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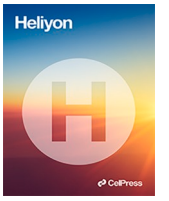

Biodiesel potential of *Cucumeropsis mannii* (white melon) seed oil: A neglected and underutilized resource in Nigeria

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ABSTRACT

A major challenge in the biodiesel industry is the availability of high-quality vegetable oil feedstocks. Thus, there is a continuous search for quality biodiesel feedstock whose production will trigger economic impact on the agricultural sector, minimize land degradation and without significant disruption to the food chain. In this work, we extracted and analysed oil from neglected and underutilized Cucumeropsis mannii seeds for their potential in biodiesel production. The oil content of *C. mannii* seed was 40.8 ± 0.56%. GC-MS analysis of the oil revealed the presence of 47.0% saturated fatty (predominantly palmitic acid, stearic acid) and 53.0% of unsaturated fatty acids (predominantly oleic, linoleic and erucic acids). The physicochemical properties were determined and values were as follows: iodine value (111.07 ± 0.15 g/100 g), saponification value (192.03 ± 0.37 mg/kg of oil), peroxide value (2.60 ± 0.10 meq/kg), acid value (4.20 ± 0.02 mgKOH/g) free fatty acid (2.51 ± 0.02%), relative density (0.93 ± 0.02), the refractive index at 28 °C (1.46 ± 0.04) and viscosity at 30 °C (3.00 ± 0.10 mm^2/s). The fuel properties namely, cloud point, pour point, flash point and caloric value were determined and the values were 3.03 ± 0.11 °C, 1.00 ± 0.10 °C, 279.04 ± 0.99 °C and 31.10 ± 0.11 MJ/kg, respectively. In addition, the protein content of the defatted seed was found to be 47.4 ± 0.61 g/100 g. The defatted protein-rich cakes can be upgraded as a food additive; thus the *C. mannii* seed oil can serve as biodiesel feedstock without altering the food chain. These characteristics demonstrate the potential of *C. mannii* oil as a high-quality feedstock for biodiesel production. We envisage that its utilization as biodiesel feedstock will improve the market value of these seeds, thus supporting the economic development of local farmers in rural areas.

1. Introduction

Crude oil is currently the primary and major source of diesel which is becoming less cost-effective as reserves are depleting due largely to population growth and industrial development [1,2]. The finite nature of the crude oil reserve, the high cost of the product and the associated environmental and health hazards have motivated the move away from over-reliance on fossil fuel, thus creating

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global interest in alternative renewable and sustainable sources of fuel [3]. Different types of energy sources such as biofuels have the potential to replace fossil fuels for use in automotive industries [2]. In particular, a suitable blend of vegetable oil in the form of their fatty acid methyl esters with diesel oil or a direct replacement has been utilized as a substitute for diesel from fossil fuel [4].

Biodiesel (Fatty acid methyl esters of vegetables) is a biodegradable, non-petroleum-based alternative form of diesel produced by the transesterification of vegetable oils. One of the major challenges confronting biodiesel industries is the availability of cheap and high-quality vegetable oil feedstock [5]. To overcome this challenge, crops and other oil seed plants have to be screened for their potential as sources of biodiesel oil feedstock [6]. In doing so, the economic values associated with the use of such seed oil for biodiesel production are to be considered. Hence, in the last decades, attention has shifted towards neglected and underutilized local seeds for possible use as biodiesel feedstock [7].

Cucumeropsis mannii, a neglected and underutilized seed, can be a good candidate because the exploitation of the seed oil for biodiesel production can positively impact rural farmers' economic lives. *C. mannii*, commonly known as the white melon seed is a member of the Cucurbitaceae family. The plant is native to tropical West Africa and grows well in the tropical and sub-tropical countries where it was originally interplanted with banana, corn and cassava [8]. It produces climbing vines up to 4 m long, which are covered in stiff hairs. As a climber, it produces well when interplanted with tall-growing plants and trees, reducing land degradation associated with the cultivation of many oil crops. The limited knowledge of other possible uses of this seed apart from its consumption as a traditional soup thickener has been a major deterrent to its wider production. Its use as a biodiesel feedstock presents an opportunity for increased income to the local farmers [9]. Its development and utilization have been hampered by the availability of other closely related species used for the same purpose but that is easier to process. The plant has lacked the attention of researchers and farmers and thus, it is categorized as an orphan crop [10]. Oils extracted from the seeds of other members of the Cucurbitaceae family (*C. melo*, *C. coloncynthis*, *C. valgaris*, *C. Pepo*) have been characterized for their suitability in biodiesel production [11–14]. The images of the seed of other members of the *Cucurbitaceae* family cultivated in southeast Nigeria are shown in Fig. 1A–D, while Fig. 1E is the *C. mannii* seed]. Other seed oils such as phoenix tree seed oil [15], Semen Abutili seed oil [16] has been reported as biodiesel feedstock. Here we report for the first time the fuel properties in addition to the physicochemical and fatty acid profile of oil extracted from *C. mannii* seed cultivated in southeast Nigeria for its potential in biodiesel production. Its alternative use as a feedstock for biodiesel production would encourage its cultivation which would translate to improving the local economy. Also, the defatted protein-rich cakes remain available for food, thus the *C. mannii* seed oil can serve as biodiesel feedstock without significant disruptions to the food chain. The steps involved in the processing, extraction and analysis of C. mannii oil were presented (see Fig. 2).

2. Materials and methods

Gas Chromatography-Mass Spectroscopy (GCMS-QP2010 plus. Shimadzu, Japan), Buck 530 gas chromatographic-Flame ionization Detector (CA, USA), *n*-hexane, and other chemicals were products of Sigma-Aldrich, USA. Experiments to determine the physical,

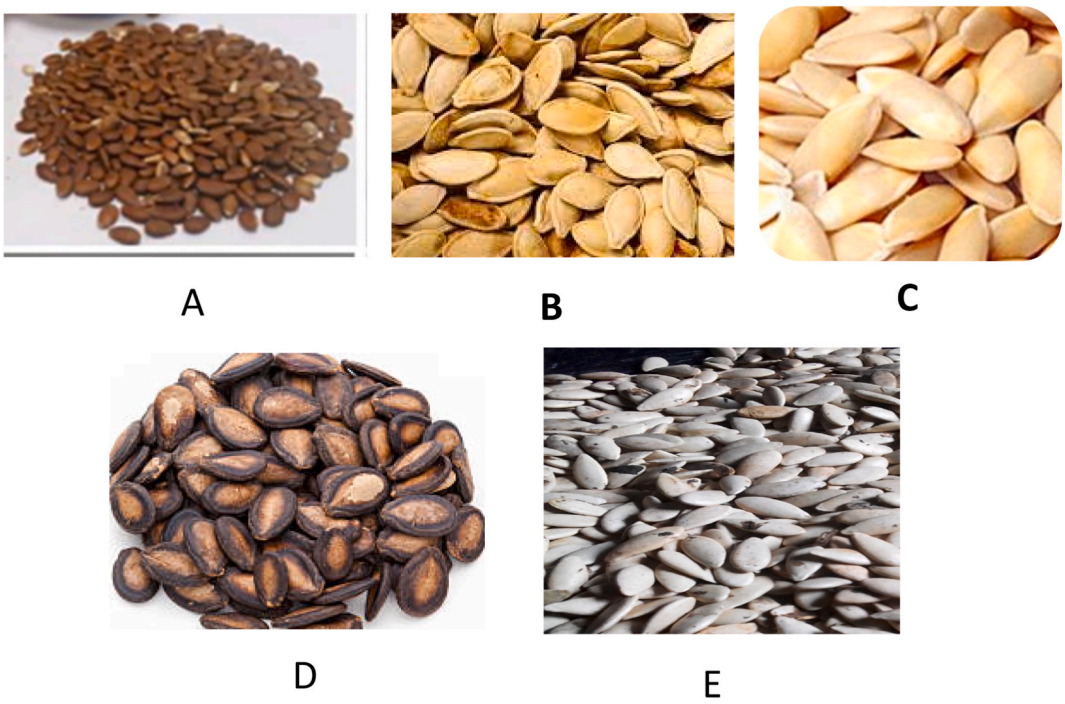

Fig. 1. Images of some members of the *Cucurbitaceae* family cultivated in southeast Nigeria. (A) *C. colencynthis*, (B) *C. melo*, (C) *C. pepo* also known as *C. moschatat* (D) *C. vulgaris* (E) *C. mannii*.

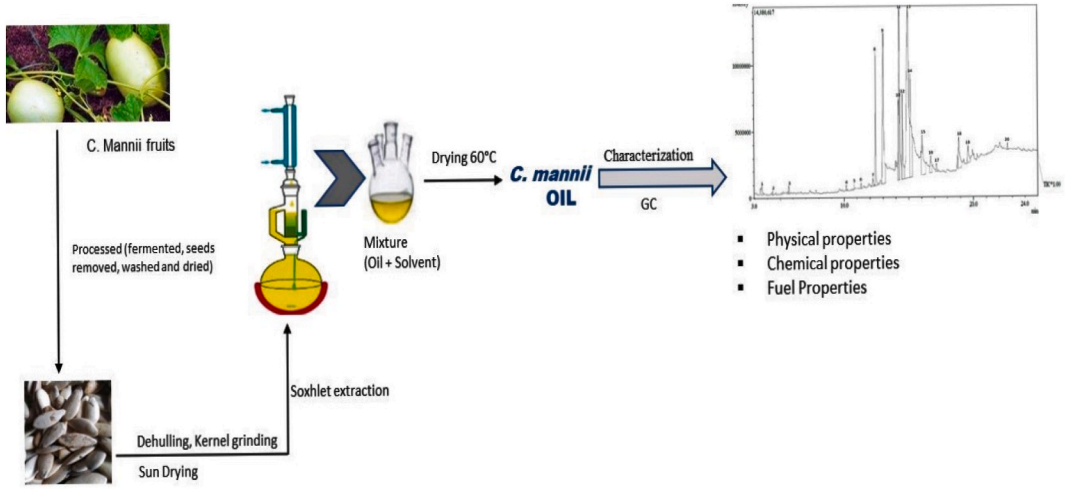

Fig. 2. Steps in *C. mannii* oil extraction and analysis.

chemical and fuel properties of the *C. mannii* oil were carried out in triplicates and the values were expressed as mean ± standard deviation.

2.1. Plant sample collection and preparation

A single batch of fresh fruits of *Cucumseropsis mannii*. (White melon) were gifts from a local farmer in the Edem-Ani community (6°51′43″N 7°20′21″E) of Nsukka LGA, Enugu State, Nigeria. The climatic condition of the area is classified as tropical savanna Köppen Aw [17], humid with an average annual temperature of 26.7 °C and average yearly rainfall of 1719 mm. The fresh fruits were broken and allowed to ferment and the seeds were washed and dried. The seed coat was removed, and the endosperms were sun-dried for seven days. The dried seed was ground using a (CAWIN, CB-B903G, 450 W, China) electric blender.

2.2. Extraction of oil from the seed using the soxhlet apparatus

To produce the C. mannii seed oil, Soxhlet extraction was carried out using *n*-hexane. A milled sample weighing 36 g was placed in an extraction thimble. The thimble and the sample were inserted into the soxhlet apparatus before adding about 200 ml of *n*-hexane in a 500 ml flat bottom flask. The set-up was mounted on a heating mantle and heated at 68 °C for 4 h. After the extraction process, the filtrate was exposed to the atmosphere and later dried in an oven at 60 °C for 24 h. The percentage oil yield was calculated using Equation (1). After two batches of extraction, the average yield was reported.

$$\%\ \text{Oil Yield} = \frac{Weight\ of\ oil\ Extracted}{Weight\ of\ Sample}\ X100 \quad \text{(Eq. 1)}$$

The extracted oil was then analysed for its physicochemical properties such as specific gravity, viscosity, refractive index, iodine value, acid value, peroxide value and saponification value. The fuel properties determined were the flash point, pour point, cloud point, the heat of combustion, water content and ash content. The fatty acid composition of the oil was determined using GC-MS analysis. The state of the oil at room temperature and the colour of the oil were noted by visual inspection.

2.3. Specific gravity determination

The specific gravity of the *Cucumeropsis mannii* oil was determined using a specific gravity bottle. A clean empty specific gravity bottle was weighed on a digital weighing balance (Virgo V600–W, Haryana, India) and the weight (w_1) was noted. It was then filled with distilled water and the weight (w_2) was noted. The water was removed and the specific gravity bottle was dried and cooled. It was then filled with *C. mannii* oil and weighed (w_3). All the determinations were at 25 °C. The relative density was calculated using equation (2) below.

$$\text{Relative Density}\ \left(\text{cm}^3\right) = \frac{W_3 - W_1}{W_2 - W_1} \quad \text{(Eq. 2)}$$

Where W_1 is the weight of the specific gravity bottle alone, W_2 is the weight of the specific gravity bottle and water, W_3 is the weight of the specific gravity bottle and sample (*C. mannii* oil).

2.4. Determination of viscosity of the C. mannii oil

The kinematic viscosity of the oil was determined using a digital viscometer (CAP2000, Lab Unlimited, Surrey United Kingdom). The appropriate spindle was selected and fixed on the instrument. The spindle was inserted into the sample to be analysed until the level mark on the spindle reaches the surface of the sample (C. mannii oil). Enter button on the instrument was pressed and the viscosity of the sample was displayed on the screen.

2.5. Flashpoint determination

The flashpoint of the fuel samples was determined as per IS: 1448 [P: 32]: 1992. A Pensky Martin Flash Point closed cup tester (ASTM D6751, Ducom Instrument, Bohemia NY USA) was used to measure the flash and fire points of the oil. The sample was filled in the test cup up to the specified level and was heated and stirred at a slow and constant rate of 250 ± 10 rpm). At every 10 °C temperature rise, a flame was introduced with the help of a shutter. The temperature at which a flash appeared in the form of sound and light was recorded as the flashpoint.

2.6. Refractive index determination

The refractive index was determined using a refractometric technique following the method of [18] using Abbe refractometer -bench type (Model: I–2S, Made by Searchtech Instruments). The oil sample was placed on the refractometer's glass slide. A few drops of the sample were introduced on the working surface of the lower refracting prism. To ensure that the oil did not drain out, it was then sealed and the box was flattened once again. The rotating arm and the collecting lens cone of the light-gathering illuminating units were rotated to make the light-intake surface of the upper light-intake prism be illuminated evenly. The prism box was turned until the telescope's crosswires' intersection and the sharp edge of the edge coincided. Then, using the eyepiece, the index of refraction was read off on the scale.

2.7. Cloud and pour points determination

The Cloud and Pour point of the *C. mannii* oil was determined as per the Indian Standard of test IS 1448 [P: 10]: [19], using the Cloud and Pour point apparatus (K46196, Koehler, Bohemia NY USA). The sample was cooled and examined periodically at temperature intervals of 10 °C. The temperature at which haze was observed at the bottom of the test jar was recorded as the cloud point of the sample. To determine the pour point, the oil sample was first heated and cooled and examined at intervals of 3 °C, the minimum temperature at which the oil was observed to flow was noted and recorded as the pour point. These were measured in triplicates and the mean values were recorded.

2.8. Determination ash content

The percentage ash content was determined using the AOAC method [20]. One gram of *C. mannii* oil was weighed into porcelain crucibles that were previously washed and dried in an oven at 100 °C, cooled in a desiccator and weighed. They were then placed inside a muffle furnace and heated at 600 °C for 4 h. After which they were removed and allowed to cool in a desiccator and then weighed. The percentage ash content was calculated using Equation (3) below.

$$\text{Ash}\ (\%) = \frac{A - B}{C} \times \frac{100}{1} \qquad \text{(Eq. 3)}$$

Where A is the weight of the crucible with ash, B is the weight of the crucible alone, and C is the weight of the original sample.

2.9. Moisture content determination

The AOAC method as described by Ref. [20] was used to determine the percentage moisture content of the oil. Porcelain crucibles were washed and dried in an oven at 100 °C for 30 min and allowed to cool in a desiccator. One gramme of the sample was placed into weighed crucibles, the crucible and the content were then put inside the oven to dry at 105 °C for 4 h. The samples were removed from the oven after this period, cooled and weighed. The drying was continued and the crucible and its content weighed at intervals until a constant weight was obtained. The percentage moisture content of the oil was calculated using Equation (4) below;

$$\text{Percentage}\ (\%)\ \text{moisture} = \frac{A - B}{A} \times \frac{100}{1} \qquad \text{(Eq. 4)}$$

Where: A is the original weight of the sample, and B is the weight of the dried sample.

2.10. Acid value determination

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of fat. The acid value was determined using the titration method as described by the association of official analytical chemists [21]. 5

g of the sample was weighed into a flask and then 50 ml of neutralized ethanol was added. The contents were mixed and boiled. It was then titrated with 0.1 N KOH to a faint pink colour that persisted for at least 15 s. The acid value (AV) was calculated using equation (5) below.

$$Acid\ value\ AV(\text{mgKOH / g of oil}) = \frac{56.1xNxTx100}{1000xG} \quad \text{(Eq. 5)}$$

Where: N is the Normality of standard KOH used, T is the titration volume, G is the weight of the sample.

2.11. Peroxide value determination

Peroxide value refers to reactive oxygen content expressed in terms of milliequivalents (meq) of free iodine per kilogram of fat. The peroxide value of *C. mannii* oil was determined according to the method of [22]. Ten grams of the sample was weighed into a conical flask and 30 ml of the acetic–acid chloroform solution was added. The flask was swirled to dissolve the sample. 0.5 ml of the potassium iodide (KI) solution was added. The flask was allowed to stand for 1.0 min and swirled occasionally. Subsequently, 30 ml of distilled water was added. It was titrated with 0.025 N thiosulphate ($Na_2S_2O_3$) solution using 1.5% starch solution as an indicator. A blank was conducted in the same way but without the sample. The peroxide value was calculated using Equation (6) below.

$$\text{Peroxide value PV } (\text{meq / kg}) = \frac{(T-B)xNx1000}{g} \quad \text{(Eq. 6)}$$

Where T is the titration volume for the sample (volume of Sodium thiosulphate ($Na_2S_2O_3$) solution used for the oil sample, B is the titration volume for blank, N is the normality of sodium thiosulphate used while g is the weight of oil sample.

2.12. Saponification value determination

The saponification value is the milligrams of base (KOH or NaOH) required to completely saponify 1 g of fats. The saponification value of *C. mannii* oil was determined according to Ref. [21]. Two grams of the oil were weighed into the flask. 25 ml of 0.5 N alcoholic potassium hydroxide ($C_2H_7KO_2$) was added to it and boiled under reflux for 1 h. The excess alkali was determined by titration with 0.5 N hydrochloric acid while the solution was still hot using 0.5 ml of 1.0% alcoholic solution of phenolphthalein as an indicator. A blank was determined under the same condition, the sample was replaced with an equal quantity of distilled water. The saponification value (SV) was calculated using equation (7) below.

$$Saponification\ value\ SV\ (\text{mgKOH / g of oil}) = \frac{56.1x(B-S)xN}{W} \quad \text{(Eq. 7)}$$

Where S is the volume in ml of standard HCl required for the sample, B is the volume in ml of standard HCl required for the blank, N is the normality of HCl, and W is the weight of oil used.

2.13. Iodine value determination

The iodine value of oil is an estimate of the degree of unsaturation of the sample, which is determined by the uptake of iodine (halogens). The iodine value of *C. mannii* oil was determined according to Ref. [23] using Wij's Reagent (Himedia Laboratories, Einhausen Germany). The reagent consisted of 16.2 g of iodine monochloride (ICl in 1000 ml of glacial acetic acid). The sample was filtered through Whatman filter paper grade 1 (11 μM). Subsequently, 0.5 g of the filtered sample was weighed into a clean flask. 25 ml of carbon tetrachloride and 25 ml of Wij's solution were added to it. The glass stopper was wetted with KI solution and used to cover the flask. The flask was swirled and allowed to stand in the dark for 30 min at 25 °C. A blank test was carried out simultaneously under the same experimental conditions. After 30 min, 15 ml of KI solution and 100 ml of water were added, and the stopper was rinsed. The liberated iodine was titrated with 0.1 N sodium thiosulphate solution while swirling the contents of the flask continuously until the yellow colour of the solution becomes faint (indicating that the liberated iodine has reacted with the double bonds in the oil sample). To remove the unreacted iodine, 1 ml of starch solution was added to react with the iodine, and the titration continued until the blue starch-iodine colour disappeared after shaking thoroughly. The Iodine value was calculated using Equation (8) below.

$$\text{Iodine value } (\text{g / 100g of oil}) = \frac{(B-T)xNx12.69}{G} \quad \text{(Eq. 8)}$$

Where B is the Blank titre (mL), T is the Sample titre (mL), N is the Normality of the standard sodium thiosulphate solution used and G is the weight of the sample (g).

2.14. Caloric value determination

Caloric Value also referred to as the heating value of biomass is the amount of energy in the form of heat that is generated when a unit mass of the substance is oxidized completely with oxygen. The caloric value of *C mannii* oil was determined following the method by the association of official analytical chemists [20]. This was carried out using a bomb calorimeter (model XRY-1A, Shanghai

Changji, China). The sample was ignited in a bomb calorimeter (under high pressure of oxygen gas). The heat energy that was released was absorbed by the surrounding water inside the bomb calorimeter. This gave rise to a temperature increase in the surrounding water and this was used to estimate the energy value of the sample. One gram of the sample was turned into the oxygen bomb calorimeter. The heat of combustion was calculated as the gross energy using equation (9) as shown below.

$$\text{Energy content} = \frac{E\Delta T - 2.3L - V}{g}\ (\text{KJ / Kg}) \quad \text{(Eq. 9)}$$

Where E is the energy equivalent of the calorimeter, ΔT is the temperature rise, L is the length of burnt wire, V is volume, g = weight of the sample.

2.15. Gas chromatography-mass spectrometry analysis of Cucumeropsis mannii oil

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis was carried out on a GC system comprising a Gas Chromatograph coupled to a Mass Spectrometer (Shimadzu GCMS-QP2010), with an ETP electron multiplier detector (Restec 14,617, USA). The following conditions were employed: Elite-1 fused silica capillary column (Agilent, Santa Clara USA, 30 × 0.25 mm ID × 1 EM df, composed of 100% Dimethylpolysiloxane), operating in electron impact mode at 70 eV; helium (99.99%) as carrier gas at a constant flow of 1.58 ml/min. A sample volume of 1 μl was employed (split ratio of 10:1) and an injector temperature of 250 °C was maintained; the ion-source temperature was 280 °C. The oven temperature was programmed from 80 °C (isothermal for 1.0 min), with an increase of 10 °C/min, to reach 180 °C, then 20 °C/min to 240 °C, (for 4 min, ending with 5 min isothermal at 280 °C. The fatty acids detected were identified based on the GC- retention time of known compounds and then compared with internal standards. The MS data were analysed using the NIST 20 mass spectral library (NIST20MF Shimadzu-multiformat version).

2.16. Determination of total protein in C. mannii seed

Total protein content was extracted following the method of [24]. 1.0 g of *C. mannii* seed was frozen in nitrogen and then grounded using mortar and pestle. The lipid content was extracted by adding 10 ml of petroleum ether and agitating for 10 min in a test tube. The process was repeated two more times, after which 10 ml of protein extraction buffer was added. The extraction buffer consisted of 50 mM of tris-HCl pH 8.8, 1.5 mM KCl, 10 mM dithiothreitol (DDT), 1.0 mM PMSF and 0.1 Sodium dodecyl sulphate (SDS) per litre. The soluble materials were separated by centrifuging for 10 min at 10,000×*g*. The supernatant was used for protein determination. The total protein concentration was determined by BCA (bicinchoninic acid) protein assay kit (Thermo Scientific, UK) following the manufacturer's instructions as described below. The kit consisted of the following; BCA reagent A: Consisting of sodium carbonate, Sodium bicarbonate, Bicinchoninic acid and sodium tartrate in 500 ml of 0.1 M sodium hydroxide. BCA reagent B: Consisting of 25 ml of 4% cupric sulphate. Standard protein: Ampules containing bovine serum albumin (BSA) at 2 mg/ml in 0.9% saline and 0.05% sodium azide.

0.1 ml of the supernatant was measured in an appropriately labelled test tube. 2.0 ml of the working reagent (50: 1 Reagent A: B)

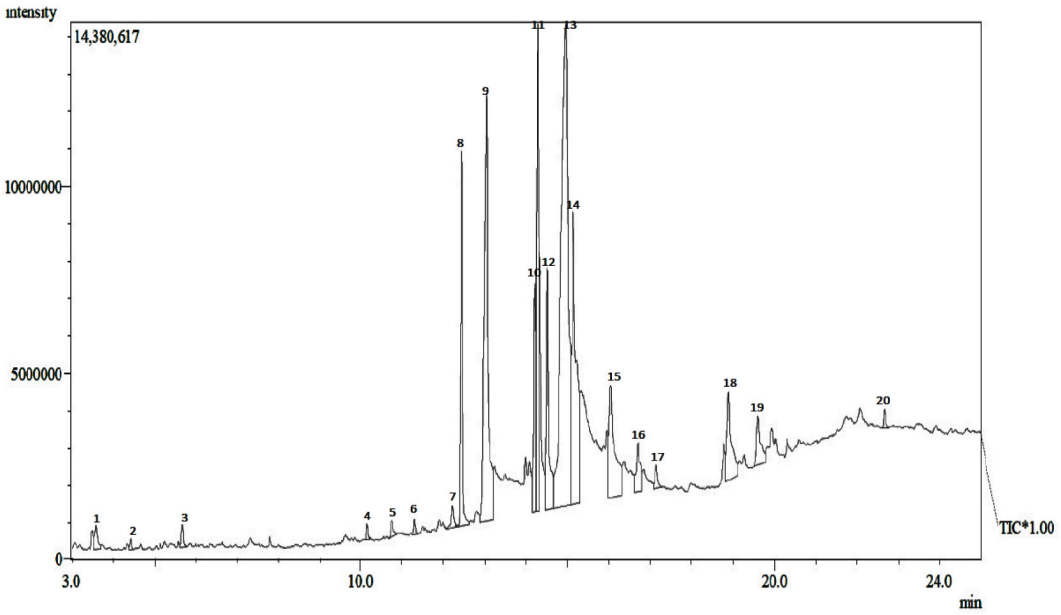

Fig. 3. Chromatogram from the GC-MS analysis of *Cucumeropsis mannii* seed oil.

was added to each tube. The tubes were incubated at 37 °C in a heating bath for 30 min and allowed to cool to room temperature and the absorbance of the purple colour developed was measured at 562 nm using a bench top visible spectrophotometer (Jenway 6300). The protein concentration was calculated from the standard curve. The experiment was carried out in triplicates.

3. Results and discussion

3.1. Extraction of oil from the seed

The oil yield of *Cucumeropsis manni* seed was found to be 40.80 ± 0.56% after 4 h s of soxhlet extraction (see Table 3). The result compares well to what was observed by other researchers who previously extracted oil from *C. mannii* [25]. reported a 38.70% yield [26], observed a 37.15% yield [27], reported 48.30% while [28] reported a higher yield of 57.26%. These slight variations can be attributed to factors such as the type of solvent used for extraction, and the method of extraction and geographical location [29]. The result indicated that *Cucumeropsis mannii* seed with oil yield of 40.80 has a relatively higher oil yield compared to other oil seeds that has been previously used in biodiesel production such as Jatropha seed, 36.70% and citrus sinensis seed, 35.60% [30], Balanite Aegyptical seed, 34.52% [31] and African star apple, 12% [32]. Therefore, with the oil content of 40.8%, the seed of C. mannii can be said contains sufficient oil to support large scale industrial production of biodiesel.

3.2. GC-MS analysis of C. mannii oil

The results of GC_MS analysis of *Cucumeropsis mannii* seed oil are shown in (Fig. 3) and Table 1. Table 2 shows the presence of 9 fatty acids in the extracted oil. The main saturated fatty acids are palmitic acid (C16:0) and stearic acid (C18:0), while unsaturated fatty acids are oleic acid (C18:1), linoleic acid (C18:2) and erucic acid (C22:1). These fatty acids detected in *C. mannii* oil, have also been reported to be the dominant fatty acids in some other seed oils that serve as feedstocks for biodiesel production. Jatropha seed oil [33], Rapeseed oil [34], castor oil [35] and palm oil [36] have been reported to contain palmitic acid, stearic acid, oleic acid and linoleic acid as the major fatty acid components similar to what is obtained from *C. mannii* seed oil [37]. stated that the dominant fatty acids in biodiesels produced from vegetable oils are palmitic, stearic, oleic, linoleic and linolenic. The percentage composition of the predominant fatty acids in the *C. mannii* seed oil is 23.5% palmitic acid, 18.11% stearic acid for the saturated and 39.83% oleic acid, 3.90% linoleic acid and 3.60% erucic acid for the unsaturated respectively. The major fatty acids reported here are similar to what was observed by other researchers [38]) reported the presence of palmitic, stearic, oleic, and linoleic acids [28], in addition to palmitic, stearic oleic, and linoleic acids also reported linolenic acids in *C. mannii* seed oil. However, the quantities of the individual fatty acid reported by various researchers varied, this can be attributed to the geographical locations and soil composition. Also, the differences in the *C. mannii* cultivar, cultivation practices, age of the seed (the period between harvest and extraction) and the extraction method employed can affect the fatty acid profile and other properties of the oil [39].

Each feedstock has its fatty acid structure/composition which strongly affects the biodiesel fuel produced from the feedstock. Analysis of the fatty acid composition of *C. mannii* oil indicated that it contained both Unsaturated fatty acids (53.0%) and saturated fatty acids (43.0%). The unsaturated fatty acids consist of 48.8% monounsaturated (predominantly oleic acid) and 4.2% of poly-unsaturated fatty acids (predominantly linoleic acid) while the saturated fatty acid was predominantly palmitic acid (23.50%), stearic acid (18.11%) [40]. observed that it is more suitable for vegetable oil feedstock used for biodiesel production to have a higher

Table 1
Fats and fat derive components of the *C.mannii* seed oil analysed by GC-MS.

Peak No	RT	Name of Compound	Molecular formula	MW (g/Mol	% Area
1	3.582	2-Hexanal, -ethyl	$C_8H_{14}O$	126.14	0.71
2	4.421	Propane −1,1-dimethoxy-2-methyl	$C_6H_{14}O_2$	118.17	0.18
3	5.668	Haxene-1,1-dimethoxy	$C_8H_{18}O_2$	146.23	0.38
4	10.148	Tetradecanoic acid methyl ester	$C_{15}H_{30}O_2$	242.39	0.25
5	10.742	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	0.44
6	11.294	Tetradecanoic acid, 1-methyl ethyl ester	$C_{17}H_{34}O_2$	270.45	0.25
7	12.209	2-Tetradecanone	$C_{13}H_{26}O$	212.37	0.50
8	12.434	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	5.97
9	13.035	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.40	15.51
10	14.209	9,12-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294.51	3.88
11	14.279	9-Octadeceniec acid methyl ester	$C_{19}H_{36}O_2$	296.52	8.95
12	14.519	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.47	5.22
13	14.956	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.50	30.88
14	15.145	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	12.89
15	16.042	1-Eicosanol	$C_{20}H_{42}$)	298.33	5.70
16	16.702	Hexadecanoic acid-2,3-dihydroxy propyl ester	$C_{19}H_{38}O$	330.51	1.99
17	17,157	Eicosanoic acid methyl ester	$C_{21}H_{42}O_2$	326.55	0.62
18	18.904	13-Docosenoic acid {13(Z)}	$C_{22}H_{42}O_2$	338.57	3.56
19	19.620	*Trans*-13-Docosenoic acid {13(E)}	$C_{22}H_{42}O_2$	338.57	1.72
20	22.680	Tetracosanoic acid methyl ester	$C_{25}H_{50}O_2$	382.70	0.39

Note: RT: retention time.

Table 2
Fatty acid composition of *C. mannii* seed oil.

Fatty Acid	Common Name	Type of Fatty Acid	% conc
Tetradecanoic Acid	Myristic acid	Saturated	0.94
Hexadecanoic Acid	Palmitic acid	Saturated	23.50
9-Octadecenoic Acid	Oleic acid	Unsaturated	39.83
9,12-Octadecadienoic Acid	Linoleic acid	Unsaturated	3.90
Octadecanoic Acid	Stearic acid	Saturated	18.11
13-Docosenoic Acid	Erucic acid	Unsaturated	3.60
Trans-13-Docosenoic Acid	Brassidic acid	Unsaturated	1.72
Tetracosanoic acid	Lignoceric acid	Saturated	0.40
Eicosanoic acid	Arachidic acid	Saturated	0.62

Total saturated = 47.0%; Total Unsaturated = 53%; Monounsaturated = 48.8%; Polyunsaturated = 4.2%.

percentage of monounsaturated fatty acids than polyunsaturated fatty acids. This is in agreement with the results obtained from *C. mannii* seed oil. This situation is more desirable because oil containing a higher concentration of polyunsaturated fatty acid exhibits poor oxidation stability due to the presence of multiple double bonds [41,42], This also negatively affects the fuel properties such as kinematic viscosity, density and cetane number [43]. On the other hand, a high concentration of monounsaturated fatty acids improves the fuel properties such as kinematic viscosity and cold flow properties [37].

3.3. Physicochemical properties of C. mannii oil

The physicochemical properties of *C. mannii* seed oil are presented in (Tables 3 and 4). The oil was light yellow in colour and liquid at room temperature. It had 0.93 ± 0.02 g/cm^3, 3.0 ± 0.10, 1.47 ± 0.04 and 4.20 ± 0.02 mg KOH/g as the relative density, viscosity at 28 °C, the refractive index at 30 °C, and acid value, respectively. While the peroxide value, iodine value, saponification value and free fatty acid values were 2.60 ± 0.10 (meq/kg), 111.01 ± 0.15 g/100 g, 192.05 ± 0.37 mg KOH/g and 2.52 ± 0.02% respectively. These values are comparable with the values reported for other seed oils which have been used for the production of biodiesel [25].

The relative density of 0.93 ± 0.02 g/cm3 obtained for *C. mannii* oil (Table 3)is comparable to 0.96 g/cm3 reported for Automation Gas Oil (AGO) [44], and the standard range by the ASTM (0.875–0.900 g/cm3) and EN14214 (0.86–0.900 g/cm$^{3)}$, for biodiesel as reported by Ref. [45]. This is an indication that *C. mannii* seed oil has the potential for biodiesel production as the relative densities of oil feedstocks were reported to decrease in value after conversion to biodiesel [45]. They observed a decrease in the densities of *Canarium schweinfurthi* seed and pulp oil from 0.97 to 0.90 and 0.88 respectively after transesterification (biodiesel production).

The viscosity value (VV) of crude *C. mannii* seed oil obtained from this work is 3.0 ± 0.01 mm^2/s (Table 3). This is lower than the values reported by Ref. [28] (5.89 mm^2/s) for *C. mannii* seed oil. The observed and that of other seed oils such as *H. crepitans* (5.91 mm^2/s) [46], and Hibiscus seed oil (3.8 mm^2/s) [47]. It is however in the range of 2.92 mm^2/s reported for soybean oil by Ref. [48], while [49] reported a VV of 2.7 mm^2/s for coconut oil. Palm oil and palm kernel oil was reported to have VV of 4.90 mm^2/s and 4.64 mm^2/s [50,51], respectively. The viscosity value obtained for *C. mannii* is lower than that obtained for Jatropha curcus (40.0 mm^2/s) and *Balanites aegyptiaca* (19.63 mm^2/s) by Refs. [52,53] *respectively.* The variations observed can be attributed to the differences in the triacylglycerol composition, degree of unsaturation, fatty acid chain length, the orientation of double bonds and the environmental temperature [54]. Viscosity reflects the flow properties (resistance to flow) of the diesel in fuel pumps, diesel injectors and distribution pipelines, particularly at lower temperatures [55]. The viscosity of oils increases with saturation and decreases with unsaturation [56]. The viscosity of biodiesels is usually higher than that of the oil it was derived from Ref. [57]. The VV of biodiesels ranges from 1.9 to 6.0 mm^2 according to the ASTMD651 standard. Therefore, the VV of 3.0 for *C. mannii* oil makes it good for biodiesel production.

A Refractive index of 1.469 ± 0.04 (at 30 °C) was obtained for *C. mannii* seed oil (Table 3). This value is in the same range as values reported for *Jatropha curcus* (1.471), *Hura crepitans* (1.36) [58], and peanut oil (1.45–1.56) [59] which have been previously used in biodiesel production. The Refractive index represents the ratio of the velocity of light in a vacuum to the velocity of light in a medium. It indicates the level of saturation and reduces with a reduction in the saturation of the fatty acids [54].

The acid value (AV) of 4.20 ± 0.02 mg KOH/g was obtained for *C. mannii* oil (Table 4) in this work. This is lower than 7.09 mg KOH/g obtained by Refs. [28,46] for *C. mannii* oil and *H. crepitans* seed oils respectively and even much lower than the AV of 35.8 mg KOH/g reported for Jatropha seed by Ref. [60]. These variations can be attributed to the nature of the feedstock, solvent, extraction

Table 3
Physical properties of *Cucumeropsis* mannii seed oil.

Physical properties of oil	Value	Equipment Used
Yield (%)	40.8	–
Colour	Light yellow	Physical observation
Relative Density (Cm^3)	0.93 ± 0.02	Relative density bottle
Viscosity at 30 °C (mm^2/s)	3.00 ± 0.10	Digital viscometer
Refractive index at 28 °C	1.46 ± 0.04	Refractometer
State at Room Temp	Liquid	Physical observation

Values are mean ± SD of three determinations.

Table 4
Chemical Properties of *Cucumeropsis* mannii oil compared to ASTM and EN 14213 Standards.

Chemical Properties of Oil	Values (This work)	ASTM standard	EN 14213 Standard
Acid Value (mgKOH/g of oil)	4.20 ± 0.02	0.5 (max)	0.5 (max)
Saponification value (mgKOH/g of oil)	192.03 ± 0.37	370 (max)	–
Peroxide value (meq/kg)	2.60 ± 0.10	–	–
Iodine value (g/100 g)	111.07 ± 0.15	–	130 (max)
Free Fatty acid (%)	2.51 ± 0.02		

Values are mean ± SD of three determinations, ASTM = American society for testing and materials, EN = European organisation, Max = maximum standard, Min. = minimum standard.

method and the age of the seed. However, the acid value (AV) for *C. mannii* oil is higher than the 2.18 mg KOH/g reported for *B. aegyptiaca* [61]. The acid value of oil affects its shelf life as well as industrial application. An oil that has a low acid value is stable over a long period and protects against rancidity [62]. The lower the acid value of oil the better it is for biodiesel production [52]. High acid value could decrease the process efficiency, particularly during the washing step in the biodiesel production process. However, this challenge can be overcome by reducing the acidity of the oil before biodiesel production through acid-catalyzed esterification of the oil [63] or by treating the oil with alkali (NaOH or Ca(OH)2 [64].

The iodine value (IV) of 111.01 ± 0.15 I_2 g/100 g) obtained for *C. mannii* seed oil (Table 4). This value is in range with values obtained for other well-known oil for biodiesel production such as rapeseed oil (107.51 I_2g/kg) and sunflower seed oil (102.02 I_2g/kg) [65], seinat oil (110.99 I_2g/100 g) and peanut oil (118.20 I_2g/100 g) [66]. This is however higher than 77.08 g/100 g reported for *Balanite aegyptiaaca* [61], 105.0 I_2g/100 g for *Jatropha curcus* [52], but slightly lower than the values reported for *Hura crepitans* (149.6 I_2g/100 g) [46]. The iodine value refers to the number of grams of iodine required to saturate the fatty acids present in 100 g of the oil or fat. It is often used to determine the amount of unsaturation in fatty acids. It can be used to estimate the oxidative stability of biodiesel. The IV of oil reportedly decreases after the transesterification of the oil (biodiesel production) [67]. According to the (EN14214) method, the international standard for the iodine value for biodiesels, the upper limit for IV is 120 g I_2/100 g. This shows that *C. mannii* oil is good for biodiesel production concerning the iodine value.

The peroxide value (PV) of 2.6 ± 0.10meq/kg was obtained for *C. mannii* seed oil in this work (Table 4). This is consistent with the value obtained for *Jatropha Curcus* seed oil which ranges from 2.1 to 3.7meq/kg [68], and Mustard seed oil 3.66meq O2/kg [65], these have been widely used as biodiesel feedstock. However, the PV for *C. mannii* oil is slightly lower than the PV recorded for other biodiesel feedstocks such as rapeseed oil (9.46 meqO_2/kg), Peanut seed oil (8.39 meqO_2/kg) and olive seed oil (6.39 meqO_2/kg) [65]. Higher PV makes an oil susceptible to rancidity which results in the polymerization of esters and the formation of gums that blocks the filters of engines [47]. Therefore, a low PV indicates oil stability to degradation which is a good pointer for quality biodiesel feedstock. The PV of biodiesel is usually higher than that of the oil from which it was derived [33]. To increase to oxidative stability of the biodiesel, the PV of the feedstock has to be minimal. With a PV value of 2.6 meq/kg, C. *mannii* seed oil is suitable for biodiesel production.

The saponification value (SV) of 192.05mgKOH/g was obtained for *C. mannii* oil in this work (Table 4). This compares well with values reported for other seed oils that have been used as biodiesel feedstock. [66], reported SV of 186.20 mgKOH/g and 187.20 mgKOH/g for Seinat seed oil and peanut seed oil respectively [52]. reported an SV value of 190.0 mgKOH/g for *Jatropha curcus* seed oil. A higher SV of 216.43mgKOH/g) and 220.19 mg KOH/g were reported for *B. aegyptiaca* oil and *Hura crepitans* respectively [46,60]. Saponification values of an oil sample correlate with the average molecular mass of the fatty acids present in the oil. A higher SV implies that higher molecular mass fatty acids are present in the oil. Seed oil with SV ranging from 130 to 220 is considered a good candidate for biodiesel production [46,67]. Thus having SV of 192.05mgKOH/g the *C. mannii* seed oil is suitable for biodiesel production.

Another important property of vegetable oil biodiesel feedstock is the free fatty acid content. The free fatty (FFA) acid content of oil affects biodiesel yield [69]. Free fatty acids are the fatty acids that are not present as triacylglycerol. It has been observed that the biodiesel yield decreases with an increase in the FFA [69] because the FFA reacts with the alkalis to produce soaps which makes separation difficult. The FFA content of the crude *C. mannii* seed oil was 2.52% (Table 4). This is below the allowed limit of 3% [28]. reported 4.512% for *C. mannii* seed oil. The observed difference can be attributed to the type of solvent used for extraction, the method of extraction, the geographical location and the age of the seed [29]. [65] reported lower values of 0.43% for mustard seed oil, 0.65% for Rapeseed oil, 0.81% for Sunflower seed oil and 1.36% for peanut oil respectively. A high FFA value of 15% has been obtained for *Jatropha curcus* seed oil [70]. Due to side reactions resulting from high FFA in biodiesel feedstock, such oil samples have to undergo pre-treatment to reduce the FFA content. This pre-treatment comes with a cost which then adds to the overall cost of biodiesel production.

3.4. Fuel properties of C. mannii oil

The fuel properties of the oil from *Cucumeropsis mannii* seed were evaluated and shown in Table 5 below. The caloric value of the oil was 31.10 ± 0.11 (MJ/kg). This value is lower when compared to values ranging from 39 to 40 MJ/kg that was reported for sunflower, grapeseed, soybean, olive and Corn seed oils that are used for biodiesel production [71]. The reduced caloric value for the *C. mannii* sample can be attributed to the relatively high moisture content (6.63 ± 0.02). The variations in the moisture content of an oil can be

attributed to the methods of extraction and the type of solvent used. However, it is above 25 MJ/kg which was reported for Canola seed oil by Ref. [42]. The caloric value is used for the estimation of the energy released by the fuel and fuel feedstocks. It represents the amount of heat that is transferred to the chambers which is an indication of the available energy in the fuel. Its value is directly proportional to the energy in the fuel or fuel feedstock.

The low-temperature properties namely cloud point (CP) and pour point (PP) for *C. mannii* oil were 3.03 ± 0.11 and 1.00 ± 0.10 respectively (Table 5). The CP and PP are referred to as the fuel's low-temperature flow properties. The CP represents the temperature at which cloud (wax) first appears as fuel is being cooled while the PP is the lowest temperature at which the fuel starts to lose its flow characteristics [72]. Both parameters are an indication of the suitability of the fuel in cold conditions. The values obtained in this study are in range with the observation made by Ref. [73], for the same oil, the PP values are always lower than that of the CP [74]. reported PP and CP values of −3.0 °C and 2.0 °C respectively for *Jatropha curcus* seed oil. [75], reported PP (9.0 °C) and CP (11.5 °C) respectively for palm olein while [76] reported much lower values for sunflower PP -19, CP -9), Mustered oil (PP -29 and CP-18) and Linseed oil (PP-16, CP -13) respectively. The flash point (FP) for *C. mannii* oil was determined. This represents the temperature at which it will form a flammable vapour that can ignite on exposure to an ignition source under controlled conditions. The flashpoint for *C. mannii* oil was observed to be 279.04 ± 0.99 °C (Table 5). This is comparable to values reported by Ref. [77] for other biodiesel feedstocks such as Coconut oil (324 °C), Soybean oil (334 °C), Groundnut oil (328 °C) and Palm oil (226 °C) respectively. The FP of 309 °C, 315 °C and 312 °C were obtained for Sunflower oil, Sesame oil and Cottonseed oil respectively by Ref. [78]. These were slightly higher than 279.04 °C obtained for *C. mannii* seed oil.

3.5. The protein content of C. mannii seed cake

In addition to the biodiesel qualities of the oil from *C. mannii*, the protein content of the seed powder that was left after oil extraction was determined. This is important to add value to the chaff left after oil extraction. The result indicated that the defatted seed powder contained 47.4 g/100 g protein. This result is consistent with the 39.4 g/100 g reported by Ref. [79] while [80,81] reported 34.5% and 31.4% protein respectively. These results indicate that the seeds of *C. mannii* are rich in protein as such the defatted seed cakes (powder) can be used in the fortification of feed.

4. Conclusion

This study has demonstrated the biodiesel potential of oil from an underexploited seed crop (Cucumeropsis mannii seed). We reported for the first time the fuel properties in addition to the physicochemical and fatty acid profile of oil extracted from *C. mannii* seeds cultivated in southeast Nigeria. As a climber which produces high yields when interplanted with tall-growing plants and trees, *Cucumeropsis mannii* cultivation for biodiesel feedstock will have minimal land degradation impact known to be associated with the cultivation of many other oil crops. Its utilization as biodiesel feedstock will increase its market value and production, thus, improving the economy of local farmers. Also, the defatted protein-rich cakes remain available for food, thus the *C. mannii* seed oil can serve as biodiesel feedstock without significant disruptions to the food chain supply. We expect that the technology developed using *C. mannii* seeds will eventually promote its reinsertion as a crop and will trigger a positive economic impact on the agricultural and transportation sectors, particularly in sub-Saharan Africa. This work, therefore, will be of interest to researchers undertaking techno-economic and environmental feasibility studies.

Author contribution statement

Benjamin. O. Ezema: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Kingsley. O. Omeje: Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Juliet N. Ozioko: Contributed reagents, materials, analysis tools or data.

Alfred Fernandez-Castane: Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sabinus Oscar.O. Eze: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Table 5
Fuel Properties of *C. mannii* oil.

Fuel Properties of Oil	Equipment used	**Values** (This work)	ASTM Standard for Biodiesel	EN14213 standard for biodiesel
Cloud point (°C)	Cloud point apparatus	3.03 ± 0.11	−3 - 12	N/A
Pour Point (°C)	Pour point apparatus	1.00 ± 0.10	−10 (max)	0 (max)
Flash Point (°C)	Pensky Martin flash point closed cup tester	279.04 ± 0.99	130 (min)	101 (min)
Caloric value (MJ/kg)	Bomb calorimeter	31.10 ± 0.11		35 (min)
Ash content (%)	Muffle furnace and digital weighing balance	0.20 ± 0.01	0.3 (max)	0.02 max
Moisture content (%)	Oven and digital weighing balance	6.63 ± 0.02	0.03 (max)	0.02 (max)

Values are mean ± SD of three determinations.

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