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Table of Contents: Volume 12 Number 16 30 August, 2017

ARTICLE

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Full Length Research Paper

Effect of sonication at different ultrasonic frequencies on the quality and quantity of fatty acids of the oil of *Chlorella vulgaris*

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In order to investigate the effect of sonication on the fatty acids of alga oil, oil of *Chlorella vulgaris* was extracted by sonication at 20, 40, 60 and 80 kHz. Oil extracted by refluxing alga cells in n-hexane was used as control. Probable changes in the structures of the fatty acids were investigated using gas chromatography and Fourier transform infrared spectroscopy (FT-IR). The retention profiles of the chromatograms were similar with differences observed only in peak heights. The FT-IR spectrum of the hexane extract was similar to the spectra of oil extracted at 20 and 40 kHz. The FT-IR spectra of oil extracted at 60 and 80 kHz were identical but different from the other spectra. Peaks at about 1560 cm^{-1} which were prominent in the 60 and 80 kHz spectra but absent from the other spectra were assigned to non-oil lipids such as steroids. It was concluded that extraction of oil from *C. vulgaris* by sonication at 20, 40, 60 and 80 kHz had no degradative effect on the structure of the fatty acids of *C. vulgaris.*

Key words: Alga, triglycerides, extraction, sonication, chromatography, spectroscopy.

INTRODUCTION

Due to the economic and environmental problems associated with the exploration and exploitation of petroleum, triglycerides have been receiving attention as sources of chemical substances and allied materials erstwhile obtained solely from petroleum. Biodiesel prepared from vegetable oils is used as fuel in compression engines. The biodiesel is used neat or blended with petrodiesel. Resins for various applications are obtained from triglycerides containing unsaturated fatty acids. Vegetable oils used as alternatives to petroleum as sources of chemicals have been obtained largely from plants. Soy, linseed, Jatropha, palm, coconut, cotton and walnut are examples of such plants. In recent times, triglycerides from algae, particularly microalgae had been identified as viable sources of fuel and chemicals (Satyanarayana et al., 2010). Microalgae have some advantages over plants. Alga biomass can double within 3 to 5 h of culturing, allowing all year round production and oil yields in excess of the best oilseed crops for the same area of cultivation.

Oil content can be as high as 70% by weight of dry biomass (Milner, 1948) and can be varied by varying the

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composition of the culture medium. Algae can be cultured anywhere with less nutrient, effort and input compared with oilseed crops. Algae cultivation does not require herbicides and insecticides (Lee et al., 2009; Christi, 2007). For functionalisation purposes, the high degree of unsaturation of fatty acids of algae oils exceeds those commonly found in seed oils. This makes algae oils attractive as raw materials for the synthesis of rigid polymers with high degree of cross-linkages (Meier et al., 2007; Bunsel and Renard, 2005; Khot et al., 2001).

In contrast to these advantages is the difficulty encountered in extracting oil from algae. Soxhlet method has been found not to be effective for extraction of oil from some microalgae due to their tough cell walls which are not easily percolated by solvents (Mercer and Roberto, 2011). Microalgae cells are so small that they pass through the pores of membranes used in holding materials to be extracted with non-polar solvents such as hexane. Attempts to overcome this problem had led to the development of several methods for the extraction of oil from algae cells. Many of the methods involved using physical means to rupture the cell wall. From the ruptured cells, oil is released into a low boiling solvent such as hexane. The quantity of hexane or solvent used in these cases is usually small compared with the quantity used in solvent extraction.

Mechanical pressing is one of the physical methods employed. This involves rupturing the cells by applying pressure (Shen et al., 2009). In milling, small beads are used to agitate and rupture the cells. Sonication entails rupturing the cells with ultrasonic waves (Budi et al., 2010; Cravotto et al., 2008). Microwaves have also been used in rupturing the cells (Miri et al., 2011). Conventional solvent extraction method has been used with hexane as the solvent of choice (Cravotto et al., 2008). Supercritical carbon dioxide has been used for extraction at high temperature and pressure leaving neat oil as the fluid vaporised at room temperature and atmospheric pressure (Mendes et al., 2005). Gradient solvent extraction using chloroform, methanol and water is the Bligh and Dyer method (Bligh and Dyer, 1959).

Many reports have been published on these methods. However, it has been observed that the effect of these methods on the chemical stability of valuable compounds prone to alteration, for example, the unsaturated fatty acids, has not been investigated (Mercer and Roberto, 2011). Such an investigation, focusing on sonication, is the purpose of this work. As earlier highlighted, sonication is one of the physical means of rupturing tough alga cells in order to aid oil extraction. Using this method, Budi et al. (2010) reported extraction of oil from *Nannochloropsis* sp. In that work, the optimum conditions for the extraction of oil from the alga cells were determined. The optimum extraction temperature was found to be 35°C and the optimum time was 8 min under agitation at 40 kHz.

There were other reports as highlighted above, but extensive literature search did not yield any report on the effect of sonication on the quality or quantity of fatty acids of oils extracted from alga cells. The effect of sonication on the fatty acid moiety of alga oil can be investigated with gas chromatography and fourier transform infrared spectroscopy. Gas chromatography has been used extensively to study composition of vegetable oils. Dispersive infrared spectrophotometer has been observed to give similar infrared spectra for different variety of oils (Wolff and Miwa, 1965). This was responsible for its unpopular choice for the study of fats and oils. However, Fourier transform infrared spectrophotometry (FT-IR) is being increasingly employed in analysis of oils. Guillen and Cabo (1997) used the subtle dissimilarities between infrared spectra of oils to provide information about their composition. They used equations obtained from frequency of absorption bands of oils and composition data to predict the proportion of saturated, monounsaturated and polyunsaturated acyl groups in oil and lard. Akintayo (2007) and Akintayo et al. (2015) employed FTIR spectroscopy in characterizing vegetable oils and their derivatives using the absorption frequencies to confirm the success of conversions.

This work reports the investigation of the effect of sonication on the quality and quantity of fatty acids of oil extracted from the cells of *Chlorella vulgaris* using gas chromatography and infrared spectroscopy as the methods of analysis.

MATERIALS AND METHODS

C. vulgaris

The sample cultured was obtained from the Department of Microbiology, University of Port-Harcourt, Nigeria. The cells were cultured in graduated 5 L white, transparent open plastic buckets using Bangladesh I mixture as the culture medium. Ten containers were used. The containers were covered with white fine-mesh nets to keep extraneous particles away from the culture. The content of each container was stirred at least thrice daily using different plastic stirrer for each container. In preparing the Bangladesh I medium, the concentration of the substances/1000 cm^3 aqueous solution were $NaHCO_3$/3.2 g, $NaNO_3$ /0.5 g, KCl/0.2 g, $MgSO_4.2H_2O$/0.1 g, $CaCl_2$ /0.008 g, $FeSO_4.7H_2O$/0.002 g, H_2SO_4 (96%)/0.1 cm^3, H_3PO_4 (85%)/0.4 cm^3, EDTA/0.008 g. The culture received about 11 h of sunlight daily. The cells received carbon from decomposing sodium bicarbonate and atmospheric carbon dioxide. During the two months culturing period, the volume of the medium was kept constant at 5 L by regular replenishment with freshly prepared medium. The cell aggregates were removed regularly, air dried at room temperature and finally dried to constant weight in an air-draught oven at 60°C. The dry cells were stored in covered plastic bottles.

Solvent extraction

1.0 g dry *C. vulgaris* was extracted at 69°C continuously by refluxing in n-hexane for 1 h. The mixture was cooled and separated by centrifugation. The solvent layer was decanted and

the residue was mixed with n-hexane and vortexed. The washing of the residue was conducted twice. The solvent-oil portions were combined and concentrated by vacuum distillation using rotary evaporator. The proportion of oil obtained, an average of three determinations with their standard deviation was 60± 0.34 of dry mass.

Sonication

1.0g dry *C. vulgaris* was measured into a beaker. 5 cm^3 n-hexane was added. The cells were agitated with ultrasonic waves supplied through a diaphragm having the same diameter as the beaker, thereby effectively covering the beaker and delivering the required energy. The ultrasonic waves were generated by a signal generator manufactured by Bridage Scientific Instrument, Nottingham, UK. The waves were delivered for five minutes at 37°C. This protocol was used for four extractions at 20, 40, 60 and 80 kHz. The oil was recovered using centrifugation and washing with solvent as described in the previous section. Oil yield for each method, as the mean of three determinations with their standard deviations were respectively 34±0.15, 36± 0.08, 41±0.17 and 45±0.16.

Fatty acid analysis

A*nalysis by gas chromatography*

Fatty acid profile was determined using GC instrument model HP 6890 powered by HP ChemStation Rev. A 09.01(1206) software. The fatty acids were determined as methyl esters. The fatty acid methyl esters (FAME) were prepared by mixing 20 mg of the oil with 2 cm^3 of toluene followed by addition of 2 cm^3, 1.5% sulphuric acid in dry methanol. The mixture was stirred and incubated at 55°C overnight. 4 cm^3 saturated solution of sodium chloride was added and vortexed. 2 cm^3 HPLC grade hexane was added followed by addition of 3 cm^3, 2% $NaHCO_3$. The mixture was vortexed. 180 µl of the upper phase was taken for GC analysis under the following conditions: split injection with split ratio 20:1; carrier gas – nitrogen; inlet temperature -250°C; HP innowax column with dimension 30 m x 0.25 mm x 0.25 µm; oven initial temperature was set at 60°C, first ramping at 12°C/min for 20 min maintained for 2 min, second ramping at 15°C/min for 3 min maintained for 8 min. The temperature of the FID detector was 320°C. The pressure of hydrogen and compressed air were respectively 22 and 35 psi. The reliability of the data generated was high with the correlation curves of the various FAME standards having correlation coefficients r = 0.99832 - 0.99927.

Infrared spectroscopy

The oil samples were analysed by FTIR using spectrophotometer model NICOLET iS5 manufactured by Thermo Scientific.

RESULTS AND DISCUSSION

60% oil was extracted from the cells by refluxing the cells in hexane for 1 h. The reflux temperature of about 69°C and the turbulence of the boiling solvent for 1 h combined to ensure efficient extraction of oil from the cells. Centrifugation, followed by vacuum distillation afforded maximum recovery of oil from the mixture of oil, solvent and residue of cells. This method introduced turbulence at elevated temperature into the solvent extraction system. Effect of turbulence on the cells is absent from conventional soxhlet extraction method. This is the probable explanation for the high quantity of oil obtained through the solvent extraction method used in this work.

Sonication was carried out at room temperature of 37°C. This temperature was used partly in compliance with the principle of green chemistry that experiments be conducted as much as possible at ambient condition and partly due to its closeness to the optimum temperature of 35°C used in extracting oil from another alga cells by Budi et al. (2010). The fixed condition of 5 min extraction time used in this experiment and the extraction temperature of 37°C were appropriate for the aim of the experiment as shown by the different features of the infrared spectra of the oil samples extracted at the various frequencies. As discussed later, the spectra showed profiles of oil with different content of extraneous substances.

Table 1 depicts the GC results of the proportions of identified fatty acids in the *C. vulgaris* oil samples obtained by the different protocols. The retention profile of all the samples is qualitatively identical. This means that there was no change in the structure of fatty acids in all the samples though they were obtained under different conditions. Docosahexaenoic acid (C22:5), detected in *C. vulgaris* oil by Otles and Pire (2001) was not detected in these samples. This is in agreement with the observation that algae are capable of producing oil with different quality depending on culturing conditions.

As earlier stated, the calibration curves of the FAMEs follow linear relationship with highly significant ($P < 0.001$) coefficient of determination ($r^2 = 0.99$) making the data in Table 1 a precise reflection of the relative quantities of the various fatty acids in the oil samples. Quantitatively, there were differences in the concentrations of particular fatty acids in the various oil samples. This may be important depending on the intended application of the alga oil. For instance, if oil sample is required for resin synthesis, unsaturated fatty acids (UFA) will be needed more than saturated fatty acids (SFA). Total UFA extracted by sonication at each of the frequencies was higher than the total UFA extracted by soxhlet. Under the experimental conditions, the highest proportion of UFA (56.11%) was extracted at 60 kHz. For use as biodiesel where higher proportion of SFA is desirable, extraction by reflux in hexane will give better oil sample. 47.04% total SFA was extracted by this method. For specific extraction of certain fatty acids, the results show the method that will give the maximum yield under the experimental conditions. The omega-3 fatty acid, linolenic acid, would be extracted in highest quantity by refluxing the cells in hexane while oleic acid will be obtained in the highest yield using sonication at 40 kHZ. While the GC data provided information on the relative quantities of fatty acids in the oil samples, the FT-IR spectra gave insight into the quality of the oil samples.

The infrared spectroscopic absorptions of C-O stretch

Table 1. Percentage composition of fatty acids in *C. vulgaris* oil samples obtained by hexane extraction and sonication at various ultrasonic frequencies.

Fatty acid	Extraction method				
	Solvent	Sonication (kHz)			
	Hexane	20	40	60	80
Lauric acid (C_{12}:0)	0.73	0.82	0.66	0.67	0.65
Myristic acid (C_{14}:0)	5.09	9.81	9.58	7.45	8.96
Hexadecadienoic acid (C_{16}:2)	2.58	2.16	2.11	2.04	1.90
Palmitic acid (C_{16}:0)	30.59	27.21	28.26	27.23	27.99
Palmitoleic acid (C_{16}:1)	4.55	3.05	3.02	3.81	3.42
Margaric acid (C_{17}:0)	2.91	1.94	1.75	2.23	1.91
Stearic acid (C_{18}:0)	3.91	2.75	2.65	3.38	3.83
Oleic acid (C_{18}:1)	26.30	37.47	37.75	35.19	34.58
Linoleic acid (C_{18}:2)	9.09	6.62	6.89	8.46	9.68
Linolenic acid (C_{18}:3)	7.92	2.78	2.75	3.42	2.38
Arachidic acid (C_{20}:0)	1.59	1.07	0.96	1.21	0.68
Arachidonic acid (C_{20}:4)	0.40	0.25	0.12	0.18	0.19
Eicosapentaenoic acid (C_{22}:3)	1.41	2.09	2.03	2.55	2.63
Behemic acid (C_{22}:0)	1.40	0.99	0.95	1.19	0.69
Erucic acid (C_{22}:1)	0.71	0.42	0.34	0.46	0.28
Lignoceric acid (C_{24}:0)	0.82	0.58	0.40	0.54	0.24
Total saturated fatty acids (SFA)	47.04	45.17	45.21	43.90	44.95
Total unsaturated fatty acid (UFA)	52.96	54.84	55.01	56.11	55.06

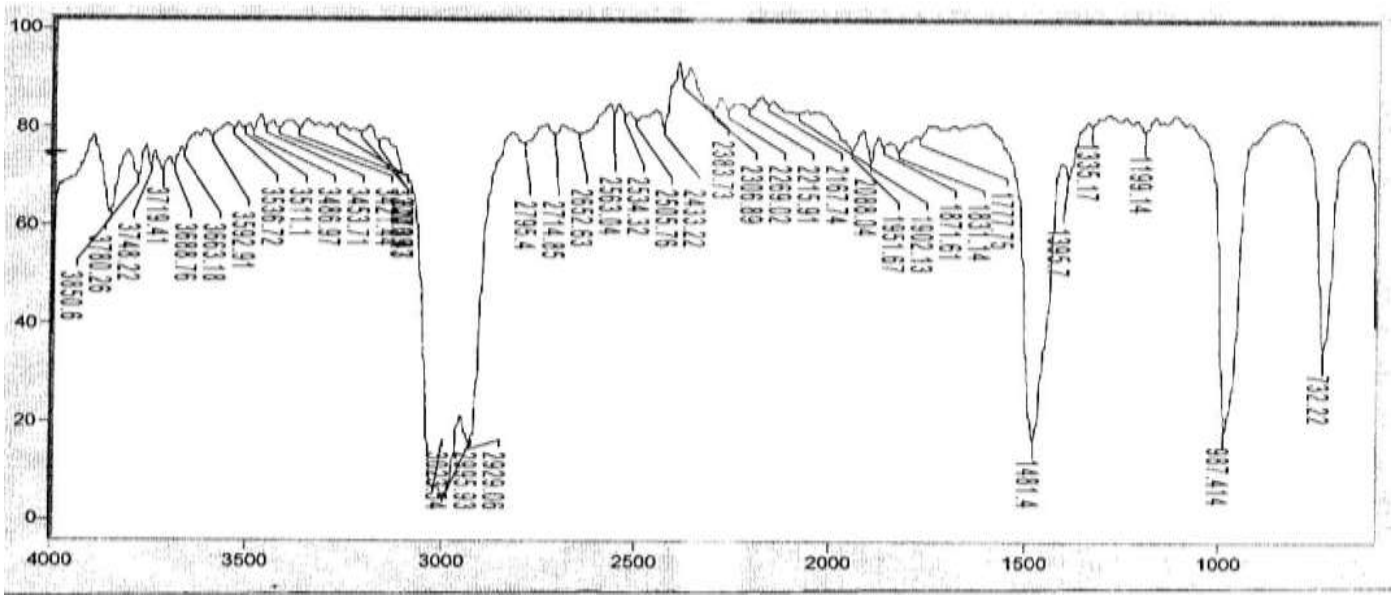

Figure 1. FTIR chart of hexane extract of *C. vulgaris* cells.

of esters corresponding to two asymmetric vibrations of O-CO (aliphatic) within 1275 to 1185 cm^{-1} and O-R stretch at 1160 to 1055 cm^{-1} have been identified as very important in FT-IR analysis of oils (Mueller et al., 2013). These absorptions are part of a band in the fingerprint region in the FT-IR charts (Figures 1 to 5). However, differences in the absorption profiles of the oil samples partition the charts into two sets. The infrared absorption profiles of oil samples obtained at 20 kHz (Figure 2) and 40 kHz (Figure 3) were similar to the sample obtained by

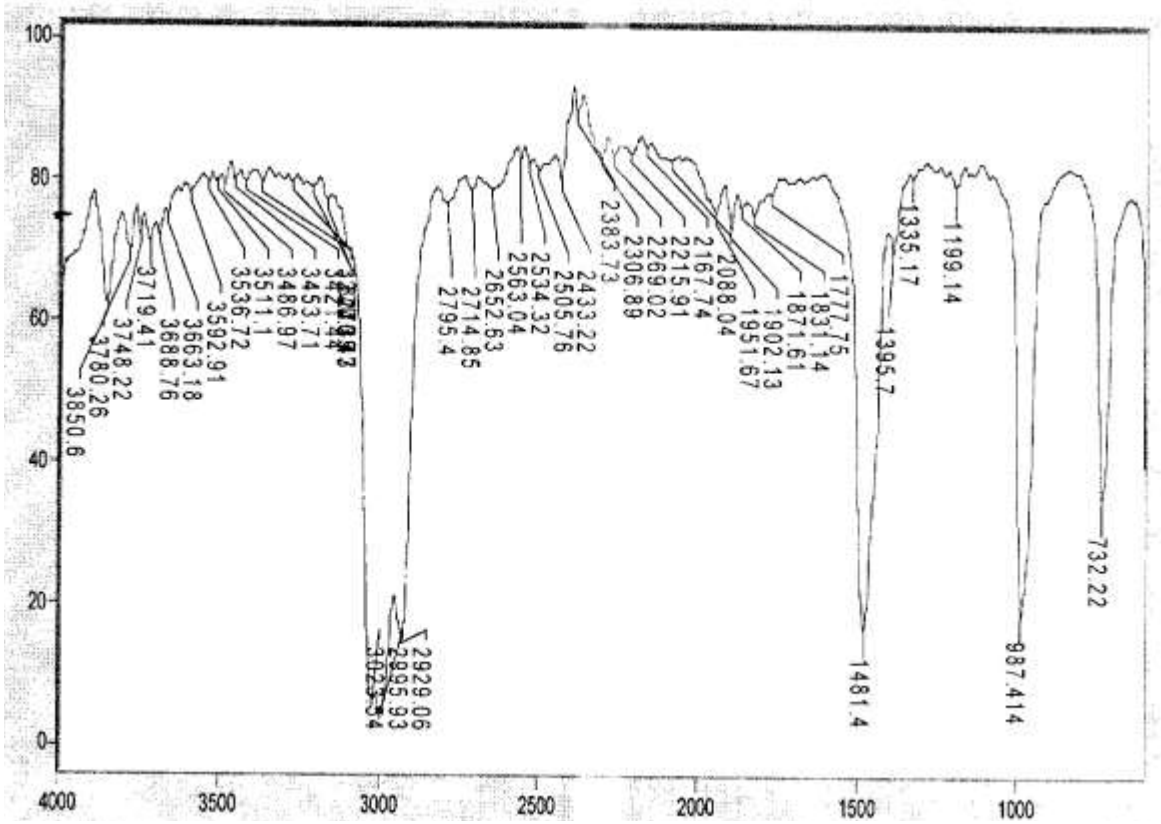

Figure 2. FTIR chart of *C. vulgaris* oil extracted at 20 kHz.

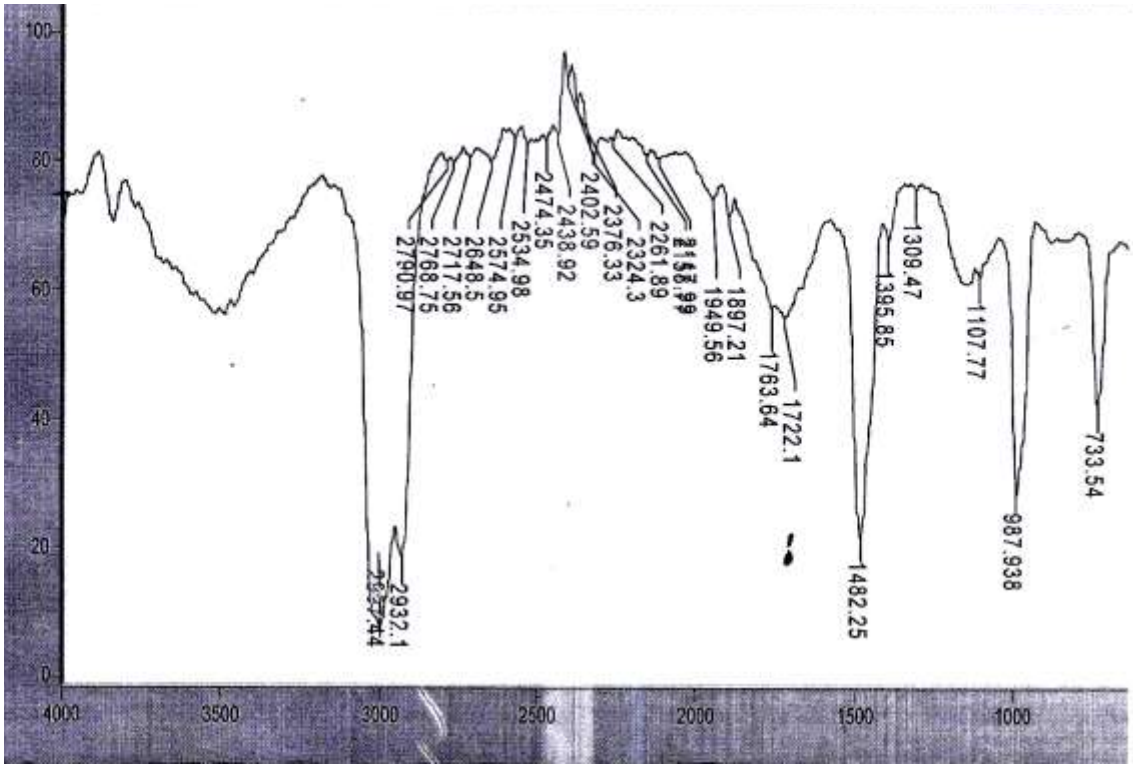

Figure 3. FTIR chart of *C. vulgaris* oil extracted at 40 kHz.

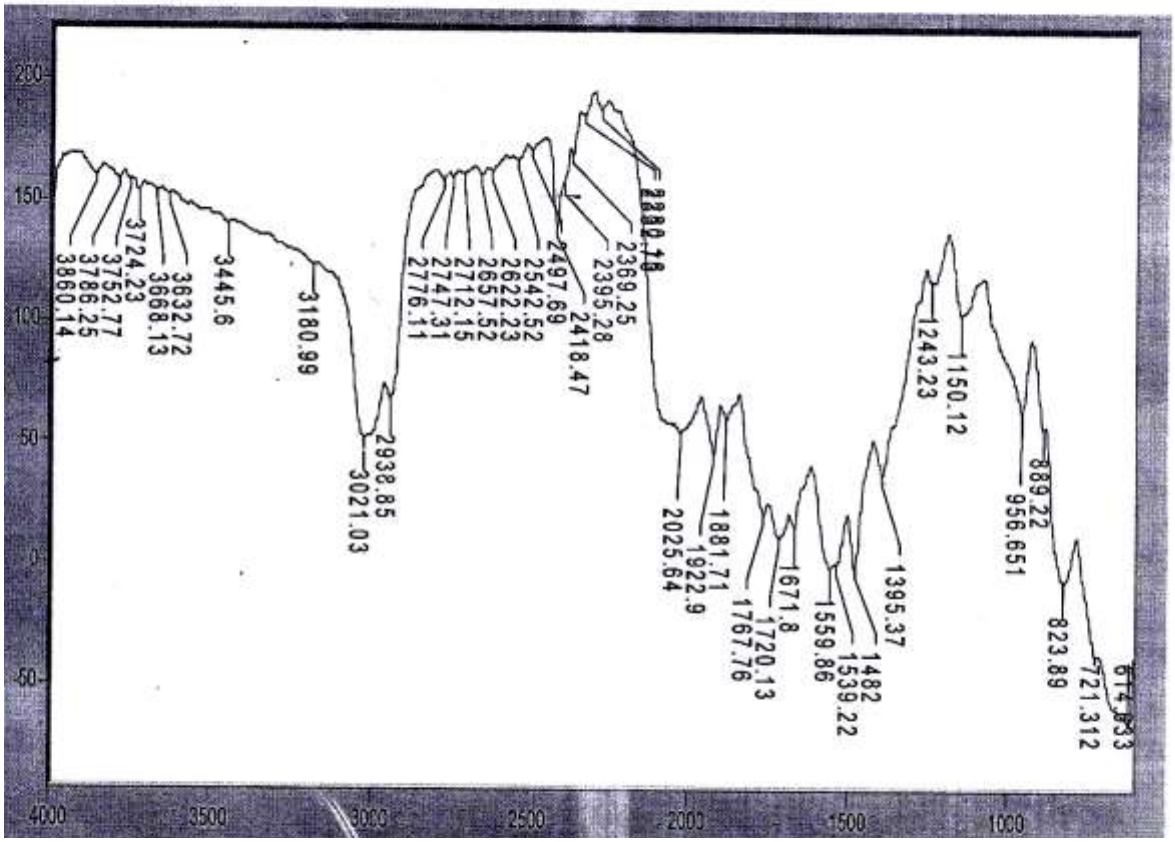

Figure 4. FTIR chart of *C. vulgaris* oil extracted at 60 Khz.

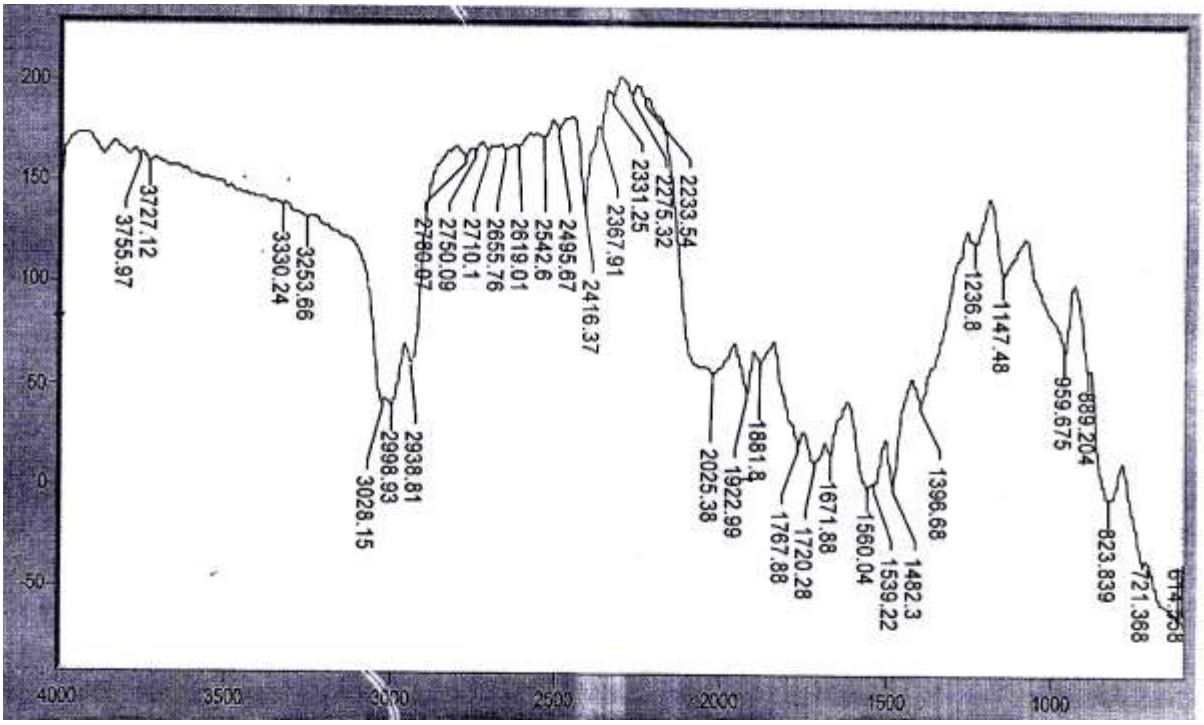

Figure 5.FTIR chart of *C. vulgaris oil* extracted at 80 kHZ.

hexane extraction (Figure 1). They showed sharp and distinct peaks at the fingerprint region suggesting absence or minimal presence of extraneous non-oil substances. The absorption profiles of oil samples obtained at 60 kHz (Figure 4) and 80 kHz (Figure 5) were similar but different from the ones obtained by hexane and by sonication at 20 and 40 kHz. At the functional group regions of the spectra in Figures 1, 2 and 3, a weak absorption occurs at the 4000 to 3100 cm^{-1} band. Absorptions of O-H stretching vibrations occur here. Such absorptions are usually of high intensity. The low intensity absorption in the Figures 1, 2 and 3 could be ascribed to the presence of small amount of volatile, low molecular weight free fatty acids. The absorption was broader in Figure 3 suggesting higher concentration of the free fatty acids in the oil extracted at 40 kHz. The absence of this band in the spectra of oil extracted at 60 and 80 kHz suggested that the volatile free fatty acids were expelled at the higher sonication frequencies. These explanations shall be investigated. The 60 and 80 kHz profiles showed broad bands with many peaks at the fingerprint region suggesting the presence of non-oil lipids. Peaks between 1450 and 1600 cm^{-1} suggested the presence of non-oil lipids particularly, compounds in which C=C is conjugated to C=O as in steroids. The characteristic C=C-C=O peaks at about 1560 cm^{-1} (Finar, 1975) which were prominent in the 60 and 80 kHz extracts were absent in the other spectra. Apparently, the higher energy at which extraction was effected at 60 and 80 kHz led to extraction of substances that were not extracted in hexane and at lower frequencies.

CONCLUSION AND RECOMMENDATIONS

Effect of extraction of oil from *C. vulgaris* cells by sonication at 20, 40, 60 and 80 kHz has been discussed. Oil extracted by refluxing alga cell in hexane was used as the standard. Using GC and FT-IR spectroscopic methods, degradation of any of the fatty acids was not observed under the experimental conditions. While oil samples extracted at 20 and 40 kHz had similar purity status as the hexane extract as shown by the FT-IR charts, other lipids apart from triglycerides seemed to be extracted at 60 and 80 kHz. As a method of oil extraction, heating alga cells under reflux in hexane for 1 h, followed by centrifugation will be further investigated. The method gave good extraction yield (60%) in this experiment. It would probably give good yield for extraction of oil from other algae if the procedure used in this experiment is followed. From this and other studies, it appears that extraction using ultrasonic frequencies above 40 kHz is not advisable particularly if triglycerides are the desired lipids. The determination of the concentration of other substances such as free fatty acids and sterols in the various samples shall be carried out to shed more light on the quality of oil extracted in hexane and at the various frequencies.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Akintayo CO, Akintayo ET, Ziegler T, Beuermann S (2015). Synthesis and characterisation of poly(hydroxylalkanethiol-ether) derivatives of *Plukenetia conophora* oil. Chem. Mat. Res. 7(4):118-128.

Akintayo ET (2007). Ethylmethyldioxirane epoxidation of *Plukenetia conophora* oil. Bull. Chem. Soc. Eth. 21(1):95-102.

Bligh EG, Dyer WJ (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Budi W, Rosli MY, Maizirwan M (2010). Ultrasound assisted extraction of oil from microalga *Nannochloropsis* sp. Int. J. Sci. Eng. Technol. 3(1):55-59.

Bunsel AR, Renard J (2005). Fundamentals of Fibre Reinforced Composite Materials. Institute of Physics Publishing, London, UK. P 102.

Christi Y (2007). Biodiesel from microalgae. Biotechnol. Adv. 25:294-306.

Cravotto G, Boffa L, Mantegna S, Perego P (2008). Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. Ultrason. Sonochem. 15:898-902.

Finar IL (1975). Organic Chemistry (2): Stereochemistry and the Chemistry of Natural Products. Pearson Education Ltd, Delhi, India, P 21.

Guillen MD, Cabo N (1997). Characterisation of edible oils and lard by fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. J. Am. Oil Chem. Soc. 74(10):1281-1286.

Khot SN, Lascala JJ, Can E, Morye SS, Williams GI, Palmese GR, Kusefoglu SH, Wool RP (2001). Development and application of triglyceride-based polymers and Composites. J. Appl. Polym. Sci. 82:703-723.

Lee JY, Yoo C, Jun SY, Ahn CY, Oh HM (2009). Comparison of several methods for effective lipid extraction from microalgae. Bioresour. Technol. 101:S75-S77.

Meier MA, Metzger JO, Schubert US (2007). Plant Oil Renewable Resources as Green Alternative in Polymer Science. Chem. Soc. Rev. 36(11):1788-802.

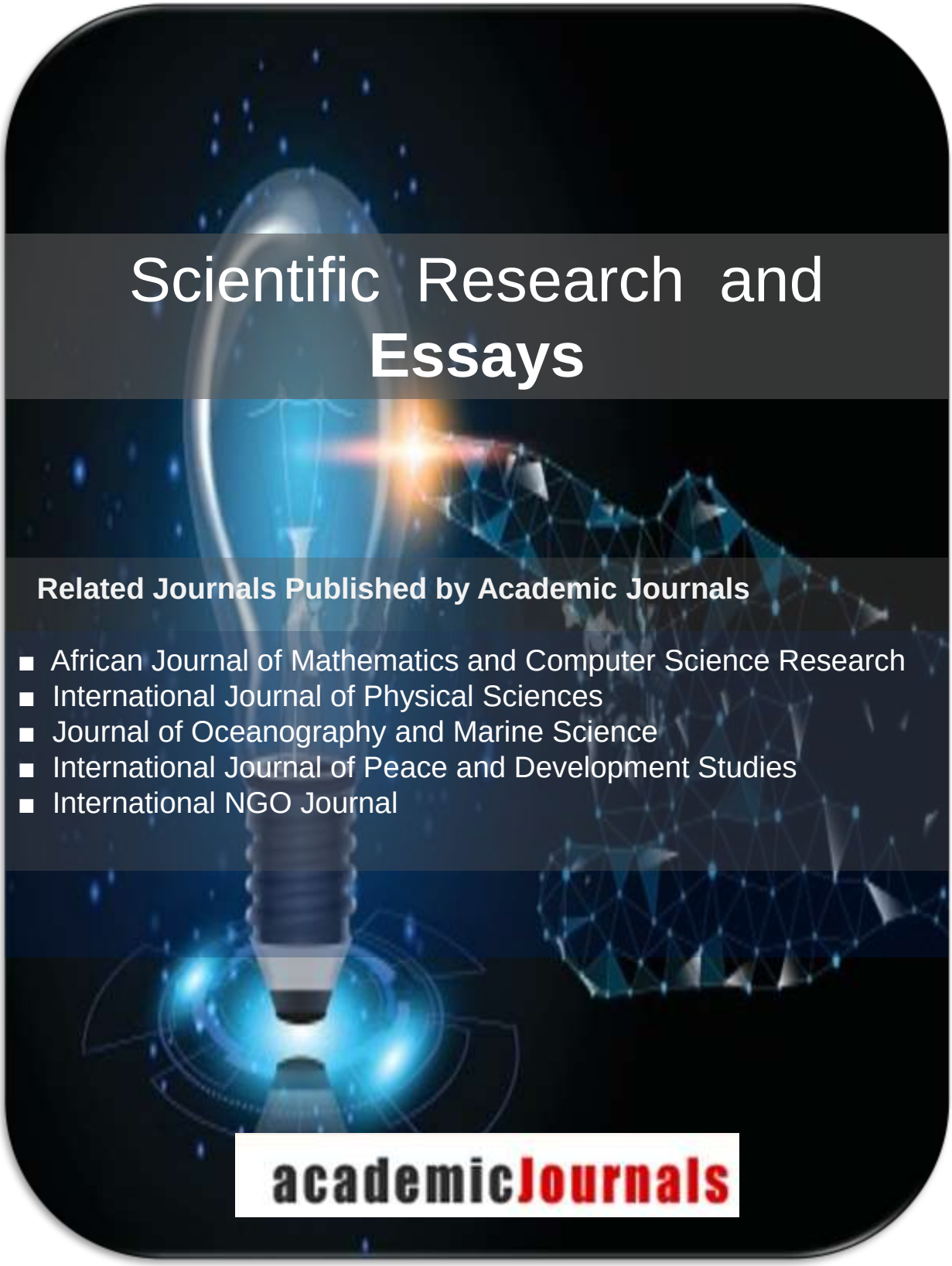
Mendes RL, Reis AD, Pereira AP, Cardoso MT (2005). Supercritical CO_2 extraction of gamma linolenic acid (GLA) from the cyanobacyerium, *Arthrospira (spirulina) maxima:* Experiments and modelling. Chem. Eng. J. 105:147-151.

Mercer P, Roberto EA (2011). Developments in Oil Extraction from Microalgae. Eur. J. Lip. Sci. Technol. 113(5):539-547.

Milner HW (1948). The fatty acids of *Chlorella*. Downloaded from jbc.org on February 9, 2012.

Miri K, Moshe C, Ami B, Aharon G (2011). Biodiesel production directly from the microalgae biomass of *Nannochloropsis* by microwave and ultrasound radiation. Bioresour. Technol. 102(5):4265-4269.

Mueller D, Marco FF, Luciano M, Adilson B, Rosana C (2013). Fourier Transform Infrared Spectroscopy (FTIR) and Multivariate Analysis for Identification of Different Vegetable Oils in Biodiesel Production. Sensors (Basel) 13(4):4258-4271.
Otles S, Pire R (2001). Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. J. AOAC Int. 84(6):1708-1714.
Satyanarayana KG, Mariano AB, Vargas JVC (2010). A review on microalgae: A versatile source for sustainable energy and materials. Int. J. Energy Res. 35(4):291-311.
Shen Y, Pei Z, Yuan W, Mao E (2009). Effect of nitrogen and extraction method on Alga lipid yield. Int. J. Agric. Biol. Eng. 2(1):51-57.
Wolff IA, Miwa TK (1965). Effect of unusual acids on selected seed oil analysis. J. Am. Oil Soc. 42(3):208-215.



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