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Potentials of Selected Microalgae Oil as a Possible Replacement for Fish Oils and Edible Oils

Adeyinka P. Ojo-Awo[1*]

[1]*Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The need to reduce the use of fish-derived oil in aquaculture to enhance the production of low-cost feeds cannot be over emphasized considering the pressure these puts on fish species and numbers. This paper reports findings of an investigation into the nutritional significance of algae oils and their potential in substituting fish oils or edible oils. A kilogram of microalgae sample was harvested from fish concrete tanks, dried and their oils were extracted using the Soxhlet extraction method. The extracted oil was subjected to gas chromatography analysis for characterization purposes. The microalgae sample yielded 7.2ml of oil per 100g sample. The chromatographic analysis of the oil showed it was composed of the fatty acids: Palmitic acid (28.2%), α-Linolenic acid (23%), Linoleic acid (22.8%), γ-Linolenic acid (16.6%), Oleic acid (2.5%) and others. The oil however lacked Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) acids, the two major polyunsaturated fatty acids in fish oils; hence this particular algae oil may not necessarily replace fish oils. The oil though exhibits promising characteristics to substitute edible oils (such as palm oil and peanut oil) for consumption and other oils for industrial uses.

Keywords: Microalgae; fatty acids; soxhlet; eicosapentaenoic; docosahexaenoic.

**Corresponding author: Email: adeyinkaojoawo@gmail.com;*

1. INTRODUCTION

Algae bear multiple advantages over traditional energy crops. They have a higher growth rate, shorter maturity rate, and higher biomass production rate than other cash crops, and require far less land than conventional crops [1]. Culture of algae does not compete for land space that could be used for food crops. Microalgae, like higher plants, produce storage lipids in the form of triacyglycerols (TAGs), thus contributing to high oil yields. The average lipid content varies between 1 and 70% but under certain conditions some species can reach 90% lipid production of dry weight [2]. Commercial and industrial algae cultivation has numerous uses, including production of food ingredients such as omega-3 fatty acids or natural food colourants (carotenoids) and dyes, foods, fertilizers, bio-plastics, chemical feedstock, pharmaceuticals, and algal fuel. They can also be used as a means of pollution control [3,4].

Fish oil is oil derived from the tissues of oily fish and contains omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as precursors of certain eicosanoids that are known to reduce inflammation in the body and have other health benefits [5]. Fish oil constitutes a major dietary ingredient in compounded fish feeds due to its essential fatty acid content, in particular omega-3 polyunsaturated fatty acids (n-3 PUFA). Recent data has shown that the aquaculture industry is solely responsible for the use of 40% and 75% of the total global production of fish meal and fish oil, respectively. Within the next decade fish oil production may not meet the required quantities for aquaculture, meaning that food grade fisheries which provide fish oil and fish meal have reached their limit of sustainability [6].

Fish do not actually produce omega-3 fatty acids, but instead accumulate them by consuming either microalgae or prey fish that have accumulated omega-3 fatty acids. They also contain high quantities of antioxidants such as iodide and selenium, which are antioxidants that protect the fragile polyunsaturated lipids from peroxidation [7]. The Omega-3 fatty acids in fish oil have been shown to be beneficial in treating hypertriglyceridemia, and preventing heart diseases. Fish oil and omega-3 fatty acids have been studied in a wide variety of other conditions, such as clinical anxiety cancer and age-related macular degeneration (AMD) [8].

Microalgae oil is a vegetarian (plant sourced) alternative to fish oil. Supplements produced from microalgae oil provide a balance of omega-3 fatty acids similar to fish oil, with a lower risk of pollutant exposure [9]. Although fish is a dietary source of omega-3, fish do not synthesize them; they obtain them from microalgae or plankton in their diets [10]. Hence, obtaining oils directly from microalgae, bypassing the "middlemen (or middlefish in this sense)" could prove to be beneficial health-wise as these oils would be free of bioaccumulated and biomagnified pollutants that the intermediate fish might have taken up in the wild. For oil-rich microalgae produced from culture, independent quality control analysis of heavy metals or other pollutants indicate these pollutants cannot be detected. Thus, one safe and probable sustainable solution for supplementing long chain polyunsaturated fatty acids (PUFA) is to derive them from microalgae sources, which are viable alternatives to fish oil. The paper reports a work done to evaluate the nutritional significance of algae oils and their potential in substituting fish oils or edible oils.

2. MATERIALS AND METHODS

2.1 Water Quality Parameter

Water samples were collected in a bottle. The bottle was dipped into the concrete tank was filled to the brim taking all precautions to prevent escape of oxygen. Physico-chemical parameters; Temperature, pH, Dissolved Oxygen, Alkalinity, Phosphate, Nitrite and Nitrate were analysed. Hanna test kit was used in determining alkalinity, pH, nitrite and nitrate parameters. Winklers Titrimetric Method was used to determine dissolved oxygen while Absorption Spectrophotometry was used in determining phosphate levels.

2.2 Sample Collection

The microalgae (MCA) sample was collected from the walls and water surface of a concrete fish tank (14.7m^3 water volume). The tank initially was used to culture catfish (*Clarias gariepinus*) but was left fallow (for about 8 months) after the harvest of the fishes. Samples of the microalgae were transported to the laboratory for identification in line with the procedure of Bellinger [11].

The samples were sun dried to constant dry weight, chopped and blended using a 400watts

capacity Sonik® milling machine. This was done so as to increase the surface area through which the extraction solvents can act in order to improve extraction yields. The samples were then packed in plastic containers preparatory to extraction of oils.

2.3 Oil Extraction

The dry mass of the microalgae (MCA) sample (100g) was used for purpose of extraction. Crude lipid extraction was carried out using the Soxhlet extraction method [12]. 250ml capacity extracting flask was dried in the oven at 105ºC, transferred to the desiccator to cool to laboratory temperature and the weight of the flask was measured. The sample (100g) was weighed into the porous thimble. 200ml of petroleum ether was measured and then added to the dried 250ml capacity flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor. The oil was extracted for five (5) hours.

The porous thimble was removed with care and the petroleum ether in the top container (tube) was collected for the recycling and reuse. The extraction flask was removed from the heating mantle arrangement when it was almost free of petroleum ether. The extraction flask with the oil was oven dried at 105ºC for the period of one (1) hour. The flask containing the dried oil was cooled in the desiccator and the weight of the dried oil was measured.

Percentage fat content extracted was determined using the formula:

$$\% \text{ Fat content} = \frac{\text{weight of lipid in grams}}{\text{weight of sample in grams}} \times 100$$

2.4 Fatty Acid Profile (Fame Analysis)

Fatty acid profile of the extracted oil was carried out using Fatty Acid Methyl Esters (FAME). 50mg of the extracted fat content of the microalgae sample was saponified (esterified) for five minutes at 95ºC with 3.4ml of the 0-5M KOH in dry methanol. The mixture was neutralized by using 0-7M HCL and then 3ml of the 14% boron triflouride in methanol was added. The mixture was heated for 5 minutes at 90ºC to achieve complete methylation.

Further characterization analysis of the oil was carried out to ascertain fatty acid composition and probably subsequent utilization in various fields. Gas Chromatography-MS was used to establish the chemical identities of fatty acids extracted from the microalgae sample.

2.5 Peroxide Value (PV)

0.75g of the oil sample was weighed into a 200ml conical flask capacity and dissolved with 25ml of 2:1 Acetic acid - Chloroform solvent and 1.0ml of potassium iodide was added and the mixture was allowed to stay in darkness for about 1 minute after which 30ml of the de-ionized water was added.

The mixture was titrated against thiosulphate solution in the conical flask using starch as indicator. The same procedure was repeated for the blank.

$$PV = \frac{100N(V1 - V2)meq/kg}{W(g)}$$

Where:

N = Normality of thiosulphate (N)
V_1 = Volume of thiosulphate used in the test (ml)
V_2 = Volume of thiosulphate used in the blank (ml)
W = Weight of sample in grams (g).

3. RESULTS AND DISCUSSION

The following species were identified; *Scenedesmus dimorphus, Ankistrodesmus* sp., *Arthrospira platensis, Spirogyra* sp. *and Chlorella sp.* After complete drying of the sample, the percentage moisture content was calculated to be 89%. Water quality parameters of the concrete tank are given below in Table 1.

The volume of the extract was recorded as 7.2ml. The oil yield obtained here was very low, compared to findings from Lee et al. [1], this could be as a result of agglomeration of the various species (both microalgae species: *Scenedesmus dimorphus, Ankistrodesmus* sp., *Arthrospira platensis, Spirogyra* sp. *and Chlorella* sp. as well as other unfavourable unidentified species) in the harvested sample or the presence of unfavourable unidentified algal species. These species tend to inhibit the tendency of the oil-producing algae species to significantly release their oil contents [13].

The extracted oil was not viscous and had a golden yellowish colour. The Percentage fat content was 2.25%, an extremely low value when compared with yields from *Ankistrodesmus* sp.

(28-40%), *Botryococcus braunii* (29-75%), *Chlorella* sp. (15-55%), and *Scenedesmus* sp. (45%) [14]. Details of the gravimetric and volumetric values of the sample are shown in Table 2. Results obtained from the extraction process showed that it was possible to obtain oils from microalgae, though, these values were low as a result of the agglomeration of the various species (both microalgae species: *Scenedesmus dimorphus, Ankistrodesmus* sp., *Arthrospira platensis, Spirogyra* sp. *and Chlorella* sp. as well as other unfavourable unidentified species) in the harvested sample or the presence of unfavourable unidentified algal species and also maybe because these species are not favourable high oil yielders.

Out of all the fatty acids obtained from the chromatography analysis, eight major fatty acids were observed as the most abundant. The major saturated fatty acids were Palmitic acid and Stearic acid while, the major unsaturated fatty acids were α-Linolenic acid (ω-3), Linoleic acid (ω-6), γ-Linolenic acid (ω-6), Oleic acid (ω-9), Palmitoleic acid (ω-7) and Petroselinic acid. The result is shown in Table 3. Saify, et al. [15] who worked on two marine fishes *Eusphyra blochii* and *Carcharhinus bleekeri* reported that Palmitic and Stearic acids were the major saturated fatty acid constituents while Oleic and Palmitoleic acids were the major unsaturated fatty acids though Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) were present.

From Table 3, it was observed that the oil sample lacked the major fatty acids; Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) usually found in fish oils that are responsible for a host of health benefits. This could due to the fact that these species investigated might not possess the all-important Fatty acids (Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)). Also the foreign species present might have inhibited the actual yields and contents of the extracted oils. This result contradicts Arterburn et al. [16] who indicated that fish and algal oils contain large quantities of long-chain polyunsaturated fatty acids (LC-PUFA), such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Hence the oils extracted from the sample may not be a good replacement for these fish oils nutritionally, though they could be employed in other functions like coating of fish feed pellets to give them an attractive flavour.

From the Table 4, it can be observed that microalgae oil sample contained high proportions of palmitic acid when compared with peanut and palm oils while stearic acid was low for all the oils. Palmitic acid is a rich source of vitamin A which is very essential for good eye functioning, it is also an antioxidant and is used as an additive in organic products. Palmitic acid is also used to produce soaps and cosmetics. The value of oleic acid for the microalgae oil was lower than those obtained in peanut oil and palm oil. The Linoleic acid value of the microalgae oil was much higher than that of palm oil, but similar to that of peanut oil. Linoleic acid is an essential acid consumed for proper health; it prevents skin scaling and hair loss.

The oxidative stability of PUFA varied widely due to fatty acid composition, physical and colloidal states of the lipids, tocopherol and carotenoids and the presence and activity of transition metals. The peroxide value is defined as the amount of peroxide oxygen per 1kg of fat or oil. Peroxide values give the measure of the extent to which an oil sample has undergone primary oxidation. *International Olive Oil Council* (IOOC) standards states that good oils should have Peroxide Values below 10meq/kg [17].

Table 1. Values of water quality parameters in sampled facilities

Parameters	Concrete Tank	Remarks
pH	7.5±0.300	Ns
Phosphate (mg/L)	10.50±0.557	Ns
Alkalinity (mg/L)	112.00±2.65	Ns
Nitrite (mg/L)	0.523±0.02	Sig
Nitrate (mg/L)	31.10±1.87	Ns
Temperature (^{o}C)	29.00±0.00	Ns
Dissolved Oxygen (mg/L)	4.10±0.00	Ns

Values in the table are Means ± Standard deviation
Sig=SignificantNs=Not significant

Table 2. Gravimetric and volumetric values of the microalgae sample (MCA)

Parameters	MCA
Weight of wet sample (g)	1000.00
Weight of dry sample (g)	110.00
Moisture Content (%)	89.00
Weight of sample used for Gas Chromatography analysis (g)	100.00
Volume of oil Extracted from 100g of samples (ml)	7.20
Weight of oil extract (g)	26.10
Weight of dried oil sample (g)	2.25
Percentage Fat Content (%)	2.25
Density (g/ml)	3.63

Table 3. Fatty acid composition of the microalgae sample

Lipid number	Fatty acid name	% Abundance
C 16:0	Palmitic acid	28.22
C 18:3 (Cis-9,12,15)	α-Linolenic acid (ALA)	22.96
C 18:2 (Cis-9,13)	Linoleicn acid (LA)	22.83
C 18:3 (Cis-6,9,12)	γ-Linolenic acid (GLA)	16.61
C 18:0	Stearic acid	4.01
C 18:1 (Cis-9)	Oleic acid	2.54
C 16:1 (Cis-9)	Palmitoleic acid	1.39
C 18:1 (Cis-6)	Petroselinic acid	1.34
Others Fatty Acids		0.10
Total		100.00

(Other fatty acids: C6:0, C8:0, C10:0, C12:0, C14:0, C14:1, C20:0, C20:1, C20:2, C20:3, C20:4, C20:5, C22:0, C22:1, C22:2, C22:6, C24:0 and C24:1)

Table 4. Comparison of the major percentage lipid contents (%LC) of edible oils and microalgae oil

Fatty acid name	%LCpalm oil	%LCpeanut oil	%LC MCA oil	%LC winghead shark
Myristic acid (C14)	0.5-2.0	-	-	4.9
Palmitic acid (C16)	32.0–45.0	5.0–11.0	28.2	40.4
Stearic acid (C18)	2.0–7.0	3.0–6.0	4.0	11.0
Oleic acid (C18:1)	38.0–52.0	52.0–60.0	2.5	26.5
Linoleic acid (C18:2)	5.0–11.0	13.0–27.0	22.8	0.8
EPA (20:5)	-	-	-	1.7
DHA (22:6)	-	-	-	0.2

% Lipid contentof palm oil and peanut oil adapted fromChempro.[19]
% Lipid contentof winghead shark adapted from Saify et al.*[15]*

The peroxide value of the oil sample was 4.26meq/Kg and this was in line with *International Olive Oil Council* (IOOC) standards which states that good oils (such as coconut and olive oils) should have PVs below 10meq/kg [17]. According to Austreng et al. [18], digestibility of oils increases as the fatty acid chain length increases from C18 to C22. Results obtained from the characterization of the microalgae oil showed the same trend.

Fish and algal oils contain large quantities of long-chain polyunsaturated fatty acids (LC-PUFA), such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) [15], that are important to assure normal animal development in culture, optimise growth and ultimately the production of a high quality food product with associated health benefits to the consumer.

4. CONCLUSION

Docosahexaenoic (DHA) and Eicosapentaenoic (EPA), which are the key fatty acids present in Fish oils, were found to be absent in the

microalgae oils and hence these microalgae oils are not suitable replacements for fish oils. They however exhibit promising characteristics to substitute common cooking oils such as peanut and palm oil. It was also observed that the oil would not easily go rancid or get oxidized, therefore make them "good oils" according to *International Olive Oil Council* (IOOC) standards. Further studies and experimentation are still required on these species and on other species on a larger and more technical scale (e.g. using photobioreactors for algae culture) so as to ascertain the viability of microalgae oils as possible substitute for fish oils, common edible oils, their utilization in soap making industries and maybe finally as a worthy replacement of our ever depleting fossil fuels.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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