatted flour.

2.4 Chemical Composition of the pulps and seeds

2.4.1 Proximate Analyses

Nutrient composition of the food samples was determined using the standard procedures as described by AOAC, [10]. To determine the moisture, the samples were dried to a constant weight in a vacuum oven at 100°C for 24 hr. Protein (N × 6.25) was determined by the Kjeldahl method. Crude lipid was quantified gravimetrically and calculated as percentage of oil using soxhlet extractor. Ash was determined by incineration (550°C) of known weights of the samples in a muffle furnace. Crude fiber content of the samples were determined by mixing of the fine powder of the sample with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions for ignition and dried residue remaining after digestion of the samples was considered as crude fiber. Digestive carbohydrate content was determined by difference using the formula;

% Carbohydrate = 100 – (% crude protein + % crude lipid + % crude fibre + % Ash + %Moisture).

2.4.2 β-carotene/Vitamin A analysis

The β-carotene was determined by soaking 1 g of the sample (that is the paste or pulp of the fresh fruits) in 5 ml of methanol for 2 h at room temperature under dark condition in order to get a complete extraction. The β-carotene layer was separated using hexane through separating funnel. The volume was made up to 10 ml with hexane and then this layer was again passed through sodium sulphate through a funnel in order to remove any moisture from the layer. The absorbance of the layer was measured at 436 nm using hexane as a blank [11]. The beta carotene was calculated using the formula:

Beta-carotene (µg/100g) = Absorbance (436 nm) × V × D × 100 × 100/W × Y.

Where: V = Total volume of extract; D = Dilution factor; W= Sample weight; Y = Percentage dry matter content of the sample.

Vitamin A determination was calculated using 1 µg retinol = 1 RE method used. Variation

1 µg β-carotene = 0.167 µg RE.

2.4.3 Determination of vitamin C content

Vitamin C was determined by the titration method using the protocol described by Harris and Ray, [12]. A volume of 1 mL of the oil and 90% acetic acid was titrated with a solution of 50 µM DCIP. The endpoint titration was determined when the blue colour changes to a pale pink colour. A blank titration was performed against 90% acetic acid while a standard titration was performed against 40 mg/L pure L-ascorbic acid. The amount of Vitamin C was expressed in mg/100ml of sample.

2.4.4 Mineral Determination

Minerals were assayed by atomic absorption spectroscopy [10]. The principle is based on the fact that when atoms of an element are in contact with a flame, they emit wavelengths of radiation whose intensity can be measured spectrophotometrically. The concentration of the cation to be determined was calculated from the absorbance values by linear regression equations. All determinations of metals were performed with the iCE 3000 v1.3 AAS (Atomic Absorption Spectrophotometry) instrument. Hollow cathode lamps of the different metals were used as radiation sources for the instrument. The instruction manual of the instrument was used as guide for all measurements. Calibration standards were first aspirated into the AAS to calibrate the instrument and check its linearity response. After all necessary set up, standardization and calibration procedures had been completed then the powdered samples were aspirated into the AAS instrument for precise measurement of metal concentration. The minerals determined were Potassium, Sodium, Magnesium, Calcium, Iron, Phosphorus and Zinc.

2.5 Anti-nutritional factors of pulp and seed flour

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2.5.1 Determination of Total Phenolics Content (TPC).

The total phenolics content of the pulp and seed was determined using the Folin-Ciocalteu colorimetric method described by Chlopicka *et al*, [13].

2.5.2 Estimation of tannins content

The content of Tannins was estimated by the Vanillin-HCl method of Price *et al*, [14]. Defatted *M. arboreus* flour and pulp flour (5g) were treated with acidic methanol for extraction of tannins. From the diluted extract, 1 ml was mixed with 5 ml of freshly prepared vanillin-HCl reagent, and the optical density was determined at 500 nm by using a spectrophotometer. The results were expressed mg/100 g dm using catechin as standard.

2.5.3 Determination of phytates content

Phytates content was determined according to Wheeler and Ferrel, [15]. The amount of phytate phosphorus content was calculated from the standard curve by assuming that 4:6 iron-to-phosphorus molar ratio.

2.5.4 Determination of oxalates content

To determine oxalates content in *M. arboreus* seed flour and pulp, 2 g of flour were extracted with 100 ml of boiling distilled water for 30 min, filtered, and adjusted to 200 ml. The hot water extract residue was further extracted with 150 ml of boiling 1 M HCl for 30 min, adjusted to 200 ml, and filtered. The two filtrates were combined and titrated with potassium permanganate, [10].

2.6 Determination of Fatty acids profile

The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950µl of n-hexane to 50mg of oil followed by 50µl of sodium methoxide using the method of Liuet *al*. [16]. The mixtures were vortexed for 5s and allowed to settle for 5 min. 1µl of the top layer was injected into a gas chromatograph (Model GC-14A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionisation detector and a polar capillary column (B PX 70 0 .25), 0.32 mm internal diameter, 60m length and 0.25µm film thickness (SGE Incorporated, USA) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 240°C and column temperature was 110°C held for one minute and increased at the rate of 8°C.min⁻¹ to 220 °C and held for one minute. The run time was 32 min. The fatty acid Methyl ester peaks were identified by comparing their retention time with those of standards. Percent relative fatty acid was calculated based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

Fatty acid profiling (ΣSFA, ΣMUFA, ΣUFA, ΣPUFA, Σn-3/n-6 ratio, Σ omega-3, Σomega-6, ΣPUFA/ΣSFA ratio) was done by summing different classes of fatty acid and the atherogenicity index (AI) and thrombogenicity index (TI) were calculated according to Ulbricht & Southgate , [17]. as;

$$AI = \frac{[C12:0+(4 \times C14:0)+C16:0]}{(\Sigma MUFA + \Sigma \omega 6 + \Sigma \omega 3)}$$

$$TI = \frac{(C14:0+C16:0+C18:0)}{[(0.5 \times \Sigma AGMI) + (0.5 \times \Sigma \omega 6 + (3 \times \Sigma \omega 3) + (\Sigma \omega 3 / \Sigma \omega 6))]}$$

The ratio of hypocholesterolemic and hypercholesterolemic (H/H) by the method of Santos-Silva *et al.* [18]. as;

$$H/H = \frac{(C18:1cis9+C18:2\omega6+20:4\omega6+C18:3\omega3+C20:5\omega3+C22:5\omega3 +C22:6\omega3)}{(C14:0+C16:0)}$$

2.7 Determination of physicochemical properties of oil

Evaluation of *Myrianthus arboreus* seed oil was done by measuring the following parameters: free fatty acid, iodine, peroxide, saponification, P-anisidine and TOTOX values. These parameters were measured using standard methods of AFNOR, [19].

2.7.1 Free fatty acid

One gram of the oil sample was weighed into a 250-ml Erlenmeyer flask and 12ml of ethanol added. Five drops of phenolphthalein indicator was added and the mixture shaken to dissolve the sample completely. The mixture was titrated with 0.05N KOH, shaking vigorously until a slight pink colour that persisted for 30s. The free fatty acid value was calculated using the following formula:

$$\%FFA \text{ (as oleic)} = \frac{(V \times N \times 282)}{W \times 100}$$

Where;

% FFA = Percent free fatty acid (g/100 g), expressed as oleic acid

V = Volume of KOH titrant (mL)

N = Normality of KOH titrant (mol/1,000 mL)

282 = Molecular Weight of oleic acid (g/mol)

W = sample mass (g)

2.7.2 Peroxide value

Peroxide value is the number of mg of active peroxides in 1g of oil that reacts with KI with the liberation of I_2 . 1g of the oil sample was weighed into two 250ml glass-stoppered Erlenmeyer flasks and 5ml acetic acid-chloroform solution (3:2) added and swirled to dissolve. 0.5ml of saturated potassium iodide solution was added and allowed standing with occasional shaking for 1 min followed by addition of 10ml distilled water. 0.5ml of 1% starch solution was added and the samples were slowly titrated with 0.01N sodium thiosulfate solution, with vigorous shaking to release all iodine from chloroform layer, until blue color just disappears. The volume of titrant used was recorded. (If <0.5ml of the sodium thiosulfate solution is used, repeat determination). A blank sample was prepared and titrated and the volume of the titrant recorded.

$$\text{Peroxide value} = \frac{((S-B) \times N)}{W \times 1000}$$

Where:

Peroxide value = mEq peroxide per kg of sample,

S = volume of titrant (ml) for sample,

B = volume of titrant (ml) for blank,

N = normality of $\text{Na}_2\text{S}_2\text{O}_3$ solution (mEq/ml),

1000 = conversion of units (g/kg)

2.7.3 Iodine value

The Iodine value of a fatty substance is the amount of iodine, in grams, that is taken up by 100 grams of the oil under the conditions of the experiment [19]. 0.1g of oil sample was weighed in a beaker and 4ml of carbon tetrachloride (CCl_4) was added. The mixture was shaken for homogeneity and 4ml of wjz reagent added and the mixture stored in the dark for 10 minutes, followed by the addition of 5ml of a 10% potassium iodide solution and 4ml of distilled water. This mixture was carefully titrated with a 0.01N sodium thiosulphate. Iodine value was derived using the formula;

$$IV = \frac{(V_i - V_o)}{m \times 126.9 \times C}$$

Where;

IV = Iodine value

V_i = Volume of sample

V_o = volume of blank

m = Mass of oil

C = Concentration of sodium thiosulphate

126.9 = Molecular weight of iodine in g/mol

2.7.4 Saponification value

Saponification value provides information of the average molecular weight of all fatty acids present in the oil. It will show the amount of long chain fatty acids in the oil. 1g of oil sample was weighed into a 250-ml Erlenmeyer flask and 12ml of ethanol added. Five drops of phenolphthalein indicator was added and the mixture shaken to dissolve the sample completely. The mixture was titrated with 0.5N KOH, shaking vigorously until the endpoint is reached. The endpoint was indicated by a slight pink color that persisted for 30s. The free fatty acid value was calculated using the following formula:

$$SV = \frac{(V_o - V_s) \times CHCLx \ 56.1}{m}$$

Where:

SV= Saponification value

V_o = Volume of blank

Vs= Volume of sample

CHCL= concentration of HCL

m= Mass of oil

56.1 =Molecular weight of KOH in mg/mol

2.7.5 P-anisidine value (AV)

Anisidine value was determined by the standard method of the American Oil Chemists' Society Cd 18-90 (p-anisidine value) using a Perkin Elmer UV-Visible Spectrophotometer. In accordance with the method, 0.5-1 g of oil sample was weighed into a 25 mL volumetric flask. The sample was dissolved and diluted to 25 mL of volume with isooctane. Then the absorbance of the solution (Ab) was measured in a cuvette at 350 nm with the spectrophotometer, using the reference cuvette filled with isooctane solvent as a blank. After the measurement of absorbance, 5 ml of this solution and 5 ml of isooctane was transferred into 2 different tests tubes and 1 ml of 0.25% acetic acid solution of p-anisidine was added and the mixtures shaken on a vortex. After exactly 10 minutes of incubation at room temperature, the absorbance (As) of the solution containing sample was read at 350 nm, using the second solution as blank. P-anisidine value was calculated as followed:

$$P - An = \frac{25(1.2As - Ab)}{m}$$

Where:

As is the absorbance of test solution after reaction with the p-anisidine reagent;

Ab is the absorbance of the fat solution; m is the sample weight.

2.7.6 Total Oxidation (TOTOX) value

A scientist, Holm, suggested a combined expression of peroxides and secondary oxidation products, and therefore developed the concept TOTOX value. Together this established a value of the total oxidation status in oil [20]. TOTOX value of the oil sample was determined based on the obtained peroxide and P-Anisidine values using the formula;

$$TOTOX = 2PV + AV$$

2.8 Statistical Analysis

The data were analysed using SPSS version 21.0. The mean and standard error of means (SEM) of the triplicate analyses of the samples were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means of the pulp from the seeds while the means were separated using the new Duncan multiple range test at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Chemical Composition of pulp and seed of *Myrianthus arboreus* fruit

Table 1 shows the chemical characterization of *Myrianthus arboreus* pulp and seed in percentages. The moisture content of the pulp and seed were 84.48% and 12.69% respectively. Crude protein, crude fat, total ash, total fiber and total carbohydrate were 17.22%, 6.10%, 1.04%, 70.60% and 73.63% respectively for pulp, and 24.40%, 42.05%, 3.65%, 1.50% and 29.56% respectively for seed. The pulp recorded 143.95 kcal of energy, and that for the seed was 589.67 Kcal.

Table 1: Proximate Composition of Pulp and Seed.

Proximate composition	Pulp	Seed	*Values with different letters on a row are significant
Moisture (%)	84.48 ± 0.32 ^a	12.69 ± 2.12 ^b	es
Crude protein (%)	17.22 ± 1.28 ^b	24.40 ± 2.12 ^b	with
Crude fat (%)	6.10 ± 2.74 ^c	42.05 ± 1.28 ^a	differ
Total ash (%)	1.04 ± 1.83 ^c	3.65 ± 0.82 ^c	ent
Total fiber (%)	70.60 ± 0.45 ^a	1.50 ± 0.59 ^c	letter
Carbohydrate (%)	5.03 ± 0.90 ^c	28.06 ± 0.01 ^b	s on
Total carbohydrate (%)	75.63 ± 1.81 ^a	29.56 ± 0.06 ^b	a
Energy value (Kcal/100g)	143.95 ± 2.75 ^a	589.67 ± 2.80 ^b	row
			are
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icant at $P < 0.05$; Mean ± S.E.M = Mean values ± Standard error of means of three experiments

3.1.2 Vitamin Analyses of pulp and seed of *M. arboreus* fruit

Table 2 below shows the vitamin A and C contents of the pulp and seed of *Myrianthus arboreus* fruit. The pulp recorded 237.95 mg/100g for vitamin A and 1098.04 mg/100g for vitamin C while vitamin A content was 435 mg/100g and Vitamin C was 796.5 mg/100g for the seed.

Table 2: Vitamin Analyses of pulp and Seed

Vitamin (mg/100g)	Pulp	Seed
Vitamin A	237.95 ± 0.002 ^a	435 ± 7.07 ^b
Vitamin C	1098.04 ± 1.32 ^b	796.5 ± 6.37 ^a

* Values with different letters on a row are significant at $P < 0.05$; Mean ± S.E.M = Mean values ± Standard error of means of three experiments

3.1.3 Mineral Analyses of pulp and seed of *M. arboreus* fruit

Fig 2 below presents the mineral composition for the pulp and seed of *M. arboreus* fruit. The minerals analysed in both samples were phosphorus, potassium; magnesium, calcium, zinc, sodium and iron. The composition of P, K, Mg, Ca, Zn, Na and Fe were 237.95 mg/100g, 1098.04 mg/100g, 53.46 mg/100g, 624 mg/100g, 0 mg/100g, 117.07 mg/100g and 5.01 mg/100g respectively for the pulps, while for the seeds, the values obtained were 435 mg/100g, 796.5 mg/100g, 980 mg/100g, 27.05 mg/100g, 7.81 mg/100g, 3.75 mg/100g and 445 mg/100g respectively.

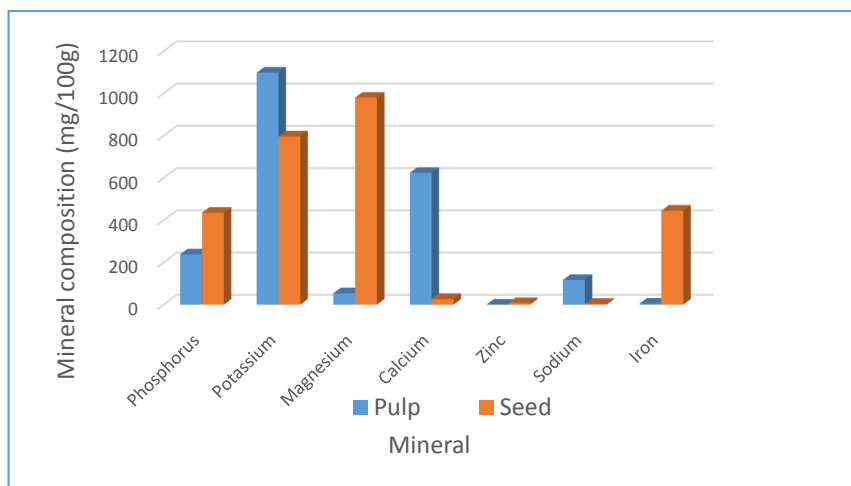


Fig. 2. Mineral Analyses of pulp and seed of *M. arboreus* fruit

Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments

3.1.4 Antinutrients Composition of pulp and seed of *Myrianthus arboreus* fruit

The antinutritional components of *M. arboreus* fruit pulp and seed are summarized on Table 3. The results show Total phenolic content of pulp and seed to be 1113.71 mg/100g and 355.71 mg/100g respectively. Phytate, oxalates and tannins contents were 14.09 mg/100g, 30.54 mg/100g and 15.45 mg/100g respectively for pulps while for seeds the values were 20.81 mg/100g, 70.79 mg/100g and 24.53 mg/100g respectively.

Table 3: Antinutrients Composition of pulp and seed

Antinutrients (mg/100g)	Pulp	Seeds
TPC	1113.70 \pm 0.15 ^a	355.71 \pm 0.05 ^b
Phytate	14.09 \pm 0.28 ^b	20.81 \pm 0.06 ^a
Oxalate	30.54 \pm 0.02 ^c	70.79 \pm 0.01 ^b
Tannin	15.45 \pm 0.13 ^b	24.53 \pm 0.07 ^a

* Values with different letters on a row are significant at $P < 0.05$; Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments

3.1.5 Fatty Acid profile of *Myrianthus arboreus* seed oil

Table 4 shows the fatty acid profile of the *Myrianthus arboreus* seed oil. The major unsaturated fatty acids present in the oil were Oleic acid (34.87%) and Linoleic acid (8.93%) while the major saturated were Lauric (25.02%), Caprylic acid (5.50%), Stearic (4.76%), Capric (4.43%) and Palmitic (2.06%) acids. Total saturated fatty acids account for 45.06% while total unsaturated fatty acids recorded 54.94% of the total seed fatty acids and 46.01% of these are monounsaturated while 8.93% are polyunsaturated.

Table 4: Fatty Acids of *Myrianthus arboreus* seed oil

Fatty Acids	Common Name	IUPAC (Systematic name)	(%)
C8:0	Caprylic acid	Octanoic acid	4.43
C10:0	Capric acid	Decanoic acid	5.50
C12:0	Lauric acid	Dodecanoic acid	25.02
C14:0	Myristic acid	Tetradecanoic acid	0.18
C15:0	Pentadecanoic acid	Pentadecanoic acid	0.18
C15:1	Pentadecenoic acid	14-Pentadecenoic acid	8.93
C16:0	Palmitic acid	Hexadecanoic acid	2.06
C16:1 (9)	Palmitoleic acid	Hexadecenoic acid	2.21
C18:0	Stearic acid	Octadecanoic acid	4.76
C18:1(9)	Oleic acid	Octadecenoic acid	34.87
C18:2(9,12)	Linoleic acid	Octadecadienoic acid	8.93
C18:3(9,12,15)	Linolenic acid	Octadecadienoic acid	0.00
C20:0	Arachidic acid	Eicosanoic acid	2.92
ΣSFA	Saturated Fatty acids		45.06
ΣMUFA	Monounsaturated fatty acids		46.01
ΣUFA	Unsaturated fatty acids		54.94
ΣPUFA	Polyunsaturated Fatty acids		8.93
Σ(ω-6)	Omega-6		8.93
UFA/SFA	unsaturated/saturated ratio		0.82
PUFA/SFA	polyunsaturated/saturated ratio		0.20
AI	Atherogenicity Index		0.51
TI	Thrombogenicity index		0.25
H/H	Hypocholesterolemic/hypercholesterolemic ratio		19.52

*Values with different letters on a row are significant at $P < 0.05$; Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments

3.1.6 Physicochemical properties of *Myrianthus arboreus* seed oil

Table 5 shows the results for the oil quality indices of oil extracted from the seeds of *M. arboreus* seeds. These indices were determined in order to know the safety of the oil for consumption. The acid value, free fatty acid, saponification value, iodine value, peroxide value, P-anisidine value and total oxidation value obtained all fell within the recommended Codex Alimentarius commission range for safety.

Table 5: Physicochemical properties of *Myrianthus arboreus* seed oil

Oil quality parameters	<i>Myrianthus arboreus</i>	Codex standard
Acid index (mgKOH/g)	5.61 \pm 0.39	\leq 4.0
FFA (mgKOH/g)	2.82 \pm 0.19	1.5
Saponification value(mgKOH/g)	196.62 \pm 0.38	188-194

Iodine value (g/100g)	171.45 ± 2.99	104-120
Peroxide value (meq/kg)	2.04 ± 0.72	≤ 15
P-anisidine value	3.80 ± 0.11	≤ 20
Totox value	7.88 ± 1.55	≤ 40

*Values with different letters on a row are significant at $P < 0.05$; Mean ± S.E.M = Mean values ± Standard error of means of three experiments

DISCUSSIONS

The moisture content obtained from the pulp (84.48%) is significantly higher than that obtained for the seed (12.69%). This result is high when compared to 6.23±0.09% of Malaysian-grown tropical almond nuts [21] and 9.88% of *Lepidium sativium* seed collected in morocco [22]. However, for the pulp the moisture content was found to be 84.48 ± 0.32%. This results are similar to 91.1% and 89.23% from *Myrianthus arboreus* pulp harvested from Belabo and Doume in Eastern Cameroon respectively [23]. The moisture content has as effect on the shelf life, texture and taste of a food product. The lower moisture content in the seed implies it has a longer life span whereas the pulp like most fruit pulps with high moisture content, cannot be kept for long unless if well preserved.

Comment [u11]: There is no need of Standard Mean Deviation in the discussion.

Proteins play a major role in our body due to their many metabolic and regulatory functions in biochemical processes. The results obtained for the protein content of *Myrianthus arboreus* pulp 17.22% was lower than that of the seeds 24.72%. These results are similar to those of Edmond *et al.*[24] showed that *M. arboreus* fruit harvested in Cote D'Ivoire had a protein content of 13.62% and 25.38% for the pulp and seeds respectively. This content (24.72%) in seeds is higher than that of cereals [25] but lower than 39.24% obtained for soya beans [26]. The pulp content (13.62%) was similar to jack fruit pulps of 18.35% [27], lower than 19.74 % and 20.96% obtained for *Myrianthus arboreus* pulp from Belabo and Doume localities in East Cameroon respectively [23] and also close to those of Ene-Obong *et al.* [28]who showed that *M. arboreus* fruit pulp harvested in Nigeria had a protein content of 18.74%. these differences could be attributed to different climatic conditions, and soil composition of the various study sites.

Fats are necessary in diets for the absorption of fat soluble vitamins. The fat content is significantly greater in the seeds (42.05±1.28) than in the pulps (6.10±2.74) processes. This value is very similar to the 6% recorded for *M. arboreus* pulp from Doume, lower than that harvested from Belabo in Eastern Cameroon [23] and higher than 2.28% obtained for pulps from Ivory Coast [29]. The value obtained for the seeds in this study is greater than 30.31% in soya bean obtained by Bayero *et al.*[26] and 13.30% obtained from cotton seeds by Bosede & Efomah, [30]and lower than 52.11% for Indian almond nuts reported by Barku *et al.*[31]. These results in line with other reports on *M. arboreus* seeds, affirms it as an oilseed with a rich source of fats, a good oil source which could be used as an alternative edible oil for cooking and for industrial applications.

The ash content gives an indication of the mineral content. This study observed a slight difference in the ash content between the seeds (3.65% ± 0.82) and the pulps (1.04±0.24). This suggests that the mineral content of the seeds is higher than that of the pulps. Similar results (3.78%±0.04) were obtained for *Terminalia catappa* seeds [21]. However these values were higher compared to those obtained for *Myrianthus arboreus* fruits from Cote D'Ivoire which recorded 0.66% and 2.27% for pulp and seeds respectively [29].

Fiber is important in the diet as it aids in digestion by increasing peristalsis in the small intestines. The fiber content of the pulps obtained in this study is very high compared to the values obtained in a previous study [23]. Studies have shown that a diet low in fiber is undesirable as it could cause constipation and that such diets have been associated with diseases of the colon. The value for the seeds was higher than the crude fiber obtained for almond nuts [32] and lower than that obtained for most legumes like cashew nuts [33] and soya beans [26] which were of the range 5-7%.The carbohydrate values obtained in this study were in close range to values obtained for *Myrianthus arboreus* in a previous study, which recorded 70.58% and 17.54% for pulp and seeds respectively

[24] but was higher than values for pulps from Doume 42.28% and Belabo 42.4% [23]. Carbohydrates are responsible for the sweetness of the nectar from the fruit and they give the fruit a sweet taste and pleasant aroma. The high content in the pulps could facilitate the transformation of *M. arboreus* into food products such as: jams, syrups, chips and juices.

The caloric value of the seeds was significantly greater than that of the pulps. This justifies the greater proportion of energy sources like fat, carbohydrates and proteins found in the seeds. The energy value of the pulps is lower compare to (339.7 Kcal/100g) obtained by [23] on *Myrianthus arboreus* pulps from Doume East region of Cameroon and *Myrianthus arboreus* pulps powder (168 Kcal/100g) obtained by Due *et al.*[29]. All these differences can be attributed to the differences in temperature, humidity, soil composition and climatic conditions from one study area to another. However, the general trend indicates a high energy value of *Myrianthus arboreus* seeds.

Minerals are vital components of food and they fulfil a wide variety of functions in the body. They regulate body processes. The mineral analysis of *M. arboreus* almonds and pulps (Fig 2) indicated that sodium (Na), iron (Fe), calcium (Ca), magnesium (Mg), phosphorous (P), potassium (K) and zinc (Zn) were present in appreciable amounts. The most abundant minerals present in the pulps were K, Ca, P, and Na while the least abundant were Mg and Fe, no Zn was detected in the pulps. On the other hand, the most abundant minerals in the seeds were Mg, K, Fe and P while Ca, Zn and Na were the least abundant amongst the minerals analyzed. All the mineral elements present in this study were found to be higher than those of cashew nuts, almond nuts and *M. arboreus* from previous studies [34-35].

Potassium is the key mineral required in the body for cellular functions due to its significant function of controlling the volume of fluid within a cell: therefore, an increase intake of K can help relax blood vessels and promote the excretion of salts of the body hence decreasing blood pressure. The mineral analysis for this study revealed that K was the most abundant mineral present in the pulp (1098.04 mg/100g) of *M. arboreus* and the second most abundant in the seeds (796.5 mg/100g). The very high content of K in the seeds and pulp suggests that *Myrianthus arboreus* fruit pulp and seeds are suitable for consumption by hypertensive people. Mg was found to be highest mineral component in the seeds. Magnesium is important in its role as an activator of many enzymes systems and maintains the electrical potential in nerves. *Myrianthus arboreus* pulp and seeds contains large amounts of magnesium and an adequate serving would satisfy the Recommended Daily Allowance (RDA) for Mg.

Calcium plays a role as a secondary messenger helping in muscle contraction, heartbeat, blood clotting and nerve function. Calcium was found to be present at significant levels in the pulp and also relatively high in the seeds. WHO/FAO recommends an intake of 400–500 mg per day of calcium for adults and hence a serving of about 100g of *Myrianthus arboreus* pulp can supply an adult's daily calcium need. An implication that the consumption of *M. arboreus* fruit can supplement other sources of dietary calcium especially when consumed mainly as snack. Phosphorus together with calcium in the body contributes in controlling body fluids and its presence in the body as phosphates salt in bones indicates that it would serve as good source of mineral for bone formation.

Sodium is vital in maintaining the body fluid volume, osmotic equilibrium and acid-base balance and it constitutes 2 % of the total mineral content of the body. The results shows that sodium content was considerably higher in the fruit pulp than in the seeds. This suggests that, a significant consumption of *M. arboreus* pulp will provide the RDA of sodium.

Iron is a constituent of haemoglobin, myoglobin, and a number of enzymes, which catalyzes oxidation, and reduction processes in the cell. A deficiency of iron in the diet could lead to anaemia. The results shows the iron content in the seeds was higher than that for the pulps. The high iron content in the seeds and the relatively good level in the pulps makes *Myrianthus arboreus* seeds and pulp a good source of Iron.

Zinc acts as a cofactor for many enzymes in vital biochemical metabolic processes such as DNA and protein synthesis. However, an excessive high Zn content in plant based foods can affect the absorption and metabolism of other minerals like Ca, Mg, P and Fe. It may disrupt the balance of

these minerals in the body, potentially leading to imbalances or deficiencies. The Zn content obtained in this study is very less when compared to other elements as this metal element was not found in the pulp but a comparative low amount was found in the seeds.

Vitamins are required by the body to perform functions such as boosting the immune system, regulating hormones, and aids in the prevention of deficiency diseases. The vitamin composition of the pulps revealed that Vitamin C with 1098.04 mg/100g had the highest concentration, followed by Vitamin A 237.95 mg/100g. Vitamin C concentration was greater than 228 mg/100g and 257 mg/100g obtained from cashew (*Anacardium occidentale*) and guava apple (*Hibrido de psidium guajava*) pulps respectively [36]. However, in the seeds, Vitamin C and Vitamin A concentrations were 796.5 mg/100g and 435 mg/100g respectively. These values are higher when compared to 2.18 mg/100g and 38.20 mg/100g for Vitamin A and Vitamin C respectively on fresh *Myrianthus arboreus* from Nigeria as reported [37]. The relatively high Vitamin C and Vitamin A contents in the pulp and seeds obtained in this study suggests that its consumption can provide sufficient amount of Vitamin C and β -carotene which can be particularly beneficial for individuals with both vitamin deficiencies or are at risk of developing one.

The content of antinutrients gives an idea about the bioavailability of the constituent elements of the pulp and the seeds of *Myrianthus arboreus*. The total phenolic content (TPC) gives an indication of the antioxidant activity of the sample. The major components are polyphenols, which have anti-inflammatory properties. The TPC of almonds and pulps of *Myrianthus arboreus* fruit was 1113.70 mg/100g and 355.71 mg/100g respectively. Similar results were obtained for *M. arboreus* pulps and seeds [29]. Polyphenols are beneficial in that they have antioxidant potentials, there by protect the body's cells from damaged caused by harmful free radicals. However, their effectiveness and benefits depends on the specific polyphenol compounds and the concentration found in foods. The high value of TPC recorded in this study suggests that, consuming *Myrianthus arboreus* fruits with their seeds will make available a substantial amount of beneficial phenolic compounds in the body.

Phytate at higher concentration >5% are detrimental as they prevent the absorption of minerals such as Mg, Ca and Fe which are important for many biochemical processes. The phytate content in this study recorded 14.09 mg/100g for pulp and 20.81 mg/100g for seeds. These results were very close to values obtained for *Myrianthus arboreus* pulps and seeds in previous studies [23, 29]. Oxalates had the highest concentration of 30.54 mg/100g and 70.79 mg/100g for both pulps and seeds respectively, Eating large amount of food high in oxalate is dangerous. That is, oxalate crystals accumulate at the kidney causing kidney stones and renal failure especially in enteric hyperoxaluria patient. This result is far lower than results obtained in a previous study, which recorded 54.38 mg/100g and 260.7 mg/100g for *M. arboreus* pulp and seeds respectively, harvested in Ivory Coast (Due *et al.*, 2019). However, the oxalate content in this study was higher than the ones harvested in Eastern Cameroon [23] but far lower than values obtained for Jackfruit from Eastern Cameroon [27].

The tannin content in the pulp was 15.45 mg/100g while that for the seeds was 24.53 mg/100g. These values were in close agreement to values obtained by Due *et al.*[23] but higher when compared to values obtained for pulps by Bemmo *et al.*[23] but lower than those obtained for tropical almond seeds by Akpakpan & Akpabio.[38]. Tannins are known to interfere with the absorption of some minerals like Fe and Zn by forming insoluble complexes that can precipitate, making them difficult for the body to break them down and absorb thereby reducing bioavailability of these essential minerals. And making them less accessible for absorption. The result recorded for tannins, oxalates, and phytates showed that these antinutrients were found to be within the recommended level (2-5%) as reported by Tijjani, [39]. Although, the concentration is within the acceptable range, care should to be taken during consumption and food formulation.

The major unsaturated fatty acids present in the oil were Oleic acid (34.87%) and Linoleic acid (8.93%) while the major saturated were Lauric (25.02%), Caprylic acid (5.50%), Stearic (4.76%), Capric (4.43%) and palmitic (2.06%) acids. Total saturated fatty acids account for 45.06% while total unsaturated fatty acids recorded 54.94% of the total seed fatty acids and 46.01% of these are monounsaturated while 8.93% are polyunsaturated. Linoleic acid, an omega 6 fatty acid, have been

known to prevent high blood pressure and serves as a structural component of the plasma membrane. Oleic acid is one of the most important unsaturated fatty acids in human food because of its antioxidant, antibacterial and anti-inflammatory properties. The high percentage of unsaturated fatty acid (54.94%) in *Myrianthus arboreus* seed oil is an indication that this oil can help reduce the risk of heart disease, lower bad cholesterol and improve overall cardiovascular health.

The percentage of UFA/SFA was 0.82. A UFA/SFA ratio of 0.2 has been associated with high cholesterol levels and with high risk of coronary heart disorders, while a ratio as high as 0.8 is associated with desirable levels of cholesterol and reduced coronary heart diseases [40]. This indicates that the oil may have a cholesterol-lowering potential tends to suggest that this seed has the potential to be used in the dietetic management of certain coronary heart diseases. PUFA/SFA is an indicator used to evaluate lipid quality. It is recommended by the British Department of Health that the minimum value is 0.45 [41]. The levels recorded for this study was less than 0.45, indicating that the oil may not be of good quality.

The atherogenicity index (AI) and thrombogenicity index (TI) are proposed to evaluate risky factors that are implicated in coronary heart disease development [42-43]. Lower values of AI and TI suggests that an oil might be beneficial to cardiovascular health. The AI value (0.51) was relatively high when compared to the so-called Eskimo diet (0.28). The TI value (0.25) obtained was low which indicates a very low incidence of coronary heart disease [17]. The very high ratio of the Hypocholesterolemic/ hypercholesterolemic (19.52) suggests a relatively greater hypocholesterolemic potential of the oil.

The acid value gives an indication of the total amount of free fatty acid in the oil due to enzymatic activity. It is used to evaluate the acidity of an oil which sometimes reflects its degree of oxidation. Its maximum acceptable level in refined oils is 0.6 mgKOH/g oil, while for cold pressed and virgin oils it is 4.0mgKOH/g oil [44]. The result showed an acid index of 5.61 mgKOH/g and free fatty acid value of 2.82 mgKOH/g. these values are slightly higher than the recommended codex of a maximum acid value of 4.0 mg KOH/g for virgin oils. Free fatty acid is very important in determining the use of oil for industrial or edibility purposes. The value obtained is within the allowable limit for edible oils (0-3) [32]. The oil could therefore be used as edible oil. Low acid value in oil indicates that the oil will be stable over a long period of time and protect against rancidity and peroxidation. This value was higher compared to 0.35 mgKOH/g for soybeans oil, 0.85 mgKOH/g groundnut oil, 0.48 mgKOH/g olive oil, 1.34 mgKOH/g sesame oil and 2.67 mgKOH/g palm oil [45]. Since the acid value and FFA value obtained for this study is slightly higher than the maximum permissible acid level of 4mgKOH/g fat or oil required for edible virgin fats and oils, this *M. arboreus* seed oil is suitable for consumption after undergoing some refining processes to improve its quality for industrial purposes.

Saponification value is a measure of oxidation during storage, and also indicates deterioration of the oils. An increase in saponification value in oil increases the volatility of the oils. It enhances the quality of the oil because it shows the presence of lower molecular weight components [46]. It has been reported by Pearson,[47] that oils with high saponification values contain high proportion of lower fatty acids. High values normally over 200 are obtained with fats and hydrogenated oils, while oils tend to have values below 195. The saponification value obtained in this study was 196.62 ± 0.38 mgKOH/g. Kyari, [48] reported that SV for palm oil is 200 (mgKOH/g sample), for groundnut is 193 (mg KOH/g sample) and for coconut oil is 257 (mg KOH/g sample). However this result is higher than 148.3 mgKOH/g obtained by Tia & Digbeu, [49] on dried *Myrianthus arboreus* seeds, similar to 195 mgKOH/g obtained on soybeans and less than 212.9 mgKOH/g of *Treculia africana* seeds and 246 mgKOH/g *Cocos nucifera* seeds [50]. Since high saponification values of fats and oils are due to the predominantly high proportion of shorter carbon chain lengths of the fatty acids [51] suggests low level of impurities and that the oil may be useful industrially for soap, shampoo and paints production [52-53]. Variation in oil quality values is determined by several physical and chemical parameters that are dependent on source of oil, processing and storage conditions [54].

The iodine value of oils measures the degree of unsaturation of a particular vegetable oil. Previous studies have shown that the greater the degree of unsaturation, the higher the iodine value and the greater the possibility of the vegetable oil to undergo oxidative rancidity. The iodine value obtained of

M. arboreus seed oil in this study 171.45 ± 2.99 g/100g agree with the values reported by Séverin *et al.*, [55] and by Katou *et al.* [9] for *Myrianthus arboreus* seed oil harvested from Cote D'Ivoire (170.62 ± 0.03 g/100g and 171.84 ± 4.35 g/100g respectively). This high IV in this study suggests that the oil may be more susceptible to rancidity by oxidation.

Peroxide value (PV) is the most common indicator of lipid oxidation/rancidity. The PV of the oil studied was 2.04 ± 0.72 meq/kg. High values of PV are indicative of high levels of oxidative rancidity of the oil and also suggest absence or low levels of antioxidants. It gives an idea on the primary oxidation state of oils and fats by giving the concentration of hydroperoxides present [56-57]. The CODEX [45] states a permitted maximum peroxide level of not more than 10 meq/kg, and values higher than 10 to 20 meq/kg are generally interpreted as rancidity. Thus, the low peroxide value recorded for this study indicates the resistance of the oil to peroxidation during storage suggesting therefore that, this oil was fresh with little or no oxidation.

P-anisidine value measures the products of hydroperoxide decomposition, especially aldehydes, and ketones. These compounds have a negative effect on human health and also affect oil taste, texture, smell and stability [58]. TOTOX value on the other hand represents the total oxidation load to which the oil is exposed. Generally, the recommended levels should not be more than 19.5 mEq/kg and 20 mEq/kg for TOTOX and P-anisidine values respectively [59]. In this study the TOTOX and p-anisidine values were 7.88 mEq/kg and 3.30 mEq/kg respectively. These values are within the acceptable range. This was therefore a clear indication that the oil was stable and not rancid.

UNDER PEER REVIEW

4. CONCLUSION

Based on the results of this research, the fruits of *Myrianthus arboreus*, possessed enormous nutritional and energetic potential due to its biochemical composition. The pulps and almonds of *Myrianthus arboreus* from Cameroon have nutritional properties which are clearly acceptable compared to the well-known fruits. These nutrients would therefore give them advantages for their application in the food industry. The analysis of the antinutritional factors results showed that the studied samples had a very low content in phytate and oxalates. Fatty acid analysis showed that *M. arboreus* contains a high percentage of unsaturated fatty acids. Oils containing small amounts of saturated fatty acids and large amounts of monounsaturated fatty acids are highly favourable in the human diet. Also, its seed has high oil yield (> 40%), which is comparable to the oil yields of some commercial seed oils. The physicochemical analysis and quality assessment suggest that its seed oil is consistent with most of the parameters with FAO/WHO values and CODEX standards. The quality evaluation show that the seed oil is of good quality and fit for consumption. The results obtained from this study can be used to promote the sustainable cultivation of *M. arboreus* tree in the mountain region of Cameroon for large-scale oil production.

CONSENT

Not Applicable

ETHICAL APPROVAL

Not applicable

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