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Alternative Solvents for *Moringa oleifera* Seeds Extraction

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ABSTRACT
Moringa oleifera is a versatile plant and has wide applicability. Two critical factors that make the oil more readily available for use are its ease of processing techniques and production rate. Solvent extraction using various solvents is common and is usually more efficient than mechanical presses. Hence investigating the suitability of three solvents-hexane, Isopropyl Alcohol (IPA) and Petroleum Ether (PE) in the extraction of moringa seeds from northern (sample 1) and southern (sample 2) parts of Nigeria was carried out using a soxhlet extractor between 2 and 12 h. The percentage yield of oil from the two samples was found to be dependent on the solvent used, the residence time and the source of the seed sample. Petroleum ether gave the highest yield of 49.38 and 37.57%, next was hexane with 44.94 and 34.71% while isopropyl alcohol gave 36.39 and 28.43%, for samples 1 and 2, respectively. For all solvents, sample 1 produced higher oil yield. The percentage oil yield increased with time reaching an optimum at between 8-10 h. From chromatographic analysis, besides other trace components, the predominant fatty acids present in the *Moringa oleifera* oil include oleic, stearic, palmitic, linoleic and palmitoleic acids. The overall composition of the oil indicated higher levels of unsaturated than saturated acids with oleic acid having the highest percentage composition of 68.8% in all the extracted oil samples. The results obtained from this investigation showed that the alternative solvents (IPA and PE) considered can potentially substitute n-hexane in *Moringa* oilseed extraction.

Key words: Solvent extraction, *Moringa* oil, soxhlet extractor

INTRODUCTION

The use of vegetable oils as raw materials for industrial applications has awakened the interest of many researchers from all works of life hence, identification, characterization, development, applicability and cost effective production methods of such useful vegetable oils have been on the increase. A good number of oilseeds have been successfully developed and are currently in use for domestic and oil processing applications with many more still under-developed. Others still, have been identified but lack of information on their chemical composition limits their applications (Adejumo *et al*., 2013). In addition, due to the increasing world population pressure and its growing demand for oils/fats derived biofuels, it has become very imperative to take advantage of more and more vegetable oils being explored to meet the world's global needs on food and energy (Nielsen, 1994). *Moringa* tree originated from Agra and Oudh in the Northwestern region of India which is South of the Himalayas (Mughal *et al*., 1999; Nielsen, 1994). Among the 13 known species, *Moringa oleifera* is a high valued plant which is versatile, adaptable, easy to cultivate and self-propagating with very fast growth rate (Mayde, 1986) 50-70 kg of pods per year may be harvested from each *Moringa* tree given favorable environmental and climatic conditions (McCabe *et al*., 2005). The life span of *Moringa*

trees is only about 20 years on the average (Seth *et al*., 2007) and up to 40% oil can be extracted from it. Its fatty acid profile indicates about 70% oleic acid. Hence, *Moringa* oil can be used as an edible vegetable or cooking oil with very little tendency to deteriorate and become rancid (Von Carlowitz *et al*., 1991). *Moringa* is a good source of protein and many vital minerals. After oil extraction the seed cake remaining may be used as fertilizer (WIIAD., 1992). Over the years, a number of independent and corporate studies have been carried out to situate appropriate characterization and composition of *Moringa* seed oil (Mayde, 1986). The gas liquid chromatography of *Moringa oleifera* seed oil shows a variety of fatty acids with higher level of unsaturated fatty acids relative to saturated fatty acids. Oleic acid was predominantly the highest unsaturated fatty acids with about 74.93% (Morton, 1991). The *Moringa* oil contains all other fatty acids as contained in olive oil except linoleic acid. It has proven to be a good and acceptable substitute for olive oil (Mayde, 1986). In the past, oils were generally extracted by wrapping seeds in cloth and then using devices operated by stones and levers to exert pressure on them (Gandhi *et al*., 2003). *Moringa* oil like most other oils can be extracted by solvent extraction or by expulsion through the application of heat and mechanical pressure (Wan and Wakelyn, 1997). The use of solvents for extraction is effective as it is capable of extracting most of the oil contained in the cells of the oilseed. Its main disadvantages include the general expensive nature of the equipment used and the hazardous nature of the solvents that may often lead to fire explosion relatively (Nielsen, 1994). Although solvent extraction is relatively faster and less expensive compared to mechanical extraction process. Generally, the higher the temperature of the extraction process, the higher the rate of oil extracted and this is due to increased oil solubility in the solvent medium (Johnson and Lusas, 1983). In order to meet the world's rising demand for vegetable oils for domestic and industrial applications, it becomes imperative to source for suitable solvents which are readily available in the country at relatively cheaper costs so as to replace hexane. Over the years, hexane has been the most commonly used and preferred solvent for the extraction of oils from seeds. The reason may be because of its availability at a reasonable cost and its viable functional properties for oil extraction. Some of such properties are its non-reactivity with oil, oil micelle and extracting equipment coupled with its high solvent power for fatty acids at relatively low temperatures. However, the desire for an environmentally friendly, less hazardous, non-flammable and more efficient solvents has remained a strong motivation of interest and continued search for alternate solvents to hexane. Moreover, n-Hexane, the main component of commercial hexane with CAS registry number 110-54-3 has the following hazardous characteristics. Overall toxicity 3, overall flammability 3, destructive to skin and eye 1, inhalation reference exposure level 7000 μg m^{-3}. Normal hexane critical effects are neurotoxicity and electrophysiological alterations in humans. Its hazard index target is the nervous system (Raitta *et al*., 1978; Seppalainen *et al*., 1979). Hence, this study is aimed at finding alternative extraction solvents that are less hazardous, environmentally friendly, non-flammable and cost effective as possible replacement for hexane.

MATERIALS AND METHODS

Materials: *Moringa* seeds were collected in their harvested state from plantations in Kaduna State (sample 1) and Oyo State (sample 2) both from the North and South West of Nigeria respectively. The three extracting solvents used: Isopropyl Alcohol (purity 99.7%, BDH analyzed), petroleum ether (purity 40-60°C, J.T. Baker analyzed) and hexane (purity 99.7%, Sigma-Aldrich analyzed). All chemicals were used without further purification.

Apparatus/equipment: Soxhlet extractor (J. SIL Borosilicate), mass balance (Pioneer, Ohaus), oven (Vision Scientific), programmable refractometer (ABBE, 203 X), Agilent 6890 N (gas chromatograph, flame ionization detector), mortar and pestle (500 mL size) and a blender.

Experimental methods: The seeds were first dehulled, cleaned, sun dried and oven dried to a constant weight. They were then crushed using a mortar and pestle and finely pulverized using a blender.

The extraction procedure described by Barminas *et al*. (2001) and Aluyor *et al*. (2009) was employed to obtain the quantity of oil used for the investigation. The extraction was done at varied times of between 2 and 12 h using 240 mL of hexane, isopropyl alcohol and petroleum ether solvents, respectively. The amount of oil obtained was then weighed and stored for further analysis.

The physicochemical properties of the oil obtained from each solvent extraction were determined by methods described below.

Saponification Value (SV) of the extracted *Moringa* oil sample was determined by dissolving 1 g of the oil in 50 mL of 0.5 M ethanolic KOH in a 250 mL flat bottom flask and the mixture refluxed for 1 h. One milliliter of phenolphthalein indicator was added to the cooled contents of the flask and then titrated with 0.5 N hydrochloric acid (HCL). A blank determination was also carried out under the same condition and saponification value determined using Eq. 1 as follows:

$$S.V = \frac{Z \times M \times 56.1}{W} \quad (1)$$

Where:
SV = Saponification value of oil sample
Z = Difference in titre value of blank solution and oil sample in KOH
M = Strength of hydrochloric acid solution
W = Weight (g) of oil sample used

The factor 56.1 is the molar mass of potassium hydroxide (KOH).

To ascertain the iodine value, 0.1 g of the oil was weighed into 250 mL Erlenmeyer (iodine) flask. Twenty five milliliter each of carbon tetrachloride and hanous solution were well premixed in a flask and added to the oil in the iodine flask. The contents of the flask were well shaken to ensure complete mixing. This was then titrated using 0.1 M sodium thiosulphate solution using starch indicator. Meanwhile a duplicate solution was prepared (without the oil) and titrated with the same sodium thiosulphate and starch indicator until color changed to permanent pale yellow.

Iodine Value (I.V) was determined using Eq. 2 as follows:

$$I.V = \frac{Z \times M \times 126.9}{W} \quad (2)$$

Where:
IV = Iodine value
Z = Difference in titre value of blank solution and oil sample in hanous solution
M = Strength of sodium thiosulphate solution
W = Weight (g) of oil sample used

The factor 126.9 is the molar mass of iodine.

To find the acid value 1 g of the oil sample was dissolved in a mixture of 50 mL of 95% neutralized ethanol and 50 mL of benzene in a conical flask. The contents of the flask were shaken to dissolve the free fatty acids. This was immediately titrated 0.1 N potassium hydroxide while swirling using phenolphthalein as indicator. The end point was the appearance of a pale permanent pink colour. The acid value determined using Eq. 3 as follows

$$A.V = \frac{Z \times M \times 56.1}{W} \quad (3)$$

Where:
AV = Acid value of oil sample
Z = Titre value of KOH required to neutralize the oil solution
M = Strength of KOH solution
W = Weight (g) of oil sample used

The factor 56.1 is the molar mass of potassium hydroxide (KOH).

The Specific Gravity was measured by means of a cleaned pycnometer. The pycnometer was filled with cooled distilled water and was stoppered. It was kept in the water bath for 30 min. It was removed and properly wiped dry of water and the weight was measured. The water was thrown away, oven dried and the pycnometer was filled with oil that has been previously dried over sodium sulphate. It was stoppered and kept in the water bath for 30 min. It was then removed, wiped dry of water and then weighed again.

The specific gravity of the oil sample determined using Eq. 4 as follows:

$$\text{Specific Gravity (SG)} = \frac{\text{Weight of a given volume of oil}}{\text{Weight of an equal volume water}} \quad (4)$$

where, volume of oil sample used was 50 mL.

To find the refractive Index, the programmable refractometer was first standardized using pure distilled water whose refractive index at 20°C is 1.3330. The surface of the prisms was cleaned up with ether. Then two drops of the oil were applied at the lower prism and the prism was closed and held in place firmly. Water was passed through the jacket at 45°C. The jacket was then adjusted and with the help of the light source the readings were taken. The temperature of the prism was also read and taken.

For the peroxide value, 5 g of the oil was put in a 250 mL Erlenmeyer flask fitted with a glass stopper. Thirty milliliter of a mixture of glacial acetic acid and chloroform (3:2) was added and mixed to dissolve the oil. 0.5 mL of KI solution was added to the mixture and mixed for exactly 1 min. Thirty milliliter of water was titrated with 0.01 N sodium thiosulphate, the titrant was added slowly while shaking continuously until the yellow color was almost discharged. Five milliliter of starch was added and the titration continued, while shaking the mixture vigorously, until the blue colour was discharged. The same steps were repeated for a blank solution.

The peroxide value was calculated using Eq. 5 as follows:

$$P.V = \frac{1000 \times Z \times N}{W} \quad (5)$$

Where:
PV = Peroxide value
Z = Difference in titre value (mL) between sample and the blank solution
N = Normality of sodium thiosulphate
W = Weight of the oil sample used

Chromatographic analysis: This involved analyzing the extracted *Moringa oleifera* oil to determine the components of the oil. The standard procedures followed are described below:

Trans esterification of fatty acids to Fatty Acid Methyl Esters (FAMES): The 0.5 g of *Moringa* oil was refluxed with 5 mL of 0.5 N potassium hydroxide methanolic solution for 5 min. After the reflux, 15 mL of ammonium chloride and sulfuric acid in methanol solution was added and heated for 3 min and after the mixture cooled down, 10 mL of hexane was added and a solvent fraction was recovered using separating funnel. Then 1.5 mL of the solvent fraction containing Fatty Acid Methyl Esters (FAMEs) was dried over sodium sulfate and centrifuged at 13000 rpm for 5 min. After the centrifugation, the resultant solution was subjected to GC analysis.

The FAMEs were then analyzed on an Econo-Cap™ EC™-WAX Capillary Column (length 30 m, internal diameter 0.2 mm, phase Polyethylene glycol, film 0.25 μm, Alltech, Deerfield, IL) in an HP 5MS series gas chromatograph equipped with a flame ionization detector and an automated injector (Agilent tech model 6890A8, Wilmington, DE). Samples were injected at an initial oven temperature of 80°C held for 3 min. Then the column temperature was increased at a rate of 4°C/min to 200°C. The injector and the Flame Ionization Detector (FID) temperatures were set to 250°C. Helium 99 technique was used as the carrier gas.

Peak identification was performed by comparison of retention times of standard solutions to that of individual fatty acid standards. Fatty acids were expressed as percentage of total fatty acids.

RESULTS

Effect of extraction time on oil yield: Extraction time is one of the major factors that must be considered in solvent extraction. With the time factor, the optimum residence time required for optimum oil yield in the sohxlet apparatus is determined. Results obtained (Fig. 1-3) indicated as expected that the oil yield is time dependent.

Effect of source of seeds on yield of oil: Two *Moringa* seed samples 1 and 2 were, respectively extracted under the same set of conditions.

Effect of different solvents on yield of oil: In this study, *Moringa* oil was extracted with hexane, isopropyl alcohol and petroleum ether at times of between 2 and 12 h. The results are indicated in Fig. 1-3. The results revealed for each solvent used that the optimum oil extraction was reached in 8 h while the overall maximum oil extracted, respectively from the two seed samples was obtained in 12 h. The average percentage oil recovered from sample 1 were 44.94, 36.39 and 49.38% and from sample 2 they were 34.71, 28.43, 37.57% for hexane, isopropyl alcohol and petroleum ether, respectively.

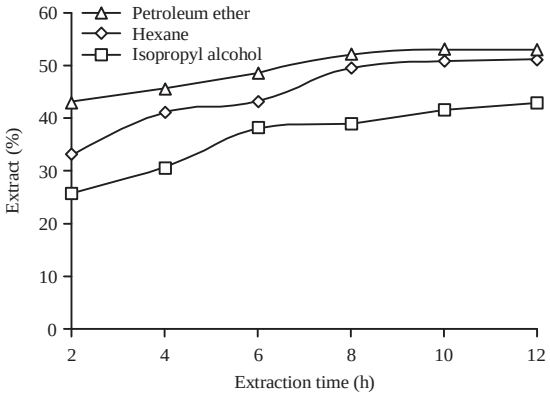

Fig. 1: Percentage oil extracted from *Moringa* sample 1 using three extracting solvents at varied extraction times

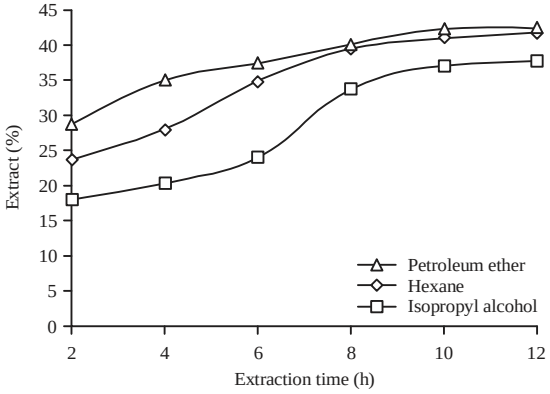

Fig. 2: Percentage oil extracted from *Moringa* sample 2 using three extracting solvents at varied extraction times

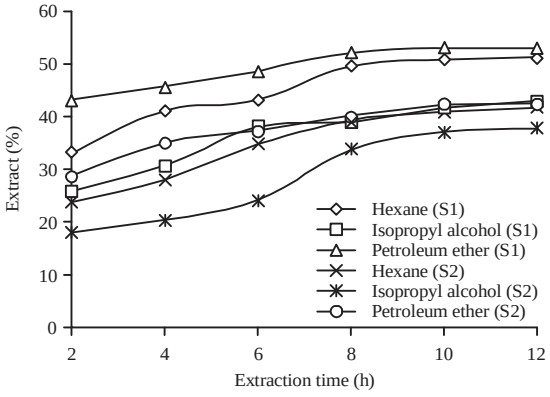

Fig. 3: Comparison of the yield of oil from seeds from different locations using the three solvents

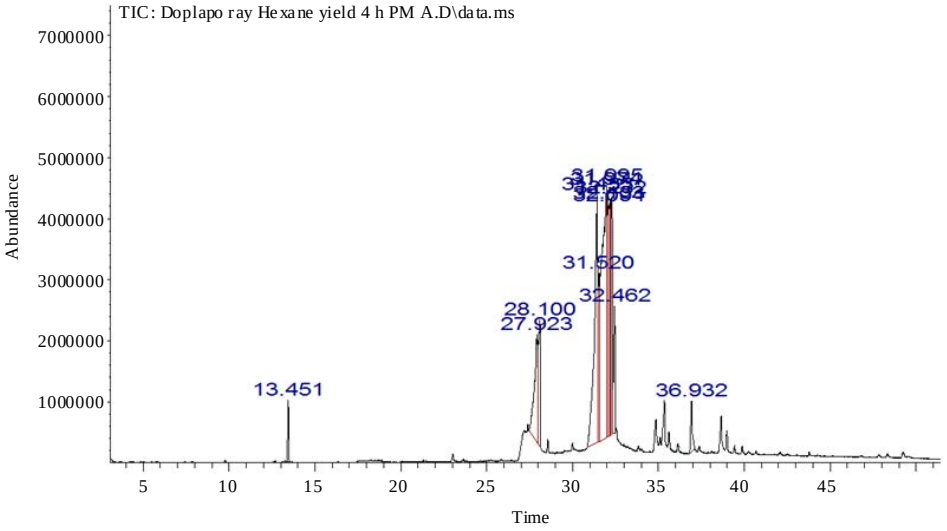

Fig. 4: Chromatographic spectrum of *Moringa* seed (sample 1) oil eluted with hexane solvent

Table 1: Physio-chemical properties of *Moringa* seed oil

Solvent used	Acid No.	S-value	p-value	I-value	Sg	Refractive index
Hex	8.98	216.76	2.10	83.75	0.90	1.4599
Hex	7.58	228.55	0.60	78.68	0.91	1.4579
IPA	7.21	260.32	0.86	91.37	0.91	1.4555
IPA	7.01	228.39	0.72	85.53	0.90	1.4522
PE	8.61	228.22	2.08	86.02	0.91	1.4556
PE	8.12	201.88	1.06	85.03	0.92	1.4543

Hex: Hexane, PE: Petroleum ether, IPA: Isopropyl alcohol, p-value: Peroxide value, S-value: Saponification value, I-value: Iodine value, Sg: Specific gravity, Acid No: Acid number

Table 2: Fatty acid composition of *Moringa* oil from *Moringa* seed samples 1 and 2 using three extracting solvents

	Sample No.								
	1	2	1	2	1	2	Literature values		
Fatty acids	Hex (%)	Hex (%)	IPA (%)	IPA (%)	PE (%)	PE (%)	Foidl *et al*. (2001)	Von Carlowitz *et al*. (1991)	Anwar and Rashid (2007)
Palmitic	18.33	18.53	18.73	7.95	81.67	10.70	5.45	5.9	6.9
Palmitoleic	6.00	-	7.56	-	-	0.36	1.48	1.1	1.1
Oleic	68.80	63.74	52.19	55.89	2.47	4.51	72.9	72.9	67.7
Stearic	5.16	5.77	8.28	18.72	6.05	-	5.42	5.1	8.3
Elaidic	-	52.96	-	6.27	-	4.51	-	-	-
Linoleic	-	-	0.38	-	5.63	64.35	0.76	0.6	0.4
Gondoic	-	-	1.10	-	-	-	-	-	-
Arachidic	-	-	3.28	-	-	-	3.39	3.6	4.7
Behenic	-	-	2.30	-	-	-	6.88	7.3	7.4
Others	1.35	11.9	6.20	17.14	-	19.98			

Hex: Hexane, IPA: Isopropyl alcohol, PE: Petroleum ether, FA: Free fatty acid

Characterization of the *Moringa* oil extracts: The *Moringa* oil samples obtained from the two *Moringa* seed samples 1 and 2 using the three extracting solvents were characterized for Saponification Values (SV), Iodine Values (IV), Peroxide Values (PV) and Refractive Index Values (RIV). The results are shown in Table 1. The chromatographic analysis of the fatty acids content of the oil samples are shown in Table 2 and Fig. 4-9.

DISCUSSION

Effect of extraction time on oil yield: From Fig. 1-3, it was observed that the oil extracted from the two *Moringa* samples generally increased with increasing time and in the order of all three solvents used in the extraction process. The increasing oil trend indicates that the oil yield has a direct linear relationship to the extraction time. This means that increasing the extraction time will bring about a higher yield of oil up to the optimum point. This was expected as prolonged intimate contact between seed samples and the respective solvent will result in more oil extraction. A steady increase in the yield of oil was obtained within the interval of 2-8 h. But there was no significant oil yield after 8 h. The difference between the overall maximum at 12 h and the optimum value at 8 h as obtained from the respective seed samples, extracting solvents and times was only nominal. This also was expected because

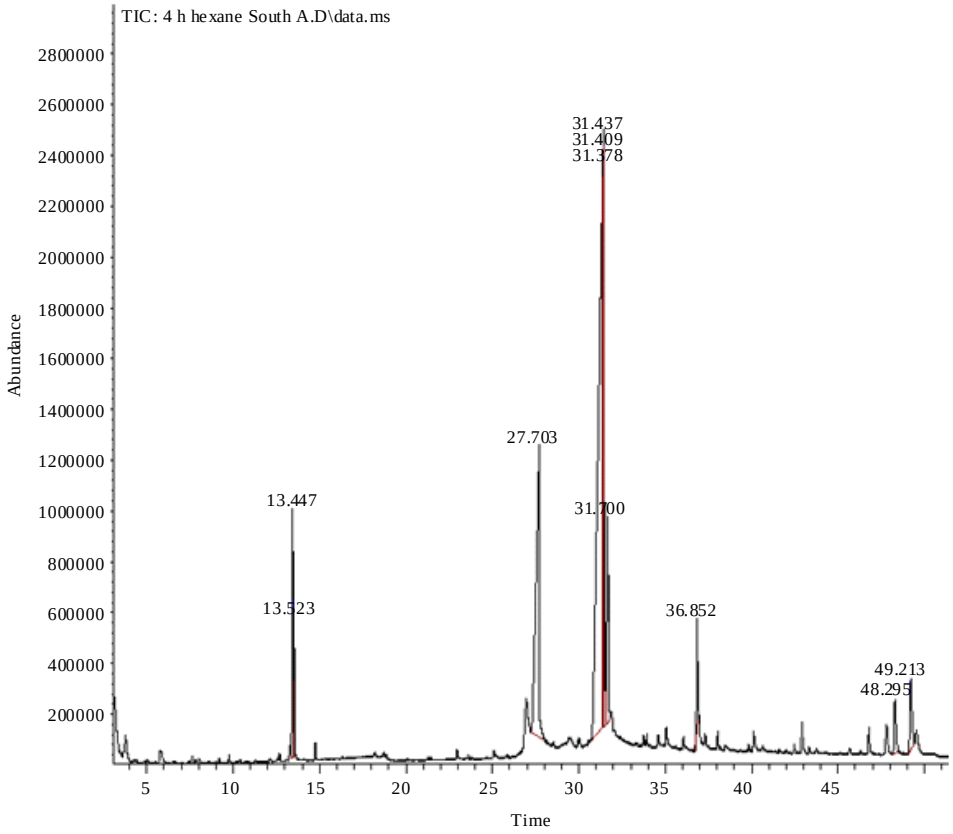

Fig. 5: Chromatographic spectrum of *Moringa* seed (sample 2) oil extracted with hexane solvent

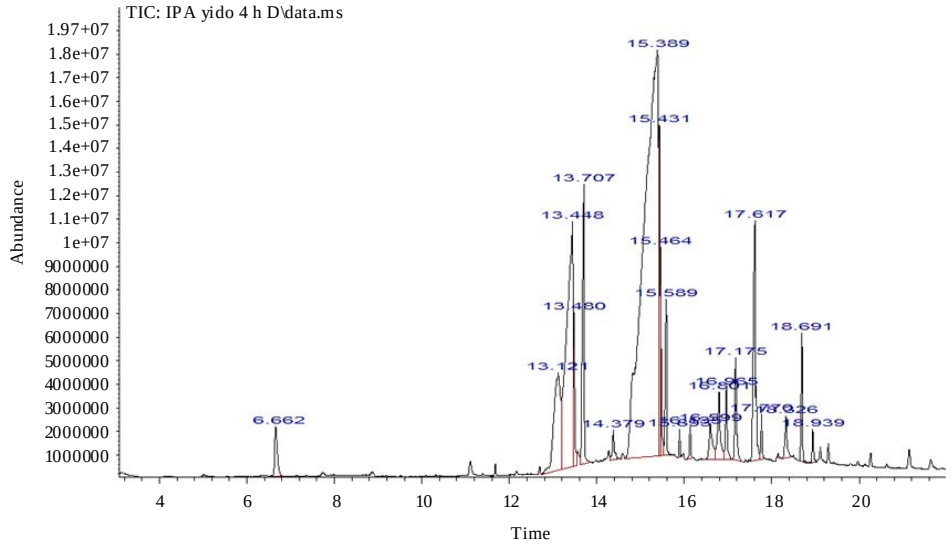

Fig. 6: Chromatographic spectrum of *Moringa* seed (sample 1) oil extracted with isopropyl alcohol solvent

after the optimum point, any further increase in extraction time resulted in decreasing oil yields until the seed sample was completely spent. For sample 1 the additional oil yields after 12 min using hexane was 1.6%, with IPA it was 2 and 0.9% using petroleum ether. Whereas, for sample 2 using hexane it was 2.4%, with IPA it was 3.6 and 2% using petroleum ether. This means that if the cost of solvent extraction (i.e., time and energy) is to be considered, the optimum conditions must be used. The nominal oil yield after this optimum point bears a negative cost on the overall extraction cost. Hence, extraction

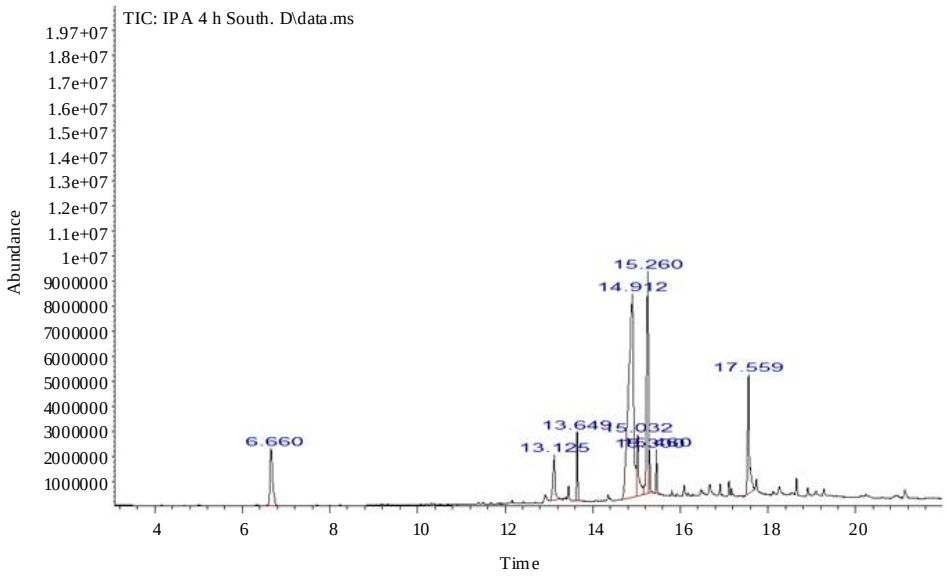

Fig. 7: Chromatographic spectrum of *Moringa* seed (sample 2) oil extracted with isopropyl alcohol solvent

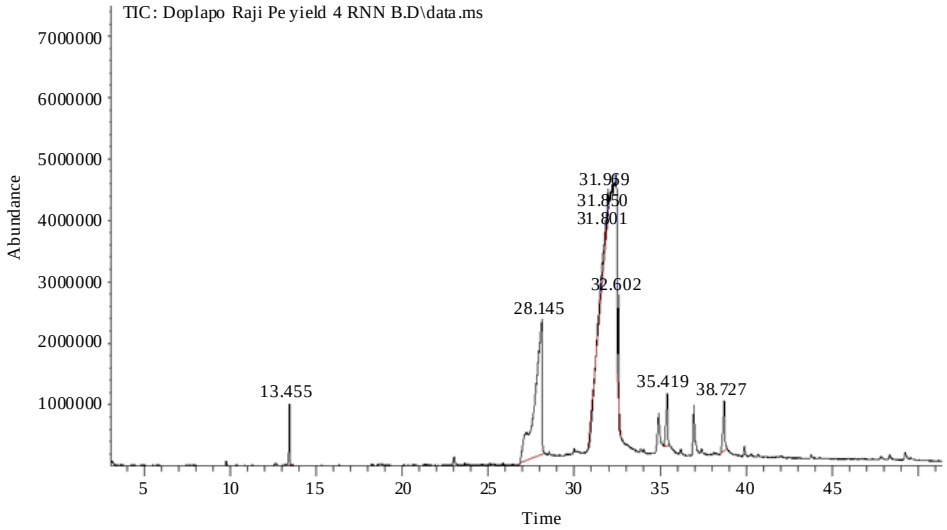

Fig. 8: Chromatographic spectrum of *Moringa* seed (sample 1) oil extracted with petroleum ether solvent

time may be successfully optimized within 8-10 h. The high rate of extraction observed at the early stages, may be due to the high solubility of the oil in the freshly charged solvent and the high concentration of the oil at the solid surface. The freshly charged solvents (oil lean), created a positive gradient or the needed driving force that resulted in higher mass transfer rate of oil in to the extracting solvents. After the optimum point has been reached, the extracting solvents (oil rich) give rise to lower driving force or slower extraction rates.

Effect of source of seeds on yield of oil: The percentage yields of oil from samples 1 and 2 (samples from the North and South of Nigeria, respectively) have been indicated in Fig. 1-3. The yield from sample 1 was observed to be higher than that of sample 2 for all solvents used. From Fig. 3, sample 2 (southern Nigeria) gave lower oil yield as depicted by the three lower curves compared to the upper curves of seed sample 1 (from the North). This suggests that geographical location affects oil yield and may vary from one location to the other. The differences may be attributed to prevailing weather and environmental conditions, soil type, nature of seeds, oil content and handling of harvested seeds. The southern part of Nigeria is known to have higher annual centimeters of rainfall than the northern part. Hence, seeds from the south will tend to contain higher moisture level which may in turn lead to lower oil concentration in the seeds.

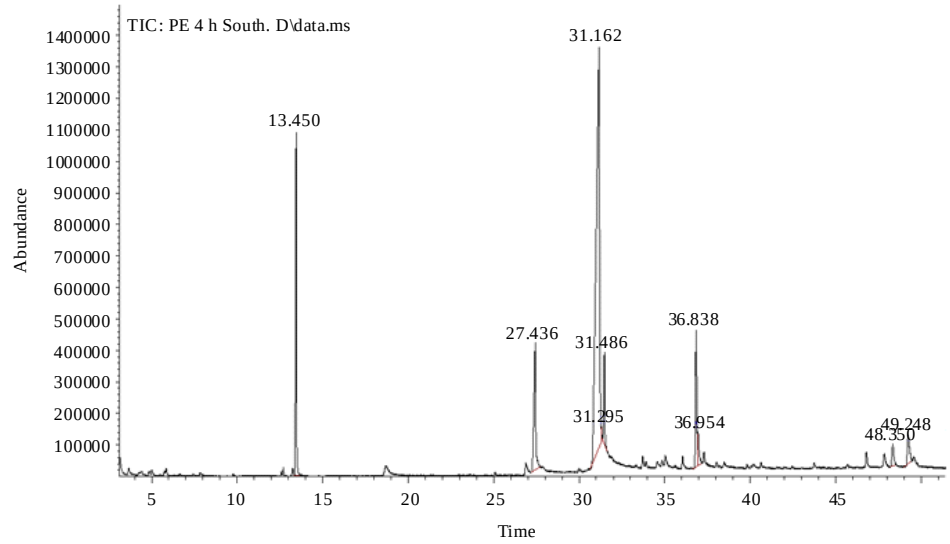

Fig. 9: Chromatographic spectrum of *Moringa* seed (sample 2) oil extracted with petroleum ether solvent

Effect of different solvents on yield of oil: Figure 1-3 indicate the results of extraction of *Moringa* seed samples using three different solvents – hexane, isopropyl alcohol and petroleum ether within a time range of 2-12 h. It was observed that maximum oil was extracted in 12 h but the optimum extraction was reached within 8 h for each solvent. The mean maximum oil recovered from sample 1, as given in earlier, were 44.94, 36.39 and 49.38% for hexane, isopropyl alcohol and petroleum ether, respectively while from sample 2 they were 34.71, 28.43 and 37.57% in the same order. Petroleum ether gave the highest oil yields, followed by hexane. Separation of the extracted oil from isopropyl alcohol resulted in the formation of a light orange jelly-like product on the surface of the oil which is believed to be responsible for the low oil yields using IPA. Charring of the oil was also noticed during solvent (IPA) recovery process that. This may be due to the fact that IPA being an alcohol is not an inert solvent to vegetable oils compared to the other two relatively inert solvents. At higher temperatures, by-products resulting from possible hydrolysis or trans-esterification of the oil in the presence of IPA may have been formed. From the physicochemical analyses carried out for the extracted oils using the three solvents, it was observed that the quality of the oils obtained was about the same. The only obvious difference was in the color of the oil extracted with IPA which was darker than the oils extracted using hexane or petroleum ether. Thus Isopropyl alcohol use for the extraction of oil for consumption should be properly investigated and analyzed to determine its ultimate application. For petroleum ether, its oil extract could be directly used for biodiesel production or for some other industrial applications because it is not a food grade solvent.

Characterization of the *Moringa* oil extracts: The *Moringa* oil samples obtained from the two *Moringa* seed samples 1 and 2 at a constant extraction time of 4 h each was selected for the characterization studies (Table 1-2 and Fig. 4-9).

Physicochemical properties of the oil from *Moringa* samples: Table 1 gives the summary of the physicochemical properties of the oil extracted from the two *Moringa* seed samples using hexane, isopropyl alcohol and petroleum ether as the extraction solvents.

The extracted oil saponification values obtained were 216.76, 260.32 and 228.22 mg KOH g^{-1} oil from sample 1 and 228.55, 228.39 and 201.88 mg KOH g^{-1} oil from sample 2 using hexane, isopropyl alcohol and petroleum ether as extracting solvents respectively. The seed sample 1 from the North had oil extracts with higher saponification values while seed sample 2 from the South had lower but close values. They compared favourably with the range of saponification values reported in literature for different samples of *Moringa* (181.1-252.34) (Adejumo *et al*., 2013; FAO/WHO., 2009) except for oil sample 1 extracted with IPA that gave SV of 260.32 mg KOH g^{-1}.

The acid values obtained were 8.98, 7.21 and 8.61 mg KOH g^{-1}, for oil from *Moringa* sample 1 using hexane, isopropyl alcohol and petroleum ether, respectively while 7.58, 7.01, 8.12 mg KOH g^{-1} were obtained for oil from *Moringa* sample 2 using the solvents in the same order. The values obtained were relatively higher than those quoted in literature 3.8-5.0 mg KOH g^{-1} (Ogbunugafor *et al*., 2011; Natural Sourcing, 2012), but closer to 5.78-7.28 (FAO/WHO., 2009). Higher acid values indicated that the *Moringa* seed samples 1 and 2 may already have been aged to

some extent before their extraction. Also, improper handling and storage of the extracted oil may have initiated the breakdown of the unsaturated fatty acids by hydrolysis or oxidation.

The peroxide values of the oil samples 1 and 2, varied with all the parameters of extraction. They were 2.10, 0.60, 0.86 for sample 1 and 0.72, 2.08 and 1.06 for sample 2 but fell within the literature values 8.1 meq kg^{-1} (Ogbunugafor *et al*., 2011) and the standard specifications (10 meq kg^{-1}) by FAO/WHO (2009). In general, as rancidity develops the peroxide value increases. The lower peroxide values are only indicative of higher stability to oxidation. Therefore, the best oil samples in terms of resistance to rancidity are the oil yield extracts using IPA (North and South) and that using hexane for south.

The iodine values obtained from oil sample 1 were 83.75, 91.37 and 86.02 g g^{-1} while for sample 2 they were 78.68, 85.53 and 85.03 g g^{-1} using Hexane, IPA and Petroleum ether, respectively. The range of iodine values as quoted in literature are in the range of 65.75-69.45 g g^{-1} of oil (Anwar and Rashid, 2007; Foidl *et al*., 2001) 66.63-72.40 g g^{-1} (Adejumo *et al*., 2013). The values gotten were significantly less than the values obtained from this research but fell within the standard values (80-106 g g^{-1} of oil) (FAO/WHO., 2009). These iodine values gave the indication of high level of unsaturation in the *Moringa* oil samples. The iodine values were slightly higher in oil extracts from sample 1 than sample 2. Higher iodine value of oil may render it unstable, free for oxidation and susceptible to hydrolysis which ultimately results in reduced shelf life of the oil.

The specific gravities of the oil from the two seed samples 1 and 2 ranged between 0.9-0.92. A comparison with the literature value of oil (0.89737-0.9066 at 20-25°C) showed that the results obtained from the extracted *Moringa* samples are slightly higher than the acceptable limits. However, they fell within the specification range of 0.9-1.16 (FAO/WHO., 2009).

The refractive index values obtained fell within the range of 1.4522-1.4599 which is slightly above the literature value, 1.4570, the oil extract from *Moringa* seed sample 2 using hexane gave a refractive index of 1.4579 which is very close to literature values of 1.4574 Pakistani breed, 1.454 Kenyan breed (Lalas and Tsaknis, 2002) and 1.474 South Eastern Nigerian breed (Ogbunugafor *et al*., 2011).

Chromatographic analysis of *Moringa* seed oil: Fatty acids content of *Moringa* oil (Table 2) was obtained from the chromatographic analysis displayed in Fig. 4-9. Table 2 compares the abundance or percentage of the fatty acid compositions of *Moringa* oil as obtained from the two *Moringa* seed samples 1 and 2. The results were compared with those reported in literature (Table 2). *Moringa* oils extracted from both seed samples were found to be composed mainly of Palmitic, Palmitoleic, Stearic, Oleic and Linoleic acids. Palmitic and Oleic acids dominated the fatty acid groups especially for oils obtained using hexane and isopropyl alcohol extractions. From Table 2 and the chromatogram (Fig. 4-9) significant variations from quoted literature values in the fatty acid contents were observed. These may be attributed to the extracting solvent medium and origin of the *Moringa* seed sample. Palmitic acid was common and similar for all categories of values obtained but significantly different from the three literature values quoted. Elaidic acid has not been reported in literature as a component of *Moringa* oil. Elaidic acid is a monounsaturated fatty acid that has the same structure as oleic acid except that it is a trans-fatty acid which is the major fatty acid in margarine and fried foods. Saturated fatty acids such as stearic acid seem to be the only acid that correlates with literature value. Palmitoleic acid is trivially present in the extract from seed sample 2 using hexane and almost present in negligible quantity in sample 2 when petroleum ether was used; however, these were relatively higher than those quoted in literature (Anwar and Rashid, 2007).

Linoleic acid composition obtained from seed sample 1 using IPA was relatively close to the literature value. It was noticed that linoleic acid was absent from the sample extracted with hexane for both seed samples 1 and 2. Arachidic acid and Behenic acid were only detected in the oil extract from seed sample 1 using IPA solvent. Both were slightly lower in content when compared to literature values. Gondoic acid was also detected in the oil from seed sample 1 extracted with IPA but absent in all others including literature references (Anwar and Rashid, 2007).

CONCLUSION

The extraction and characterization of *Moringa* seed oil using three different extracting media reveal that.

The yield of oil from *Moringa* seeds by solvent extraction is dependent on residence time, type of solvent used and geographical source of seeds.

The percentage yield of the extracted oil increased with time reaching an optimum between 8-10 h with the highest oil yield reached at 12 h for each sample.

The mean percentage of oil yield from seed sample 1 (from the North) were 44.94, 36.39 and 49.38% while from seed sample 2 (from the South) were 34.71, 28.43 and 37.57% for Hexane, Isopropyl Alcohol and Petroleum Ether, respectively.

Higher percentage oil yield was recovered from seed sample 1 than those from seed sample 2. Petroleum Ether gave the highest oil yield, followed by Hexane then Isopropyl alcohol. The Saponification value, acid value, specific gravity and refractive index were similar for both samples. Peroxide values were low which indicate that the oil is less liable to oxidative rancidity at room temperature.

The primary fatty acids present in the sample *Moringa oleifera* are oleic acid, stearic, palmitic, linoleic and palmitoleic acid aside from others in trace amount.

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