Alkali-aided Extraction of Oil from Green Macro Alga - *Cladophora rupestris*: A Novel Method for Extracting Oil from Alga Cells

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ABSTRACT

Alkalization is presented in this report as a novel addition to the methods that can aid extraction of oil from alga cells. Different concentrations of aqueous solution of sodium carbonate were infused into samples of dried and powdered thalli of macro alga - *Cladophora rupestris.* The alkalized samples were exhaustively extracted with hexane in Soxhlet extractors. Oil samples from the alkalized cells were analysed by GC. Oil sample from unalkalized (natural) cells was used as the control. Different quantities of oil and different quantities of fatty acids were obtained from the different modes of extraction. The GC spectra of the various samples have identical retention time but different peak heights for each fatty acid methyl ester. The report concludes that alkalization as carried out in the work does not alter the structure of the fatty acids. It observes that a figure is not valid as the oil content of a biomaterial if the mode of extraction is not stated. It recommends that a convention which will indicate the mode of extraction must be developed for reporting oil contents. It highlights the expediency of optimizing the extraction process for improved profit margin.

Keywords

Alkalization, Extraction, Oil, Alga, Chromatography, Nutraceuticals.

1. INTRODUCTION

The exploitation of fatty acids as renewable sources of resins, chemicals, fuel, foods and pharmaceuticals is growing [1]. Because of these important applications, research into the chemistry of fatty acids is increasing in scope and depth. The fatty acids are obtained from triglycerides or oils which are usually extracted from oilseeds and algae. The advantages of obtaining oil from algae over oilseeds as well as the difficulties associated with the extraction of oil from algae have been discussed in another paper [2]. As a result of the difficulties in extracting oil from algae cells using conventional solvent extraction method otherwise called Soxhlet extraction; many physical, chemical and biological methods have been developed [3]. The study of the effect of the various methods on the structure of the fatty acids as suggested by Mercer and Roberto [4] has been initiated with the extraction of oil from the cells of *Chlorella vulgaris* using sonication at 20kHz, 40kHz, 60kHz and 80kHz [2]. An observation with high profit potential for industries was the extraction of different quantities of oil as well as different quantities of the same fatty acid at different conditions of sonication. The difference in proportion of oil extracted from the same substrate by different methods suggests that quantity of oil extracted depends on the degree of weakening of the cell wall. Based on this suggestion, more methods to aid extraction of oil from alga cells can be developed. A means of reducing the time required for cooking vegetables and legumes which is used traditionally by the *Yorubas* is the addition of a small amount of alkali in the form of sodium carbonate mineral, trona (*kaun in Yoruba).* When cooking jute or saluyot leaves, *Corchorus (ewedu in Yoruba, rama ayoyo in Hausa/Fulbe),* a small amount of trona is usually added. Giving empirical backing to this practice, Bergeson et al.[5] found out that alkaline ash filtrate reduced the time required to cook dried black beans (*Phaseolus vulgaris L.)* by 18%. Wanjekeche et al.[6] discovered that the time required to cook *Mucuna* beans in alkaline solution was shorter than the time required to cook the beans in water. Improvement in taste and texture was also reported for the beans cooked in alkaline medium. The increase in flavour, viscosity and tenderness observed when cooking is aided by addition of trona to food substances can logically be ascribed to the release of contents of the cells as the cell wall ruptures under alkaline action. Among the contents released are triglycerides which usually make food items more palatable [7]. This researcher with several years of experience in cocoa processing had always linked the traditional use of trona to the Dutch treatment - a practise in cocoa processing whereby the nibs to be pressed for cocoa butter in a hydraulic press were alkalized with sodium carbonate or potassium carbonate before roasting. Though this process is carried out to enhance the flavour and colour of cocoa powder and chocolate [7], the quantity of butter obtained from alkalized nibs is always higher than the quantity obtained from unalkalized (natural) nibs of the same batch. It can be inferred from these observations that alkalization can assist in obtaining relatively higher proportion of oil from cells with tough cell walls.

Because of the toughness of the cell walls and the high oil content of oil producing *Chlorophyta* division of algae, members of the division are ideal candidates for oil extraction studies. Considerable report is available in literature on species such as *Chlorella* [8] and *Nannochloropsis*[9] among others. A green alga which is found in the fresh waters of the riverine area of south-west Nigeria is *Cladophora rupestris* about which literature report is very scarce. The very scarce account includes one on the biotechnological potential of the lipid extract which was predicated on microbial activity, nutritional value and bio-plastic content [10]. However, the oil content of the alga was not stated. Extensive web search on several search engines did not yield any result on the oil content of *C. rupestris.* The alga belongs to the *Chlorophyta* division of algae which are known to be oil producers [11].

Due to its availability and dearth of facts on its oil content, *C. rupestris* was chosen for this study. Alkalization was investigated as the extraction method. The effect of alkalization at different concentrations of alkali on the quantity and quality of fatty acids in the extracts was also investigated.

2. MATERIALS AND METHODS.

2.1. *C. rupestris*

The thalli of the green alga were collected in June during the raining season in the fresh water of the riverine area of Okitipupa, a city at the southern tip of Ondo State, south-west Nigeria. The alga was identified at the herbarium of Ekiti State University. The thalli were washed free of extraneous matter in distilled water, drained, and air-dried in the laboratory. The air-dried thalli were ground into bits and further dried to constant weight at 105^0C in an air-draught oven. The dried thalli were pulverised and stored in a desiccator.

2.2. Alkalization of Cells and Extraction of oil

100g of powdered sample of *C. rupestris* was infused with 20g of 5% sodium carbonate solution at 80^0C for thirty minutes. The alkalized sample was vacuum-dried and then dried to constant weight at 105^0C in an air-draught oven. The dried sample was stored in a desiccator. This procedure was repeated for three other 100g portions of the powdered alga cells but the portions were alkalized respectively with 10%, 15% and 20% sodium carbonate solutions. Soxhlet apparatus was used in the exhaustive oil extraction process. 5g sample of dried alkalized cells weighed into a thimble was placed in the Soxhlet extractor. The sample was repeatedly extracted with hexane for a total period of 24hrs. By this time, the solvent had become colourless in the extractor. The solvent was recovered in a rotary evaporator. Three extractions were carried out for each grade of the alkalized cells. The same procedure was used to extract oil from unalkalized or natural cells - the control.

2.3. Fatty Acids Profile of the Oil

GC was used to analyse the oil samples. The model of the instrument and procedure used in preparing the methyl esters for analysis was as published in an earlier report [2]. The methyl esters were analysed under the following conditions: split injection with split ratio 20:1; carrier gas – nitrogen; inlet temperature – 250^0C; HP INNOwax column with dimension 30m x 0.25mm x 0.25µm; oven initial temperature was set at 60^0C, first ramping at 12^0C/min for 20min maintained for 2min, second ramping at 15^0C/min for 3min maintained for 8min. The temperature of the FID detector was 320^0C. The pressure of hydrogen and compressed air were respectively 22psi and 35psi.

The reliability of the data generated was high with the correlation curves of the various FAME standards having coefficients r = 0.99847 – 0.99962.

3. RESULTS AND DISCUSSION

3.1 Oil Content

Table 1: Proportion of oil extracted from natural and alkalized cells of *C. rupestris.*

	Natural	Alkalized (sodium carbonate solution)			
		5%	10%	15%	20%
Oil Content (g/5g sample)	0.54±0.01	0.51±0.01	0.82±0.01	0.63±0.01	0.57±0.02
% Oil	10.8	10.2	16.4	12.6	11.4

Determination of oil content of *C. rupestris* was carried out on natural (unalkalized) alga mass and samples alkalized with 5%, 10%, 15% and 20% sodium carbonate solutions. The control was the sample of oil extracted

from unalkalized alga mass by conventional Soxhlet method. It is referred to as 'Natural' in Table 1. It yielded lower quantity of oil than the alkalized cells except the cells alkalized with 5% solution of sodium carbonate. The lower yield of the 5% mode might be due to increase in ionic character of the cell contents due to formation of sodium compounds while the cell membrane was not sufficiently weakened to facilitate extraction. The higher yield of the other alkalized modes compared to the natural can be attributed to greater weakening of the cells of the alga by the alkali. From the 10% to 20% modes, the decrease in the quantity of oil extracted can be attributed to increase in ionic character of the oil due to increasing hydrolysis of oil to sodium salts. This would reduce the quantity of oil extracted into the non-polar hexane. It can therefore be suggested that in an alkali-aided extraction process, the quantity of oil extracted from the cells varies inversely with the degree of hydrolysis of cell contents to sodium salts, and directly with the extent of weakening of the cell membrane by the alkali. This researchable assertion is justified by the figures in Table 1 each of which is an average of three determinations. Macro alga *C.rupestris* is not a rich source of oil compared with the micro algae in the same *chlorophyta* division. The oil contents of many species of the division are widely reported in literature. Depending on the method used, the extraction of different quantities of oil from the same biomass has been reported [12]. However, the implications of the observation have not been highlighted. *The first implication is that a universal convention must be developed for reporting oil content.* It is not absolutely correct to state a number or figure as the oil content of a biomass without stating the method or mode of extraction. If any of the values in Table 1 is reported as the oil content of *C. rupestris*, the figure will be misleading. Yet, each of the figures is precise as indicated by the low standard deviations. Using a convention, the oil content obtained from the hexane extract and the figure obtained by aiding the extraction with 5% $Na_2CO_{3(aq)}$ can be reported as 10.8: HEX and 10.2: 5% $Na_2CO_{3(aq)}$ respectively. Conventions can be developed to cover all methods of oil extraction.

The second implication is that oil processors should carry out preliminary studies to determine the mode of extraction that will give the highest yield of oil. This will be of great benefit to oil processors particularly processors of alga cells. The higher the quantity of oil that can be extracted from a material, the higher the gain. In Table 1, the range is 0.31g or 6.2%. This difference can translate to significant profit margins in extraction plants where several metric tons of materials are processed daily. This fact is highlighted in Table 3.

3.2 Fatty Acids Profile

Table 2. Gas chromatography profile of the fatty acids of the various oil samples extracted from *C. rupestris.*

Fatty acid		Retention time (min)	Natural	Alkalized (sodium carbonate solution)			
				5%	10%	15%	20%
Lauric acid	(C20:0)	12.83	1.28	1.69	1.5	1.88	1.77
Myristic acid	(C14:0)	14.44	10.56	8.48	8.6	8.76	9.21
Hexadecadienoic acid	(C16:2)	15.03	3.01	2.98	3.13	3.13	3.26
Palmitic acid	(C16:0)	16.04	28.10	28.95	27.48	29.96	31.53
Palmitoleic acid	(C16:1)	16.67	5.12	4.41	4.92	4.43	4.43
Margaric acid	(C17:0)	17.37	0.00	0.34	0.83	0.43	0.75
Stearic acid	(C18:0)	18.06	4.52	5.53	6.42	5.38	4.99
Oleic acid	(C18:1)	18.94	23.53	21.89	21.94	22.35	21.32
Linoleic acid	(C18:2)	19.52	9.41	10.51	11.98	9.62	9.28
Linolenic acid	(C18:3)	20.65	6.29	6.83	6.69	6.21	6.23
Arachidic acid	(C20:0)	21.91	2.69	1.67	1.35	1.55	1.4
Arachidonic acid	(C20:4)	22.36	0.36	0.4	0.19	0.36	0.18
Docosatrienoic acid	(C22:3)	22.60	2.18	2.69	3.29	3.05	2.95
Behenic acid	(C22:0)	23.97	1.60	1.86	1.12	1.44	1.47
Erucic acid	(C22:1)	24.79	0.63	0.8	0.32	0.61	0.54

Lignoceric acid	(C22:1)	25.62	0.69	0.97	0.23	0.84	0.67

The linearity of the calibration curves of the FAMEs has high significant ($P < 0.001$) coefficient of determination ($r^2 = 0.99$). Therefore, the data in Table 2 reflects precisely the difference in the proportions of the same fatty acids in the oil. The retention times of each fatty acid in all the spectra are identical. This means that the various modes of extraction have no effect on the structure of the fatty acids. In an earlier work [2], the significance of the difference in the proportions of the same fatty acid obtained from different modes of extraction was discussed. The process used in that work was sonication. Differences are also observed in the present work. The peak heights of each fatty acid in the spectra are different. Application of this observation in oil processing will bring significant benefits to processors and consumers. Oils are often traded on the proportion of certain essential fatty acids. For instance, DHA 40% alga oil is a premium product. The combination of quantity of oil (Table 1) and quantities of fatty acids (Table 2) extracted under a mode are used to estimate the quantity of each fatty acid that would be extracted from one metric ton (1000kg) of *C. rupestris* (Table 3). For example, the estimate (1.4kg) for lauric acid in Table 3 under 'Natural' was obtained from: 1.28% x (0.54/5) x 1000. The general expression is *1000ab* where '*a*' is the proportion of a particular fatty acid and '*b*' is the quantity of oil extracted per unit weight of alga cells.

Table 3: Amount of fatty acids (in kg) per 1000kg of *C. rupestris.*

Fatty acid		Natural	Alkalized (sodium carbonate solution)			
			5%	10%	15%	20%
Lauric acid	(C20:0)	1.4	1.9	2.6	2.4	2.1
Myristic acid	(C14:0)	11.5	9.3	14.6	11.4	11.1
Hexadecadienoic acid	(C16:2)	3.3	3.3	5.3	4.1	3.9
Palmitic acid	(C16:0)	30.6	31.8	46.7	38.9	37.8
Palmitoleic acid	(C16:1)	5.6	4.9	8.4	5.8	5.3
Margaric acid	(C17:0)	0	0.4	1.4	0.6	0.9
Stearic acid	(C18:0)	4.9	6.1	10.9	6.9	5.9
Oleic acid	(C18:1)	25.6	24.1	37.3	29.1	25.6
Linoleic acid	(C18:2)	10.3	11.6	20.4	12.5	11.1
Linolenic acid	(C18:3)	6.9	7.5	11.4	8.1	7.5
Arachidic acid	(C20:0)	2.9	1.8	2.3	2.0	1.7
Arachidonic acid	(C20:4)	0.4	0.5	0.3	0.5	0.2
Docosatrienoic acid	(C22:3)	2.4	2.9	5.6	3.9	3.5
Behemic acid	(C22:0)	1.7	2.0	1.9	1.9	1.8
Erucic acid	(C22:1)	0.7	0.9	0.5	0.8	0.6
Lignoceric acid	(C24:0)	0.8	1.1	0.4	1.1	0.8
Summary						
Total Fatty Acids		109	110	170	130	119.8
Total SFA (% of Total)		53.8(49.4)	54.4(49.5)	80.8(47.5)	65.2(50.2)	62.1(51.8)
Total MUFA		31.9	29.9	46.2	35.7	31.5
Total PUFA		23.3	25.8	43	29.1	26.2
Total UFA(% of Total)		55.2(50.6)	55.7(50.6)	89.2(52.5)	64.8(49.8)	57.7(48.2)

Omege-3 Acids	8.6	9.7	15.7	11.2	10.3
Omega-6 Acids	11.4	12.9	21.8	13.8	12.1
Ω-6/Ω-3	1.3	1.3	1.4	1.2	1.2

The same biomass that yielded oil containing 6.9kg linolenic acid under the natural mode of extraction gave 11.4kg of the essential fatty acid when the biomass was alkalized with 10% sodium carbonate solution. PUFAs like linolenic acids are important natural products used in nutrition and pharmaceuticals [13]. *Now, such nutraceuticals can be more profitably extracted from sources from which they have been obtained in low quantities. This is the third implication.* This implication can be appreciated further when the degree of saturation or unsaturation of the oil has appreciable effect on application. Worldwide, production of alga oil is increasing due to the reasons given at the introductory section of this paper. For preparation of biodiesel, feed oil should be of high degree of saturation. Though the highest quantity of oil was extracted using 10% Na_2CO_3 mode, the proportion of total UFA is high. UFAs are prone to rancidity and degradation. Therefore, oil samples containing them are not ideal for preparing biodiesel. Conversely, oil obtained using 15% or 20% Na_2CO_3 will give biodiesel with better qualities since they contain higher SFAs and lower UFAs [14]. However, in manufacturing polymers, oil with high degree of unsaturation is needed for rigid highly cross-linked products [15]. Oil extracted with 10% Na_2CO_3 solution is the best for polymers.

This section shall be concluded with a comment on the ratio of omega-6 to omega-3 fatty acids - a ratio with vital health implications. The proportion of omega-6 to omega-3 isomers of linolenic acid was not determined in this work. However, the proportion was estimated using I:10 of the quantities of the acid obtained. 1:10 was the approximate ratio of values from the report earlier cited [10]. The ratio of omega-6 to omega-3 fatty acids is low for all the modes of extraction. All the values are more than 1 compared to the less than 1 values obtained by Stabili et al. for two samples of thalli obtained at two different periods [10]. However, these values are far less than 10, the uppermost limit recommended by W.H.O for good health [16]. High values of the ratio have been discovered to promote cardiovascular, autoimmune and inflammatory diseases and cancer [17]. There are other differences in the data obtained in this work and those obtained by Stabili et al. [10]. These variations might be due to differences in extraction method, differences in analytical procedure or the usual characteristic ability of algae to produce different quantities and qualities of oils depending on the environment and nutrient [18]. The fatty acids identified here which were not reported by Stabili et al. are hexadecadienoic acid, erucic acid and docosatrienoic acid. However, eicosapentaenoic acid, docosadienoic acid and docosahexaenoic acid which are not identified in this work were reported by Stabili et al. In all the extracts, linoleic acid is the dominant PUFA as against linolenic acid reported by Stabili et al.

4. CONCLUSION AND RECOMMENDATION

Alkali-aided extraction of oil from *C. rupestris*, referred to as alkalization has been presented in this report as a new addition to the methods developed to improve extraction of oil from alga cells. The method is simple and effective. More oil was extracted compared to the quantity extracted with the conventional solvent extraction method. The chemicals used in the extraction are not toxic. Therefore, oil extracted by the method can be used for producing nutraceuticals. Extraction of different quantities of the same fatty acid at different modes of the method of extraction is of high industrial significance. The GC profiles of the fatty acids indicate that the different modes did not affect the structure of the fatty acids.

Processors should endeavour to determine the optimum condition for obtaining the highest quantity of oil as well as the highest quantity of specific fatty acids from a biomass. Oil content of biomass should be stated with the condition of extraction. A standard international convention for doing this should be developed.

5. CONFLICT OF INTEREST

The author declared that he had no conflict of interest.

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