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STUDIES ON THE BIOLUBRICANT PROPERTIES OF *MORINGA OLEIFERA* SEED OIL: CORRELATING VISCOSITY AND FATTY ACID COMPOSITION

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ABSTRACT This study examined the characteristics of *Moringa oleifera* seed oil as a biolubricant. The study covered the physicochemical and rheological characteristics of the seed oil. The seed yielded 37.9% oil with the following physicochemical properties: peroxide value (1.655 meq kg^{-1}), saponification value (154.5 mg KOH g^{-1}), and acid value (9.724 mg KOH g^{-1}).The rheological characteristics of *M. oleifera* oil showed the oil was solid at 10.1°C and exhibited flow properties typical of vegetable oils in the temperature range 30 to 70°C however it exhibited thermal instability at 90°C as indicated by the entirely shear thinning properties of the oil attributed to polymerization of the oil to gum. The oil had oleic acid (72.73%) as the principal fatty acid. The high viscosity of M. oleifera oil (0.06078 Pas, 30°C) correlates with its high percentage of long chain fatty acids (C ≥16 = 99.86%), and the high oleic acid composition indicates a potential for application as a biolubricant.

Keywords: *Moringa oleifera* oil, biolubricant, physicochemical, rheological, fatty acid composition

INTRODUCTION

There has been growing interest in the use of vegetable oils as lubricants and hydraulic fluids due to the toxicity and environmental issues arising from conventional petroleum-based fluids. Biolubricant is an alternative lubricant different from mineral oil lubricant as it is prepared from non-conventional energy resources and is nontoxic, biodegradable and eco-friendly. Several vegetable oils have been applied as biolubricant and additives including canola, rapeseed, castor and palm (Hsien, 2015). Vegetable oils with high oleic contents are considered to be the best alternative to substitute conventional mineral oil-based lubricating oils and synthetic esters (Singh & Chhibber, 2013).

Moringa oleifera commonly referred to as "*Moringa*" is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family *Moringaceae*. It is commonly known as the horse-radish or drumstick tree and is native to the sub-Himalayan region of northwest India. The tree itself is rather slender, with drooping branches and ranges in height from 5 –12 m and the fruits (pods) are around 50 cm long. Fully matured, dry seeds are round or triangular in shape, the kernel surrounded by a light wooded shell with three papery wings (Abdulkarim *et al*., 2005). It is known as Ben oil tree in English; *'Okwe oyibo'* in Igbo, *'Gawara' or 'Habiwal'* in Hausa and *'Adagba maloye'* or '*Ewe Igbale*' in Yoruba, all people of Nigeria. It grows rapidly in most regions and climatic conditions of Nigeria. *M. oleifera* is an important food

commodity which has had enormous attention as the 'natural nutrition of the tropics' (Anwar *et al*., 2007).

There have been several reports in literature on extraction and characterization of *M. oleifera* seed oil. Extraction methods reported included mechanical press (Anwar *et al.,* 2006; Eman & Muhamad, 2016), solvent extraction (Anwar & Bhanger, 2003; Abdulkarim *et al.* 2005; Anwar *et al.,* 2006) and enzymatic extraction (Abdulkarim *et al.* 2005; Latif *et al.,* 2011), the yield and physicochemical properties of the oil varied with method of extraction. *M. oleifera* seed oil has been reported to have high degree of unsaturation with oleic acid (67.8 - 85%) as the most prominent fatty acid (Tsaknis *et al*., 1999; Abdulkarim *et al.* 2005; Anwar *et al*., 2006; Nzikou *et al.,* 2009). Studies on the viscosity of the oil as a function of temperature (Nzikou *et al.,* 2009) and melting behavior (Mohammed *et al.,* 2003; Abdulkarim *et al.* 2005; Nzikou *et al.,* 2009) of the oil have been reported. Abdulkarim *et al. (*2007) who investigated the frying stability of *M. oleifera* seed oil observed it was more stable than canola oil, soybean oil, and palm oil when used in frying. The rheological properties of *M. oleifera* seed oil have not been well studied especially over a wide range of temperatures. In this work, we studied the rheological properties of *M. oleifera* seed oil using steady shear to investigate the changes in viscosity with temperature and oscillatory shear to determine the changes in mechanical properties with temperature. The rheological characteristics were related to the fatty acid composition of the oil.

MATERIALS AND METHODS

Extraction of Oil

Dried pods were collected from *M. oleifera* trees growing on a farmland on the campus of the University of Ibadan. The seeds were removed from the pods, dehulled and the resulting kernels dried in air at ambient temperature. The dried kernels were then blended using an electric blender. The weight of the pulverized flour was measured (838.87 g), packed in a soxhlet extractor and extracted with hexane for twenty four hours. The *M. oleifera* seed oil was then concentrated by distillation to remove the hexane. The yield was calculated as in Eq. 1.

$$Yield(\%) = \frac{Weight\ \ of\ oil\ \ extracted\ \ (g)}{Weight\ \ of\ \ pulverized\ \ \ seed\ (g)} X\,100 \qquad \text{(Eq.1)}$$

Physicochemical Tests

Saponification value

Ethanolic potassium hydroxide (0.5N) was pipetted into a conical flask containing 2.0 g of the oil. The content of the flask was refluxed for 45 min, cooled to room temperature, after which it was titrated with hydrochloric acid (0.5N) using phenolphthalein as indicator. A blank was subjected to the same condition. The saponification value was calculated as in Eq. 2.

$$Saponification\ value\ (mg\ KOH/g) = \frac{(V_b - V_s) \times C \times 56.1}{W} \qquad \text{(Eq.2)}$$

Where V_b= Titre for the blank (ml), V_s= Titre for the sample (ml) , C = concentration of HCl (0.5N), 56.1= equivalent weight of KOH, W= Weight of oil in gram (2 g)

Peroxide value

Oil (0.5 g) was added into a boiling tube, glacial acetic acid/chloroform mixture (20 ml; 2:1 volume/volume (v/v)) was added, the boiling tube was placed in boiling water for 1 min after which its content was poured into a conical flask containing KI solution (20 ml; 5 %). The boiling tube was rinsed twice with distilled water (25 ml) and content added into the conical flask. The liberated iodine was titrated with sodium thiosulphate (0.002N) solution until the amber colour lightens when 1 ml of 1% starch solution was added as indicator. The resulting blue colour was titrated to a colourless end point. A blank was subjected to the same condition. Peroxide value of the oil sample was calculated as in Eq. 3.

$$Peroxide\ value\ (meq/kg) = \frac{(V_s - V_b) \times normality\ of\ \ titrant \times 1000\ g\ kg^{-1}}{W} \qquad (Eq\,3)$$

Where, V_b = Titre for blank; V_s = Titre for sample; W = Weight of oil in gram.

Acid value

The number of mg of potassium hydroxide required to neutralize the free acids in 1g of the sample was determined by placing 0.5 g of the oil in a conical flask containing mixture of ether and ethanol (50 ml; 95% v/v). The resulting solution was titrated with 0.1N ethanolic potassium hydroxide solution using phenolphthalein as indicator. The acid value was expressed as mg KOH g^{-1} and calculated as in Eq. 4.

$$Acid\ \ value = \frac{V_s \times C \times 56.1}{W} \qquad \text{(Eq. 4)}$$

Where, V_s = Titre for KOH used; C= concentration of KOH (0.1N); 56.1= equivalent weight of KOH; W = Weight of oil in gram (1 g).

Rheological Studies

The rheological measurements were carried out on a Controlled Stress Rheometer (AR 500, TA Instruments Ltd, USA) with cone and plate geometry (40 mm, 2^o steel cone and 53µm gap) as reported in Nwokocha & Olorunsola (2016). The oil was placed on the Peltier plate by means of a spatula spoon, the gap was set and the excess oil trimmed off. The sample was allowed to equilibrate for 30 s at a given temperature before measurement.

Effect of shear rate and temperature on apparent viscosity

The effect of shear rate was determined by performing a stepped flow procedure in the shear rate range 1.0 s^{-1} to 1000 s^{-1} at different temperatures, 10 - 90^{o}C. The flow characteristics were determined according to following shear stress-shear rate rheological models (Eqs. 5-9):

Herschel-Bulkley ($\sigma = \sigma_Y + \eta \dot{\gamma}^n$) (Eq. 5)

Power Law ($\sigma = \eta \dot{\gamma}^n$) (Eq. 6)

Bingham ($\sigma = \sigma_Y + \eta \dot{\gamma}$) (Eq. 7)

Newtonian ($\sigma = \eta \dot{\gamma}$) (Eq.8)

Casson ($\sigma^{0.5} = \sigma_Y{}^{0.5} + (\eta \dot{\gamma})^{0.5}$) (Eq.9)

Where σ = shear stress (Pa); η = viscosity (Pas); $\dot{\gamma}$ = shear rate (s^{-1}); σ_Y = yield stress (Pa) and n = rate index.

Oscillation studies

Oscillation stress sweep was carried out on the oil at 10^{o}C at frequency of 1 Hz in order to locate the linear viscoelastic region. A temperature sweep was carried out at temperature 70^{o}C to 0^{o}C at frequency of 1Hz and oscillation stress 0.1 Pa (oscillation stress in the linear viscoelastic region).

Fatty acid composition

Fatty acid methyl esters (FAME) were prepared by standard IUPAC method 2.301 (Paquot, 1979). 1 g oil was weighed into 50 ml round bottom flask and 5 ml of 1M methanolic NaOH added. The sample was refluxed at 70^{o}C for 20 min, cooled, and hexane and water (10 ml of each) were added. The mixture was vortex mixed for 15

min and the upper phase (hexane layer) containing the fatty acid methyl esters was recovered and analyzed by gas chromatography (Varian Chromopack, Model- CP3380, USA). 0.2 ml of the methylated sample was injected into the capillary column (VF-1ms, 30m 0.25mm 0.25µm; Part number CP8912) in the splitless mode, (injection temperature 260^{o}C, detector temperature 260^{o}C), the carrier gas was nitrogen (flow rate 30 ml/min) and flowed through the air drier at 571^{o}C, coolable oven at 100^{o}C which increases with time and the Front FID at 260^{o}C. Fatty acids were identified by comparing with the retention times of FAME with a standard 37 component FAME mixture (Resket). The components eluting from the column were detected by flame ionization detector whose signal output was captured and recorded in computer with Totalchrom software data system. Three replicate GC analyses were performed and the result was expressed as as percentage of the sum of all fatty acids in the sample as indicated in Eq. 10.

$$\%\ Fatty\,acid = \left[\frac{Fatty\,acid\ peak\ area}{\sum total\ fatty\,acid\ peak\ areas}\right] \qquad (Eq.10)$$

RESULTS AND DISCUSSION

Physicochemical Properties

The physicochemical properties of *M. oleifera* oil are presented in Table 1. The yield of *M. oleifera* seed oil was 37.9% which makes the oil economical for commercial extraction. The yield is in the range of values reported in literature (Anwar *et al*., 2005; Abdulkarim *et al.* 2005).

Table 1. Physicochemical properties of *Moringa oleifera* seed oil

Parameters	Characteristics
Colour	Light golden yellow
Yield (%)	37.9
State .at room temperature	Liquid
Acid value (mg KOH g^{-1})	9.724±0.56
Saponification value (mg KOH g^{-1})	154.5±0.81
Peroxide value (meq kg^{-1})	1.655±0.01

Mean ± standard deviation (n=3)

The acid value obtained for the *M. oleifera* seed oil (9.724±0.56 mg KOH/g) is higher than (3.8±0.28 mg KOH/g) reported by Ogbunugafor *et al*. (2011). The oil is less corrosive than pawpaw (47.12 mg KOH/g) and orange (51.4 mg KOH/g) seed oils (Audu *et al*., 2013). The acid value is outside the range (0.00-3.00 mg KOH/g) recommended for cooking oil (Oderinde *et al.,* 2009).

The peroxide assay is a predominant test for oxidative rancidity in oils and fats. A high peroxide value for any oil shows that the oil has less resistance to lipolytic hydrolysis and oxidation. The peroxide value of *M. oleifera* seed oil (1.655±0.01 meq kg^{-1}) is low and indicates less susceptibility to oxidation (Ezeh et al., 2012). The saponification value (154.5 mg KOH/g) is lower than 164.09 -171.9 mg KOH/g (Ogbunugafor *et al*., 2011; Orhevba *et al.*, 2013) but higher than 143.76 mg KOH/g reported for African pear oil. The oil could be good for soap making. Saponification value is inversely proportional to the mean molecular weight of the glycerides in the oil (Ikhuoria & Maliki, 2007).

Rheological Properties

Figure 1 shows the viscosity shear rate flow curves of *M. oleifera* seed oil at different temperatures while the flow characteristics obtained from the curves by fitting to different shear stress-shear rate models are presented in Table 2.

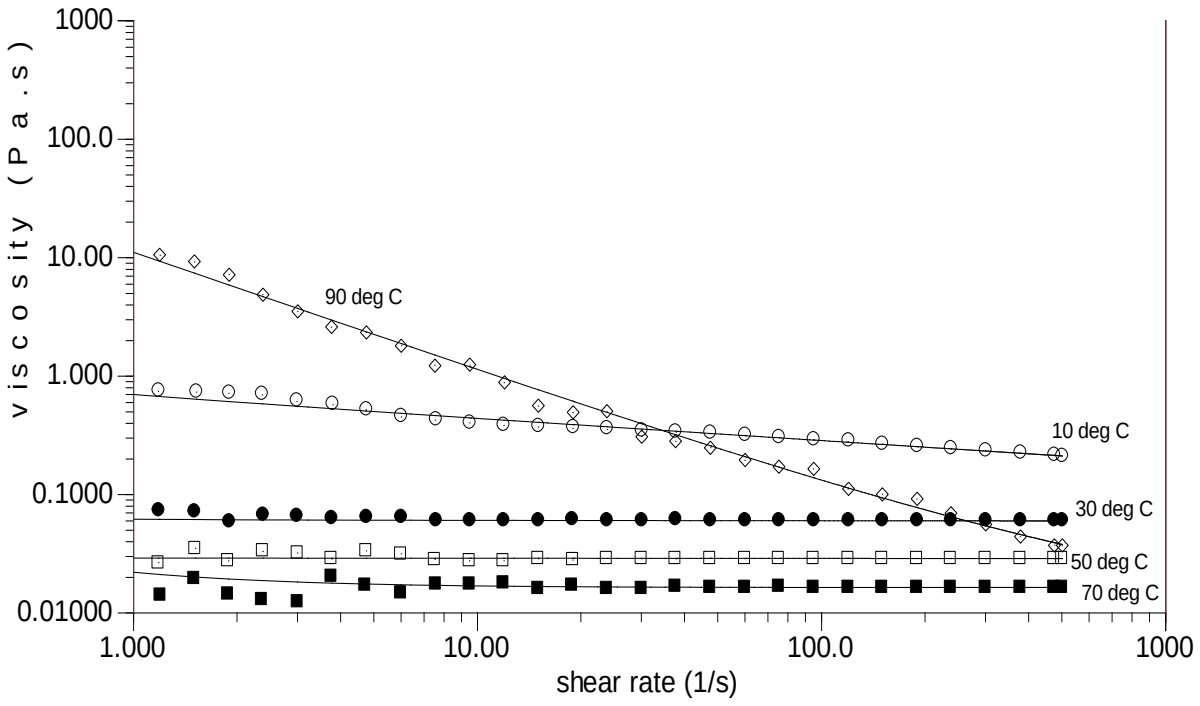

Figure 1. Viscosity- shear rate curves of *Moringa oleifera* oil at different temperatures fitted to several rheological models

Table 2. Parameters of the flow curves of *Moringa oleifera* seed oil fitted to various shear stress-shear rate rheological models

Model	Parameter	10°C	30°C	50°C	70°C	90°C
Herschel-Bulkley	σ_y	0.03131	1.198E-3	4.191E-3	5.757E-3	11.05
	η	0.6682	0.06078	0.02894	0.01634	0.05649
	n	0.8153	0.9977	0.9995	1.001	0.7956
	s.e	2.498	0.430597	0.8651	1.182	78.9488
Power law	η	0.6718	0.06082	0.02911	0.01659	11.37
	n	0.8145	0.9976	0.9986	0.9983	0.04627
	s.e	2.526	0.439333	0.8070	1.285	107.203
Bingham	σ_y	1.945	0.01011	1.839E-4	5.483E-3	11.22
	η	0.2194	0.05995	0.02887	0.01641	0.01642
	s.e	24.53	0.467213	0.8290	1.184	80.2825
Newtonian	η	0.2258	0.05995	0.02887	0.01641	0.05302
	s.e	29.72	0.562186	0.8291	1.336	557.709
Casson	σ_y	0.8723	8.185E-4	5.675E-7	3.168E-5	10.68
	η	0.1801	0.05919	0.02888	3.433E-4	1.958E-3
	s.e	12.39	1.01729	0.8489	280.7	82.2773

σ_y = shear stress (Pa), η= viscosity (Pas), n = rate index (dimensionless), s.e = standard error.

The flow curve at 10°C and 90°C were different from the other curves as they were essentially shear thinning or Non-Newtonian flow. From Table 2, it can be seen that there was a decrease in viscosity as temperature increased from 10°C to 70°C. This is because an increase in temperature results in an addition of thermal energy to a system which will increase the vibrational energy of the molecules and thereby weaken the inter- and intra-molecular associations resulting in ready flow. This is in agreement with results observed with other oils (Stanciu, 2012; Nwokocha & Olorunsola, 2016). However the change in viscosity at 90°C was unusual, instead of viscosity decreasing, it rather increased. The viscosity at 10°C was shear thinning throughout the shear rate range this is attributed to low thermal energy of the oil molecules resulting in increased intermolecular attraction and the change of state to solid- a soft solid. These associations were broken down under shear action. This can also be seen from the rate index (n) of 0.8153. It can be seen that as temperature increased up to 70°C, the rate index (n) approached unity (Newtonian). The flow profile of the oil at 90°C is one of a strongly shear thinning fluid (n= 0.7956) completely distinct from a near Newtonian behavior observed at 30 to 70°C. This transformation is indicative of thermal instability at 90°C. The transformation is attributed to thermally

induced polymerization of unsaturated carbons in the oil to a resinous mass. The reactivity and polymerization of unsaturated carbon in oil forming long chains of molecules has being remarked (Srivastava & Sahai, 2013). All the shear stress-shear rate rheological models failed to describe the flow characteristics of *M. oleifera* oil at 90^{o}C. The Casson model also failed to describe the rheological properties of the oil at 70^{o}C as seen from the high values of the standard errors of estimates which are more than 20. The high yield stress (11.05 Pa) and the shear thinning characteristic indicated by the low value of the rate index (0.7956), support the observed transformation of the oil. The instability of the *M. oleifera* oil at 90^{o}C is a limitation to its suitability as biolubricant at temperatures ≥ 90^{o}C. Nzikou *et al.*, (2009) studying the effect of temperature (5 - 45^{o}C) on the viscosity of *M. oleifera* seed oil observed a similar decrease in viscosity as temperature increased, and at 30^{o}C, the viscosity of the oil was 0.04527 Pas for Blye and Dyer (chloroform) extraction method and 0.04815 Pas for soxhlet (petroleum ether) extraction method.

Figure 2 shows the oscillation stress sweep used to obtain the linear viscoelastic region. Oscillation stress of 0.1 Pa which fell within the linear viscoelastic region- a region where the material properties of the oil were not affected by the applied stress was used for temperature sweep.

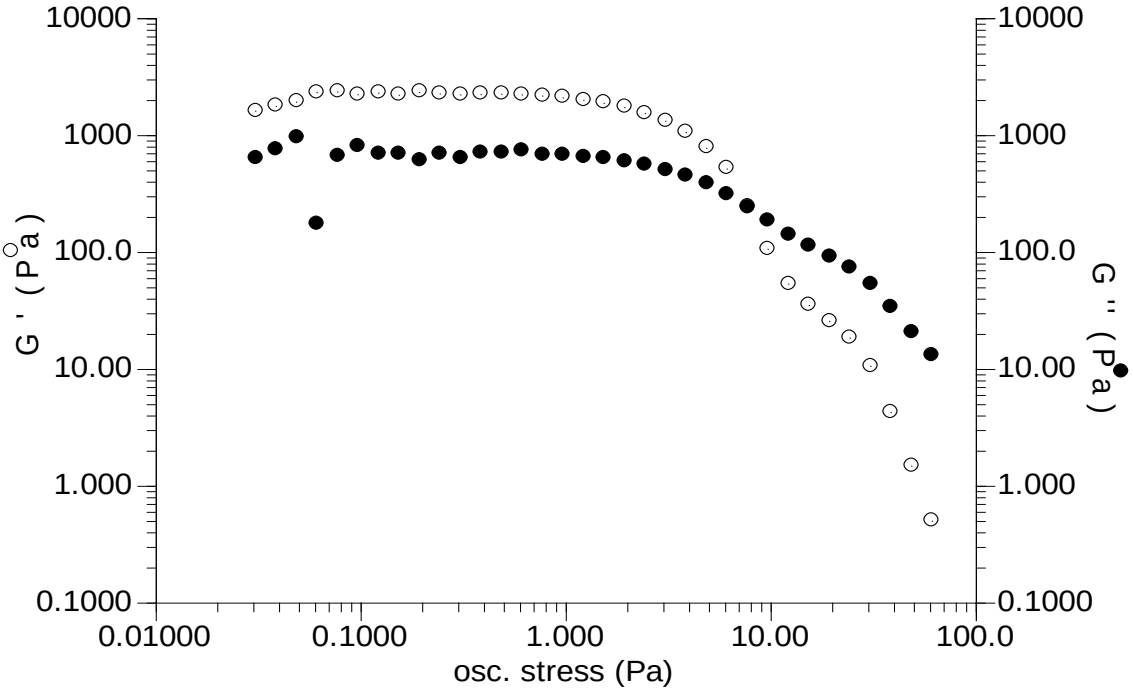

Figure 2. Elastic modulus (G′), loss modulus (G″) versus oscillation stress at 1Hz, 10^{o}C.

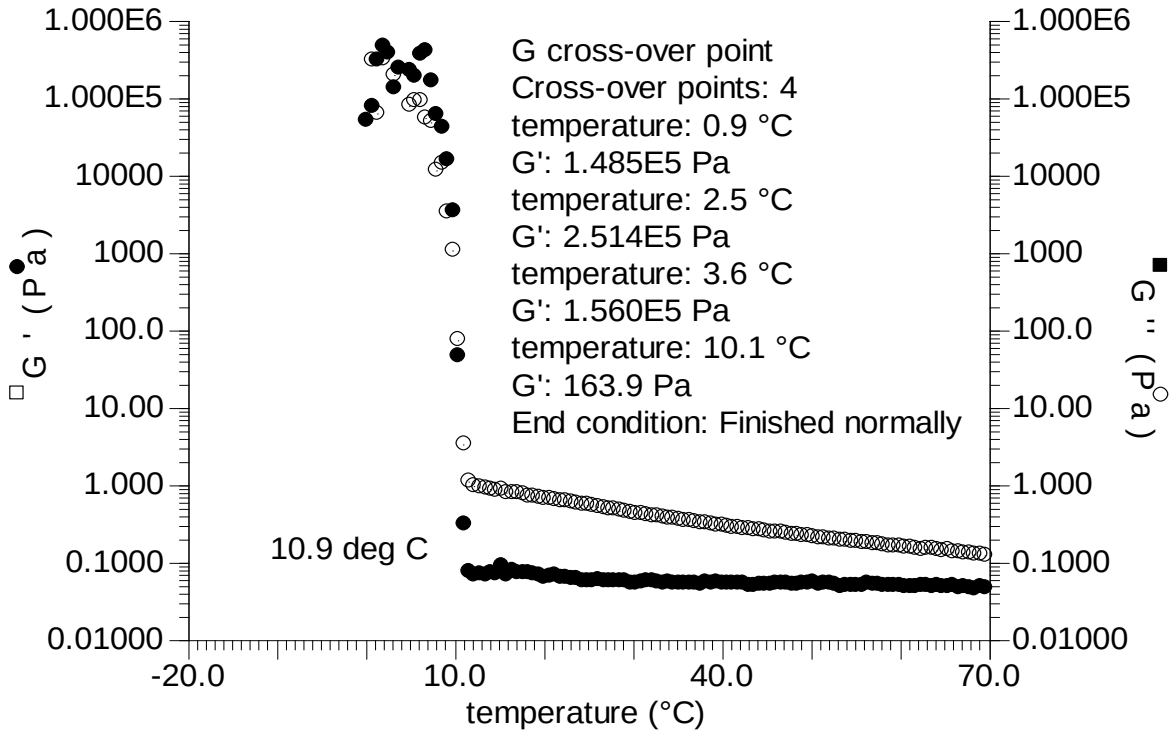

Figure 3. Temperature sweep showing elastic modulus (G′), loss modulus (G″) versus temperature at frequency of 1Hz, oscillation stress of 0.1 Pa

Figure 3 shows the elastic modulus (G′), loss modulus (G″) versus temperature for *M. oleifera* oil at frequency of 1 Hz and oscillation stress of 0.1 Pa. As temperature decreased from 70^{o}C, the distance between G′ and G″ became wider but G″ > G′ indicating the viscous nature of the oil. At 10.9^{o}C there was a sharp change in the values of the moduli with the separation becoming narrower indicating increased association between oil molecules and an approach to transition. However a crossover occurred at 10.1^{o}C (G′ = G″ = 163.9 Pa). Thus 10.1^{o}C could be taken as the melting point of the oil. Above 10.1^{o}C the oil was liquid-like (G″ > G′) but below this temperature the oil was solid-like (G′ > G″). This explains the flow characteristics of *M. oleifera* oil at 10^{o}C (soft solid) in which the oil exhibited a shear thinning property throughout. Lower temperature hardening transitions were also observed in the oil but these occurred after change of state from liquid to solid. The melting temperature of *M. oleifera* seed oil has been studied using other instrumentations: differential scanning calorimetry (DSC) (Mohammed *et al.,* 2003; Abdulkarim *et al.,* 2005; Nzikou *et al.,* 2009), WRR melting point apparatus (Ogbunugafor *et al.,* 2011). Mohammed *et al.,* (2003) reported a melting point of 20.5^{o}C for solvent extracted oil while Abdulkarim *et al.,* (2005) reported 19.0^{o}C and 18.9^{o}C for solvent- and enzyme-extracted oils, respectively. In another study, Ogbunugafor *et al.,* (2011) obtained a

melting point of 28^{o}C for the oil. In a study by Nzikou *et al.,* (2009) using DSC, they observed three different melting transitions which they attributed to the existence of different crystal forms in the oil they associated this to different degrees of saturation/unsaturation of the fatty acids. They also observed that the melting point depended on the method of extraction and scan speed in the DSC. At a scan rate 10^{o}C/min over a range (-50^{o} to 50^{o}C) they obtained the following melting transition temperatures: (petroleum ether extracted: 10.64^{o}C, -6.71^{o}C, -31.54^{o}C) and (chloroform extracted: 11.13^{o}C, -6.23^{o}C, -33.52^{o}C). The first transition in a cooling cycle for solvent extracted oil (10.64^{o}C) is close to 10.1^{o}C which we obtained by rheometry.

Fatty acid Composition

The fatty acid profile of *M. oleifera* seed oil is shown in Table 3.

The major fatty acids in order of abundance were oleic acid (72.73%), palmitic acid (7.25%), linoleic acid (5.75%) and stearic acid (4.63%). Oleic acid is the principal fatty acid in *M.oleifera* seed oil and this agrees with values (67.8 - 85%) in the literature (Tsaknis *et al.,* 1999; Abdulkarim *et al.,* 2005; Anwar *et al.,* 2006)

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Table 3. Fatty acids present in *Moringa oleifera seed* oil

Peak Name	**Fatty Acid Present**	**Retention Time**	**Fatty acid (%)**
C14:0	Myristic acid	37.228	0.14
C16:0	Palmitic acid	41.005	7.25
C16:1	Palmitoleic acid	41.757	1.50
C18:0	Stearic acid	44.925	4.63
C18:1n9c	Oleic acid	45.672	72.73
C18:2n6c	Linoleic acid	45.761	5.75
C18:3n6	Ɣ-Linolenic acid	48.846	0.51
C20:0	Arachidic acid	49.327	2.26
C18:3n3	Linolenic acid	50.235	1.46
C20:4n6	Arachidonic acid	55.311	3.47
C24:0	Lignoceric acid	63.86	0.30
Recovery			**100**

Table 4. Classification of the fatty acids in *Moringa oleifera* seed oil

Saturated Fatty Acid, SFA (%)	14.58
Unsaturated Fatty Acid, UFA (%)	85.42
Monounsaturated Fatty Acid, MUFA (%)	74.23
Polyunsaturated Fatty Acid, PUFA (n≥2) (%)	11.19
Fatty Acid (C ≥16) (%)	99.86

Table 4 shows the classification of the fatty acids in *M. oleifera* seed oil. The oil contained 85.42% unsaturated fatty acids (UFA) of which 74.23% were monounsaturated (MUFA). *M. oleifera* seed oil contained principally long chain fatty acids with C ≥ 16 of 99.86%. The thermal instability of the *M. oleifera* oil at high temperatures has been attributed to the thermal polymerization of the UFA.

Biolubricant Characteristics

The biolubricancy of a vegetable oil is determined by its viscosity and the type of fatty acids it contains. The ability of the vegetable oil to create and maintain a lubrication film between two moving metal surfaces is much dependent on the viscosity of the oil itself (Kittiwake).

The *M. oleifera* oil had a viscosity of 0.06078 Pas at 30^{o}C. This is in the range of viscosities of vegetable oils like canola, rapeseed and sunflower applied as biolubricants (Fasina & Colley, 2008; Diamante & Lan, 2014). This viscosity is higher than 4.5 x 10^{-2} Pas reported for *D. microcarpum* (C ≥ 15 =79.41%), 5.6 x10^{-2} Pas for *A. africana* (C ≥ 15 = 96%) (Nwokocha & Olorunsola, 2016). It has been reported that vegetable oils with high oleic contents are best oils as biolubricant (Singh & Chhibber, 2013). The oleic acid content of *M. oleifera* seed oil (72.73%) is high indicating a potential for application as a biolubricant. *M. oleifera* oil is a triacylglycerol with long fatty acid chains and polar groups which make it amphiphilic in character, and therefore allows it to be an excellent choice as lubricant and functional fluid. The polar ester groups in the oils possess more sites to react and adsorb with metal surfaces to provide boundary lubrication effects (Hsien, 2015). Boyde & Randley (2013) have stated that these triacylglycerol molecules in vegetable oils orient themselves with the polar end at the solid surface making a closed packed monomolecular or multimolecular layer resulting in a surface film that provides desirable qualities in a lubricant. Another advantage of long chain triacylglycerol is the very low volatility due to the high molecular weight of the triglyceride molecule and excellent viscosity properties.

From the rheological characteristics of *M. oleifera* oil, it can be seen it has limitations in application as biolubricant at low

temperatures (~10^{o}C) as the oil solidifies and forms fatty crystals and therefore would not form continuous film on metal surface. Also at temperatures ~90^{o}C the oil also fails because it polymerizes to a resinous mass. For environmental safety, *M. oleifera* oil can be used as biolubricant in machinery that loses oil directly into the environment during use like Chain saw, and in machinery used in any sensitive areas, such as in or near water (Hsien, 2015).

CONCLUSION

Moringa oleifera gave a high oil yield (37.9%), a quantity high for commercial exploitation. The oil has a melting point of 10.1^{o}C and is essentially Newtonian at 30- 70^{o}C with viscosity decreasing from 0.06078 - 0.01634 Pas with increasing temperature. It exhibited thermal instability at 90^{o}C polymerizing to gel-like material with strong shear thinning property. The major fatty acid in *M. oleifera* oil was oleic acid. The characteristics of the oil make it a candidate for biolubricant.

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