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Investigation of *Cucumeropsis mannii* N. seed oil as potential biodiesel feedstock

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ABSTRACT

Biodiesels fuels are being explored worldwide as alternatives for fossil fuels. The seeds of “Werewere” (*Cucumeropsis mannii* N., Cucurbitaceae) a fruit vegetable plant in Ghana with high oil content were analysed for their fatty acid composition as well as fuel properties to ascertain their potential as biodiesel fuel. The seeds contained 37.15% oil. The extracted seed oil by GC/MS analysis consisted of mainly linoleic acid (18:2) accounting for 58.8% of the total fatty acids. Other fatty acids detected were oleic acid (C18:1), stearic acid (C18:0) and palmitic acid (C16:0,) with contribution of 15.5, 14.1 and 11.5% respectively. Among the fuel properties measured were density (889.9 kg/m^3), cetane number (57), flash point (155 ^{o}C) and pour point (-3 ^{o}C). The measured fuel properties of the fatty acid methyl ester of the oil were comparable to both the ASTMD 6751 and the EN 14214 biodiesel standards. Fuel properties were essentially identical with those of soybean, safflower, sunflower and “egusi” biodiesel. The Kinematic viscosity was however measured to be 15. mm^2/s a value higher than most biodiesel fuels reported in the literature.

Keywords: Renewable energy, biodiesel, vegetable oil, transesterification, fatty acid methyl ester.

INTRODUCTION

The world’s increasing high energy demands and scarcity of conventional fossil fuels coupled with growing emissions of combustion-generated pollutants, and increasing costs have fostered an interest in alternative fuel sources. On the brink of reaching the peak production of non-renewable petroleum reserves, developing renewable energy sources has become more attractive.

Biodiesel is an alternative fuel diesel for internal combustion engines that, if made technically feasible, economically competitive, environmentally acceptable, and easily available, can be a viable alternative fuel option. There are many advantages to using biodiesel as a diesel fuel. Biodiesel is more environmentally friendly and improves engine performance – not only is it a derivative of renewable natural products, but it is also more biodegradable and produces lower emissions. It also has higher lubricity

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and combustion efficiency properties than gasoline and petroleum diesel (Muregesan et al., 2009).

In particular, the main advantages of vegetable oils as diesel fuel are ready availability, renewability, lower sulfur and aromatic content, and biodegradability (Balet et al., 2009) of more than 350 oil-bearing crops that have been identified, rapeseed, canola, soybean, sunflower, and palm oils are the most common sources of raw, biodiesel materials (Demirbas, 2008). Various oils such as Jatropha (*Jatropha curcas)*, Mahua (*Madhucaindica*), and Karanja (*Pongamia pinnata*) are used as a significant fuel source in different developing countries owing to their availability and therefore greater economic benefit (Allen et al., 1999; Moser, 2009).

The plant *Cucumeropsis mannii* N. a member of the Cucurbitaceae family, is both cultivated and found in the wild in Ghana, Sierra Leone, Cameroon, Gabon, Congo, and Angola (Akoh et al., 1992; Dokosi, 1998). In Ghana it is called "Werewere" among the "Twi" speaking people. It can be distinguished from "Egusi" or "Akatoa" (*Citrullus colocynthis)* also from the Cucurbitaceae family by the seeds which are larger in the latter. The only edible part of the "Werewere" plant is the seed, which in Ghana, is roasted and ground to a powder for use as a soup thickener and in vegetable preparations.

The main aim of this study was to evaluate the use of "Werewere" seed oil as a potential feedstock for biodiesel production as is the case of various oils such as canola, soybean, sunflower, rapseed , jatropha etc. The use of "Werewere" seed oil for biodiesel can be especially beneficial to the economies of West African countries since the crop is already domestically produced and has the potential of reducing dependency on imported petroleum. The fuel properties of the "Werewere oil methyl ester were determined and compared with other biodiesel fuels derived from conventional vegetable oils.

MATERIALS AND METHODS

Seeds used in the analysis

The "Werewere" seeds were purchased at the "Kotokoraba" Market in Cape Coast, Ghana in June 2012. The unroasted seeds were milled into fine particle size using a heavy-duty blender. The material that passed through 80-mesh sieve was used for the lipid extraction.

Lipid extraction

Soxhlet extraction of 400 g powdered seeds was done for 8 hours using 1 L petroleum ether (40-60 ^{o}C). The extract was concentrated to dryness under pressure to give a pale yellow oil, WSO, (148.6 g, 37.15%) which was stored in a sealed dark bottle in a desiccator at 30 °C until analysis.

Physical and chemical analysis of the extracted seed oil (WSO)

Specific gravity was determined at 30 ^{o}C using a 25 ml capacity pycnometer. Refractive index was measured with an Abbe refractometer (Atago Co. Ltd, Tokyo, Japan) equipped with a thermostated circulator. Free fatty acid content and saponification value were measured by a titration method (Sadasivam et al., 2005). The British Standards (British Standard, 1958) was used to determine unsaponifiable matter, iodine value (Wij's solution method), specific gravity and acid value. All experiments were run at least twice and the mean values were reported.

Transesterification of oil and GC/MS analysis

The oil WSO (6 g) was heated under reflux for 1 hr. in a methanol/concentrated sulphuric (VI) mixture (20:2 vol.) to covert the lipids present into fatty acid methyl esters (FAME). The cool solution was neutralized with enough 1% Na_2CO_3 solution and extracted with hexane (5 x 10 ml). The hexane extract was dried with anhydrous Na_2SO_4 and evaporated under pressure to afford the "Werewere" oil fatty acid methyl ester, WOME, in a yield of 10 ml.

The fatty acid composition of the seed oil fatty acid methyl ester (WOME) in hexane was analysed by GC coupled to an MS spectrometer. The GC (Hewlett-Packard 6890) was equipped with a flame ionization detector (FID) and a fused silica capillary BPX-70 column (60 m x 0.32 mm; 0.25 µm film thickness). The oven temperature was set at 115 ^oC, raised to 180 ^oC at 8 ^oC/min and held for 10 min. The temperature was finally raised to 240 ^oC at 8 ^oC/min, and held for 10 min. A sample volume of 1 µL was injected using spitless injection mode. The carrier gas used was helium at a flow rate of 1.6 mL/min. Identification of the unknown fatty acid methyl ester (WOME) was made by comparison of the retention times with those of pure standards of FAMEs. The fatty acids composition of WOME was reported as relative percentage of the total peak area. The mass spectra were run on HP 5972 MSD spectrometer.

Determination of fuel properties of WOME

The fuel properties of the methyl ester WOME such as density (ASTM D1298), kinematic viscosity (ASTM D445), flash point (ASTM D93), pour point (ASTM D97) distillation (ASTM D86) were determined according to relevant biodiesel test methods. The cetane number of WOME was evaluated using empirical formula on calculated cetane index reported in the literature (Ramos, 2009; Moser, 2009) based on formulas ASTM D976 or ASTM D 4737 as given below. All the data were compared with those of biodiesels obtained from vegetables such as "egusi" (*Citrullus colocynthis*, EMOME), soybean (SOME), sunflower (SUOME) and safflower (SAOME) obtained from the literature (Giwa, 2010).

(i) Calculated Cetane Index = - 420.34 + $0.016G^2$ + 0.192GlogM + $65.01(\log M)^3$ – $0.0001809M^2$ or

(ii) Calculated Cetane Index = 454.74-1641.416D +$774.74D^3$ – 0.554B + 97.80 $(\log B)^3$

Where G = API gravity, determined by Test Method D287 or D1298, M = mid-boiling temperature (^oF), determined by Test Method D 86 and corrected to standard barometric pressure, D = density at 15 ^oC, (g/ml) determined by Test Method D 1298, B = mid-boiling temperature (^oC) determined by Test Method D 86 and corrected to standard barometric pressure.

RESULTS AND DISCUSSION

Physicochemical properties of "Werewere" seed oil (WSO)

The yield of "Werewere" seed oil (WSO) obtained was 37.15%. This was slightly lower than in other studies (Akoh et al., 1992). Whereas the seeds used in this experiment were unroasted, the seeds analyzed in other studies were generally roasted before extracting the oil. Roasting has been shown to yield more oil, probably by reducing the moisture content of the kernel and allowing for easier rupture of the cell walls (Badifu, 1993; Idouraine et al., 1996). It should be also noted that heating denatures protein, leading to the release of more oil from the kernel. The quality of "Werewere" seed oil (WSO) was determined using selected markers such as saponification value, iodine value, free fatty acids, etc. as shown in Table 1. The specific gravity, refractive index, and unsaponifiable matter were 0.9158, 1.47, and 2%, respectively. These values were all in general agreement with previous studies (Badifu et al., 1991).

Unsaponifiable matters are substances in vegetable oils that are inert to alkaline treatment and nonvolatile at 80 °C oven temperature (Badifu et al., 1991). It is usually a mixture of hydrocarbons, alcohols, sterols, pigments, ketones, and fat-soluble vitamins. Saponification value signifies the average molecular weight of oil (Schafferman et al., 1998). A high saponification value indicates a greater proportion of fatty acids of low molecular weight. The saponification value was 187.23 mg KOH/g, which was slightly lower than in other studies (Achu et al., 2005; Akoh et al., 1992; Badifu et al., 1991). Yaniv et al. (1996) pointed out that saponification value depends on the neutralization of the free fatty acids in oil and complete saponification of the fatty material. It is possible that saponification did not occur to a complete extent, thereby leading to a lower value. Nevertheless, the results indicated that the oils contained higher proportions of low molecular weight fatty acids.

Iodine value indicates the degree of unsaturation of a fatty acid. A high iodine value corresponds with a high unsaturated fatty acid content. The iodine value of WSO was 128.49, a value comparable to what has been reported in other studies (Badifu, 1991; Milovanovic et al., 2005). The value was confirmed by GC/MS results which indicated that *C. mannii* has a high unsaturated fatty acid composition. Saponification and iodine values have however been shown to vary according to the region of cultivation – such was the case in “Egusi” seed oils from Cameroon, including *C. mannii* (Achu et al., 2005).

Acid value is a measure of the extent to which the glycerides are decomposed by lipase action. Increasing acidity leads to the deterioration of grains and milled products. Free fatty acids are responsible for the acidity and oxidability of oils (El-Diwani et al., 2009). The lower the free fatty acid content, the more stable the oil. The acid value and free fatty acid content of WSO were 6.89 mg KOH/g and 3.42%, respectively. These values are higher than in other reports (Badifu, 1993; Milovanovic et al., 2005). Oils with high free fatty acid content easily react to form soaps that can cause an increase in viscosity (Wright et al., 1994). Nevertheless, the free fatty acid percentage is still lower than the 5.00% free fatty acid content recommended as the maximum for non-rancid oils (Stevenson et al., 2007. A higher acid value and free fatty acid percentage implies a higher percentage of impurity. It has been shown that acid value increases with temperature (Gohari et al., 2011). Refrigerated storage (4-7 °C) can limit changes in the acid value of melon oils, even after 6 months of storage. The addition of butylated hydroxyanisole (BHA) in crude oil lowers peroxide levels and little deteriorative change when stored in under ambient conditions (25-30 °C) with less exposure to light for a period of 6 months (Ogunsua and Badifu, 1989). Since a lower peroxide level indicates a lower amount of lipid oxidation, the addition of BHA should also lower the acid value and free fatty acid content of a crude oil sample.

The percentage impurity was determined to be 3.68 a value higher than for most other vegetable oils. The high percentage of impurity may be attributed to the methods of processing, the conditions and duration of storage of the seeds and oils, and the method of extraction.

Fatty acid composition

WSO is composed of four fatty acids: palmitic, stearic, oleic, and linoleic acids. Linoleic (C18:2) and oleic (C18:1) acids were the two most prominent fatty acids at 58.8% and 15.5%, respectively. There were more unsaturated fatty acids (74.28%) than saturated acids (25.29%). The fatty acid profile of WSO was in close agreement with previous studies, with the exception of palmitic and stearic acids (Akoh et al., 1992; Badifu et al., 1991; El-Adawry et al., 2001; Stevenson et al., 2007). These studies reported palmitic acid as the predominant saturated acid, but it was found to be stearic acid in this experiment. However, it should be noted that Achu et al. (2005) have suggested that palmitic and stearic acid composition of some Cucurbitaceae oils in Cameroon, including *C. mannii*, can be affected by region of cultivation. As shown in Table 2, the fatty acid composition of WSO is similar to that of some conventional vegetable oils used for biodiesel, including soybean, sunflower, corn, and jatropha oils, with linoleic acid as the chief fatty acid followed by oleic acid, but not rapeseed and palm oils. Furthermore, the high unsaturation content is reported to considerably reduce viscosity of biodiesel fuels, a highly desirable trait in biodiesels Allen et al., 1999). Such similarity in chemical composition and oil properties suggests the potential of WSO to be used as biodiesel production feedstock.

Fuel properties of "Werewere" oil methyl ester (WOME)

Biodiesel yield was estimated after the separation and purification of the transesterified product (WOME). It was determined relative to the extracted oil (WSO). The biodiesel yield for WOME was 86.13%. It has been reported that the presence of unsaponifiable matter, such as tocopherols, phospholipids, fat soluble vitamins, and hydrocarbons, leads to reduced yields in biodiesel production (Balet et al., 2009). Furthermore, in acid catalyzed transesterification methods, the yield of methyl esters is reduced significantly as free fatty acid content increases (Demirbas, 2009). The free fatty acid (FFA) content of WOME is greater than the maximum 0.5% FFA content required for successful transesterification according to Demirbas (2009) and Schinas et al. (2009). These studies reported that a free fatty acid content exceeding this limit unavoidably leads to soap formation during transesterification.

The properties of biodiesel are largely dependent on the fatty acid profile of the oil (Goering et al., 1982). In Table 3, the biofuel properties of WOME and the methyl esters of sunflower (SUOME), soybean (SOME), safflower (SAOME) and "Egusi" (EMOME) are compared with the biodiesel standard, ASTM D 6751. This standard outlines the requirements that pure biodiesel (B100) must meet before it can be used as a pure fuel or blended with a petroleum-based diesel fuel. These biodiesels are also compared with EN 14214, an international standard that identifies parameters for biodiesel produced from rapeseed fuel stock.

The density is the weight of unit volume of fuel and is an important property for diesel fuel injection. A higher density for biodiesel results in the delivery of a slightly greater mass of fuel since fuel injection equipment operates on a volume metering system (Giwa et al., 2010). Gas oil and heavy fuel oil have densities of 835 and 930 kg/m^3 respectively. The density of WOME was measured to be 889.9 comparable to that of EN14214, SOME, SUOME and EMOME biodiesel fuels as indicated in Table 3 for comparison.

Cetane number or CN is a measurement of the combustion quality of diesel fuel during compression ignition. It is a significant expression of diesel fuel quality among a number of other measurements that determine overall diesel fuel quality. Cetane number is actually a measure of a fuel's ignition delay; the time period between the start of injection and the first identifiable pressure increase during combustion of the fuel. In a particular diesel engine, higher cetane fuels will have shorter ignition delay periods than lower cetane fuels.

Cetane number affects the engine performance parameters like combustion, stability, driveability, white smoke, noise and emissions of carbon monoxide and hydrocarbons. A higher cetane number signifies better ignition properties. Biodiesels has higher cetane number than conventional diesel fuel which results in higher combustion efficiency (Giwa et al., 2010).The minimum limit of cetane value of the fuel is 42. The value of 57 for WOME is comparable to the other biodiesel fuels as shown in Table 3. Normal modern highway diesels run best with a fuel rated between 45-55. The following is a list of cetane numbers varying grades types of compression ignition diesel fuels: regular diesel (48), Premium diesel (55), Biodiesel ,B 100 (55), Biodiesel blend, B20 (50) and Synthetic diesel (55) (Drew, 1998). Cetane number has been found to depend largely on chain length and degree of unsaturation and is higher in compounds with higher saturated fatty acid (Knothe et al., 2003).

The calculated cetane index is a useful tool for estimating ASTM cetane number where a test engine is not available for determinitg this property. It may be conveniently employed for approximating cetane number where the quantity of sample is too small for engine rating (Drews, 1998).

The Flash point of a fuel is the temperature at which it will ignite when exposed to a flame. The flash point of biodiesel is higher than diesel fuel, which makes it safer for transportation purposes (Giwa et al., 2010). The biodiesel produced from WOME has a flash point of 155 ^{o}C which is within the range for biodiesels. Flash point values of 135, 160, 180, 141 and 163 °C have been reported for palm, soybean, sunflower, pongamia and jatropha biodiesel respectively (Sarin et al., 2007).

The Cloud point is the temperature at which wax first becomes visible to the naked eye when the fuel is cooled. At temperatures below the cloud point, larger crystals fuse together and form agglomerations that eventually become extensive enough to prevent pouring of the fluid and consequently affecting the performance of fuel lines, fuel pumps and injectors. The low-temperature behavior of biodiesel is significantly influenced by molecular structure. It depends mostly on the saturated ester and unsaturated ester composition effect is negligible (Giwa et al., 2010). The pour point temperature is another property of diesel fuel which is about 3 ^{o}C higher than the cloud point. The pour point measured for WOME was -3 ^{o}C. It indicated that the cloud point of WOME could be comparable to the other known biodiesel fuels as shown in Table 3.

Kinematic viscosity is a very important fuel property which represents the flow characteristics of the fuel during storage transportation from one place to another. One of the reasons why biodiesel is used as an alternative fuel instead of pure vegetable oils or animal fats is as a result of its reduced viscosity which enhances fuel flow characteristics. In addition kinematic viscosity is an important parameter regarding fuel atomization and combustion as well as distribution (Giwa et al., 2010). Kinematic viscosity increases with fatty acid chain length and increasing degree of saturation of either the fatty acid or alcohol moiety in the fatty acid (Knothe et al., 2003). It is an important fuel property and measures the flow characteristics of fuel. The viscosity of WOME was 15.1 mm^2/s, a value quite higher than most of the biodiesel fuels. This is an indication that the WOME biodiesel unlike the others would not flow easily and might be a major disadvantage to its use as a biodiesel.

Distillation process is an indispensable tool in petroleum industries which removes small amounts of impurities, odours and coloured bodies from vegetable feedstocks to provide a colourless biodiesel. The distillation process is important as biodiesel tends to darken during storage due to oxidation. Antioxidants are added to increase shelf life during its time in storage. Distillation is to create product homogeneity, superior biodiesel and to ensure fuel quality. Normally for a good fuel 85% is recovered at 360 ^{o}C (Bachler et al., 2010; McCormick et al., 2007). With the new ASTM standards (ASTM 6751) recently revised, allowable levels of water and sediments has greatly reduced. This has been implemented to reduce particulate emissions, fouling, filter plugging and other potential engine problems. The distillation results of WOME showed that 50% of the biodiesel was recovered at 368 ^{o}C. This shows that the biodiesel fuel prepared from "Werewere" (WOME) is quite heavier than normal fuel which is a disadvantage as a biodiesel fuel.

Table 1: Physiochemical properties of werewere (WSO) seed oil.

Parameter	
Lipid content (%)	37.15
Specific gravity	0.9158
Refractive index (at 30 °C)	1.47
Saponification value (mg KOH/g)	187.23
Iodine value	128.49
Acid value (mg KOH/g)	6.89
Free fatty acid (%)	3.42
Percentage of impurity	3.68
Unsaponifiable matter (%)	2.0

Table 2: Fatty acid composition (wt%) of common vegetable oils.

Fatty acid	**Werewere**	**Soybean**[a]	**Corn**[a]	**Sunflower**[a]	**Cotton**[a]	**Jatropha**[b]	**Rapeseed**[a]	**Palm**[a]
Palmitic (C16:0)	11.5	13.9	11.8	6.4	28.7	18.2	3.5	42.6
Palmitoleic C16:1)	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.3
Stearic (C18:0)	14.1	2.1	2.0	2.9	0.9	5.1	0.9	4.4
Oleic (C18:1)	15.5	23.2	24.8	17.7	13.0	28.5	64.1	40.5
Linoleic (C18:2)	58.8	56.2	61.3	72.9	57.4	48.2	22.3	10.1
Linolenic (C18:3)	0.0	4.3	0.0	0.0	0.0	0.0	8.2	0.2
Others	0.0	0.0	0.1	0.0	0.0	0.0	1.0	1.9
Total saturates	25.59	16.00	13.80	9.30	29.60	23.36	4.40	47.00
Total unsaturates	74.28	84.00	86.10	90.70	70.40	76.64	94.60	51.10

[a]: Demirbas [2008a, 2008b, 2009] [b]: El-Diwani et al. (2009)

Table 3: Fuel properties of wome and other biodiesel fuels.

Property	**Unit**	**ASTM D6751**	**EN 14214**	**Wome**	**Some**	**Suome**	**Saome**	**Emome**
Density	kg/m^3	-	860-900	889.9	885	884	874	883
Cetane number		47 min	51 min	57	49	55	52	53
Flash point	^oC	130 min	120 min	155	171	168	176	142
Pour point	^oC	-	-	-3	-	-	--	-
Cloud point	^oC	-	-	-	1	1	2	0.5
Kinematic viscosity, 40 ^oC	mm^2/s	1.9-6.0	3.5-5.0	15.1	4.2	4.8	4.2	3.8
Ash content	%	-	-	7.45	-	-	-	-
Distillation 50% Recovered, (760 mm. of Hg.)	^oC	-	-	368	-	-	-	-

Conclusion

WSO was transesterified using methanol in the presence of sulfuric acid to produce WOME. The fatty acid compositions were as follows: linoleic acid 58.8%, oleic acid 15.5%, stearic acid 14.1% and palmitic acid 11.5% similar to that of some conventional vegetable oils used for biodiesel such as soybean, sunflower, corn and jatropha oils. The biodiesel fuel properties of WOME were comparable to ASTMD 6751 and EN14214 and the other biodiesels prepared from other vegetable oils. The kinematic viscosity and distillation process results of 15.5 mm^2/s and 50% at 368 ^oC respectively were however the major drawbacks to the use of WOME as a biodiesel. Nevertheless the other parameters determined in the present investigation indicated that WOME has a potential as a biodiesel fuel.

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