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Obtaining DHA–EPA Oil Concentrates from the Biomass of Microalga *Chlorella sorokiniana*

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Abstract: Microalgae have attracted growing interest all around the world due to their potential applications in multiple sectors of industry, such as energetics, nutraceuticals, pharmaceuticals, agriculture, and ecology. Concepts of biorefinery of microalgae lipids for biodiesel production coupled with other applications have been suggested in several studies. However, very few studies focus on overcoming the degree of unsaturation of microalgae lipids using methods of fractionation. This study presents a method for obtaining two oil fractions from microalgae *Chlorella sorokiniana* suitable for food and biofuels via urea complex formation with further production of a long-chain PUFA concentrated oil suitable for the nutraceutical industry. A DHA–EPA-rich fraction was obtained from the dry microalga biomass using a succession of extraction, urea-complexation, fractionation, and esterification with glycerol. Analytical and organoleptic methods were used to assess the quality of the final product. Results show that the urea-complexation method allowed the obtaining of two lipid fractions with different fatty acid profiles. The urea complexed fraction (UCF) contained a majority of saturated fatty acids (54.46%); thus, it could find applications in the biofuels or food industry. The non-urea complexed fraction (NUCF) was rich in polyunsaturated fatty acids (PUFA) (81.00%), especially long-chain PUFA with 16.52% EPA and 35.08% DHA. The recovery rates of EPA and DHA in the NUCF reached 59% and 87.14%, respectively. Finally, the physicochemical and organoleptic characteristics of the DHA–EPA oil concentrate were determined and found conform to the norms recommended by the WHO/FAO standards for edible oils and the Russian State Standard GOST 1129-2013.

Keywords: algal oil; extraction; purification; urea-complexation; re-esterification

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1. Introduction

Several edible oils derived from plants present interesting health-promoting effects and are essential for a balanced diet. Moreover, the composition and biological activity of these substances differ greatly from one plant species to another [1]. Dietary lipids of plant oils mostly consist of triacylglycerols (TAG)-esters of glycerol and fatty acids [2]. According to their degree of saturation, fatty acids are classified as either saturated or unsaturated, the latter being subdivided into monounsaturated and polyunsaturated fatty acids (PUFA). Oils such as coconut oil, cocoa butter, and palm oil mostly contain saturated fatty acids (SFA), while olive, peanut, and avocado oils are composed of monounsaturated fatty acids (MUFA). On the other hand, sunflower, sesame, soybean, rapeseed, corn, and cottonseed

oils include considerable amounts of PUFA. It is therefore recommended to combine the consumption of several types of healthy oils in order to make the most of their health benefits [3].

In the last decades, microalgae have emerged as a new source of vegetable oil. Algae of the *Chlorella* genus contain essential nutrients such as proteins (9–88%), lipids (5–86%), carbohydrates (6–38%), and vitamins. Indeed, some species are already considered as an alternative food source [4,5]. Studies describe the use of microalgae biomass not only for livestock feed [6] but also for improving the immune function of the human body [7]. Some of the health benefits attributed to the consumption of *Chlorella* include: reduction of atherosclerotic risk [8]; cadmium and uranium detoxification; reduction of blood sugar and cholesterol levels; and an increase in the level of hemoglobin [9]. Moreover, *Chlorella* cells synthesize a wide range of carotenoids and phenolic substances which are known for their antioxidant properties [10].

Aside from that, microalgae, as other types of biomass [11,12], can be used as a raw material for producing biofuels [13–19]. Scientists all over the world have studied lipid-rich microalgae and several technologies have been developed for extracting these biomolecules. Nevertheless, the production of microalgae-based biodiesel is considered, to this day, economically unfeasible mainly due to the high operational costs related to downstream processing [20]. One of the proposed strategies to overcome this obstacle is to improve the productivity of lipids by microalgae cells. Among the recent technologies for enhancing lipid productivity we can cite: the addition of inducers such as phytohormones; nutrient starvation; metabolic switch; and the use of stressers such as pretreatment with low-dose cold atmospheric pressure plasma [21,22]. However, these methods have shown to affect the quality of the accumulated lipids as well as the growth rates of the biomass [23]. The composition of microalgal lipids and the degree of saturation of its fatty acids must be taken into consideration as it is a crucial factor for determining its eventual usability. For instance, it is known that a high content in saturated fatty acids generally results in more stable biodiesel with high cetane numbers [24].

Despite the numerous researches carried on microalgae biotechnology for biodiesel production, only a few studies focus on the development of biorefinery approaches for producing edible lipids from microalgae, and even less for the combined production of lipids for food and biofuels. Moreover, these two applications require lipids with distinct fatty acid profiles, i.e., a higher degree of unsaturation for the production nutraceuticals and a majority of saturated lipids for biodiesel production. This bottleneck could explain the scarce biotechnological concepts proposing the combined production of algal lipids destined for bioenergetics and pharmaceuticals.

Evidence shows that it is possible to obtain microalgae biomass with a high content of unsaturated fatty acids [25–28]. Microalgae lipids and fatty acids, including omega-3 and omega-6, have attracted an increasing interest due to several beneficial health effects associated with their consumption. Long chain omega-3 PUFA, namely docosahexaenoic acid (DHA; 22:6ω3) and eicosapentaenoic acid (EPA; 20:5ω3) have been shown to offer an array of health benefits [29]. These fatty acids are abundantly found in cell membranes, especially in the brain and retina [30–32]. Studies show that these lipids play an essential role in fetal development and healthy aging [33]. Long-chain PUFA are also anti-inflammatory, anti-aggregator, and cardio-protector [34,35]. Omega-3 such as EPA and DHA are found in fish and krill oils but they are originally synthesized by microalgae [36,37]. The human body is able to convert α-linolenic acid (ALA) obtained from diet to EPA and DHA by the action of elongase and desaturase enzymes. However, their conversion rates are very low: only 0.3–10% of ALA is converted to EPA and 0–4% is converted to DHA [38,39]. Moreover, a modern diet rich in omega-6 has been shown to reduce this conversion rate by 40–50% [40]. Hence, it is important to aim for a ratio of omega-6/omega-3 lower than 4–5:1 [41]. Although it is often challenging to achieve an appropriate intake of EPA and DHA [42], it is possible to offset this lack by using dietary supplements such as oil concentrates.

There are several methods for extracting oil from biomass: cold pressing (such oils are considered most beneficial); hot pressing (the raw material is heated before the pressing in order to liquefy the oil contained in the cell); extraction (the raw material is treated with a solvent that extracts the oil. After the extraction, the solvent is subsequently removed by evaporation) [43]. A comprehensive review by N.L. Vostrikova presents a comparison of several extraction methods [27]. Microalgae oils are commonly extracted using Folch, Randall, and Soxhlet methods [43–46]. However, microalgae biomass contains high amounts of pigments that are extracted together with the lipids. Therefore, it would be appropriate to carry out a "cascade" type of extraction which includes a preliminary step for pigments extraction followed by the extraction of lipids. In a review by S. Bermúdez, several extraction and purification methods of PUFA and pigments, such as astaxanthin, phycoerythrin, and phycocyanin, are discussed [47].

Several factors are to be considered before extracting valuable compounds from microalgae. Studies show that the cultivation conditions significantly affect the growth rate and composition of microalgae biomass [48–52]. The effect of physical factors such as the light spectrum and intensity [49,50], IR, UV and laser radiation influence the cultivation rate and the composition of the biomass [51,52]. The chemical composition of the growth medium, in particular, the nitrogen concentration, affects the lipid content and fatty acid profile of the microalgae biomass [53].

The purification of oil extracts is often necessary before their consumption. For this purpose, a number of methods have been developed, including: fractionation; saponification; sorption on silica gel; cryo-phase separation; winterization; and urea complexation [47,54–58]. Such techniques are also applied for obtaining oil concentrates with higher amounts of long chains PUFA [55–58]. The resulting purified oils often lose their beneficial properties under the influence of light, heat, and oxygen generating biologically harmful oxidation products. Therefore, various preservatives and antioxidants are added before and after the purification process to extend the shelf life of oils [59]. Antioxidants of natural origins such as vitamin E (α-tocopherol), ascorbic acid, and β-carotene have been shown to be equivalently active or even more effective than synthetic preservative such as butylhydroxyanisol (BHA) [47,60]. Furthermore, BHA has raised concern among the scientific community and is suspected to be carcinogenic. Nevertheless, this additive is, to this day, widely employed in the food industry [61].

The purpose of this study is to propose a method for obtaining lipid fractions from *Chlorella sorokiniana* microalgae suitable for food and biofuels via urea complex formation with further production of a long-chain PUFA concentrated oil suitable for the nutraceutical industry.

2. Materials and Methods

2.1. Microalga Cultivation and Growth Assessment

Microalga *Chlorella sorokiniana* strain 211-8k was obtained from the Culture Collection of Algae of Goettingen University (SAG). The biomass was grown autotrophically under laboratory conditions for 10 days in a 100-L photobioreactor with constant aeration and a temperature of 22 ± 2 °C (Figure 1). The composition of the nutrient medium (containing 0.3 g/L of KNO_3) and the cultivation conditions were described in a previous study [53]. The volume of the culture was adjusted daily during cultivation using the nutrient medium in order to compensate for the evaporated water.

Microalgae growth was controlled by the determination of the total dry weight of the biomass. For this purpose, 10 mL of samples collected daily (in triplicate) were vacuum filtered on pre-dried and weighed paper filters with a pore size of 0.45 μm. After that, they were washed 3 times with distilled water to dissolve salts. The filters were then dried at 95 °C in a SNOL 200/200 oven till reaching constant weight. The dry cell weight was then calculated as follow:

$$DCW = m_2 - m_1 \quad (1)$$

where: DCW is the dry cell weight, g; m_2 is the weight of the dried filters with the filtered biomass; m_1 is the weight of the pre-dried filters.

The cell concentration was then calculated by the formula:

$$C = \frac{DCW \cdot 1000}{V} \quad (2)$$

where: C is the cell concentration, g/L; V is the volume of the filtered sample, mL.

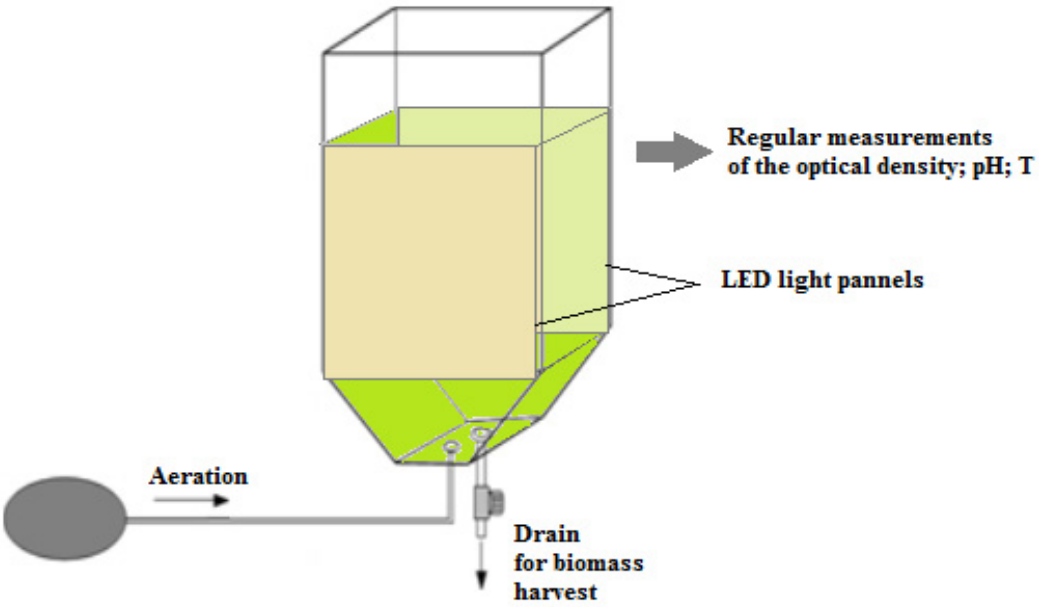

Figure 1. Scheme of the 100-L photobioreactor for microalgae cultivation.

2.2. Biomass Harvest and Drying

At the end of the cultivation process, the biomass was harvested by centrifugation at 3500 rpm for 5 min, and the pellet was washed 3 times with distilled water then freeze-dried using the AK-50N lyophilizer (Proflab, Saint-Petersburg, Russia).

2.3. Cell Wall Disintegration and Algal Oil Extraction

To increase the yield of lipid extraction, the lyophilized biomass was subjected to mechanical disintegration using the Silent Crusher M Homogenizer (Heidolph Instruments, Schwabach, Germany). For this purpose, 3 g of dry biomass was placed in a centrifuge tube and mixed with 10 mL of the extraction solution (hexane: ethanol) at a ratio of 9:1 (*V*/*V*). The disintegration was performed at 10,000 rpm for 5 min with continuous cooling using a water bath maintained at 6 °C. The extraction of the algal oil was carried out in a Soxhlet extractor using the Büchi E-812 SOX extraction unit (Flawil, Switzerland). The disintegrated biomass was recovered by filtration in a cellulose thimble; 90 mL of the extraction solution (hexane: ethanol, 9:1) was used to conduct the extraction. The algal oil was recovered after 15 cycles of extraction followed by 30 min rinsing step. The extracts were dried under vacuum on a rotary evaporator. The extraction yields were determined gravimetrically as described previously [62]. The next experimental steps were carried out in short order according to the scheme presented in Figure 2.

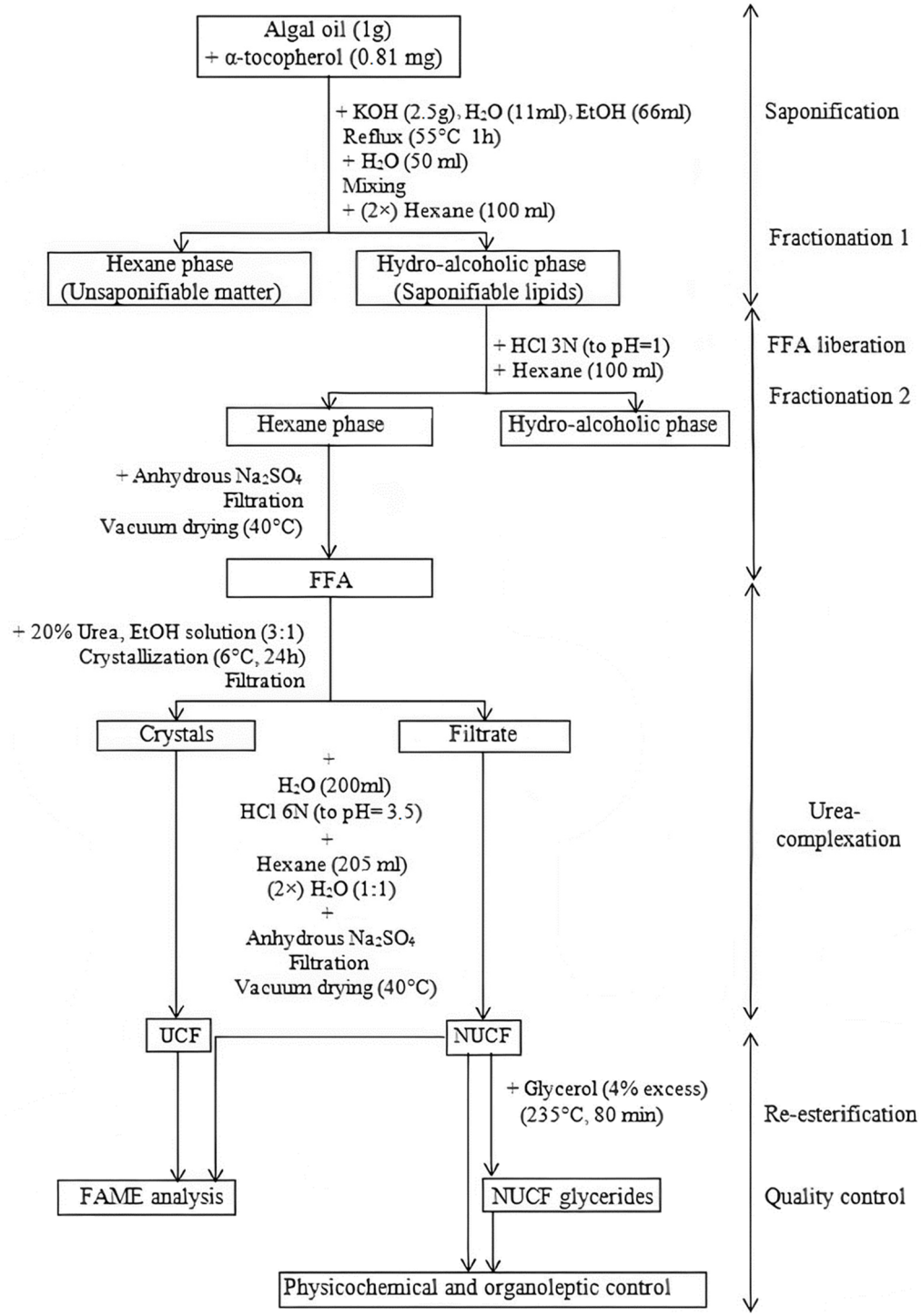

Figure 2. Scheme for the DHA–EPA concentration and purification of algal oil.

2.4. Obtaining Free Fatty Acids from Algal Oil

In order to minimize the process of lipid oxidation, α- tocopherol was used for the conservation of the algal oil at a concentration of 81 mg per 100g oil [60]. The algal oil (1 g) was first saponified with a mixture of KOH (2.5 g), water (11 mL) and ethanol (66 mL) and placed in a reflux apparatus for 1 h at 55 °C. After the saponification, 50 mL of distilled water was added to the mixture and the unsaponifiable matters were extracted into hexane (2 × 100 mL) and separated. The polar layer containing the saponifiable lipids was acidified to pH = 1 using a 3 N solution of hydrochloric acid. The acidified mixture was transferred in a separation funnel and the free fatty acids were extracted into hexane (100 mL). The hexane

layer was dried over anhydrous sodium sulfate and the free fatty acids were recovered in a rotary evaporator at 40 °C and weighed [61,63].

2.5. Separation of Long Chain PUFA by Urea Complexation

The separation of the long-chain PUFA from the free fatty acids was carried out using a urea complexation method described by Senamayake et al. [64]. A urea solution in 95% ethanol (20%, *w*/*v*) was added to 450 mg of the free fatty acids at a ratio of 3:1 (m/m) urea to fatty acids [64] and mixed at 55 °C till a homogeneous solution was obtained. The solution was then stored for 24 h in a cold room maintained at a temperature of 6 °C for the crystallization process. The resulting crystals were separated from the non-urea complexing fraction by vacuum filtration on a Nylon 66 Membrane filter with a pore diameter of 0.45 µm (SUPELCO). Next, 200 mL of distilled water was added to the filtrate and the solution was acidified to pH = 3.5 using a 6 N solution of hydrochloric acid. Subsequently, 205 mL of hexane was added, and the two fractions were vigorously mixed by magnetic stirring for 1 h. The fractionation process was then carried out in a separation funnel. The hexane layer was separated and washed twice with an equal volume of distilled water, then dried over anhydrous sodium sulfate. The non-urea complexed fractions (NUCF) were then dried in a rotary evaporator at 40 °C and their yield was determined [61].

The crystals of urea were treated using the same technique: addition of distilled water (200 mL) and acidification with 6 N hydrochloric acid. The urea-complexed fraction (UCF) of fatty acids were extracted into hexane, washed twice with distilled water, dried at 40 °C in a rotary evaporator, and then weighed [61,63].

2.6. Analysis of the Fatty Acid Methyl Esters (FAMEs)

The fatty acid composition of the lipid samples (algal oil, NUCF and UCF) was determined after converting them to methyl esters according to GOST 31665 2012. The analysis of the fatty acid profile was carried out by gas chromatography on a Kristal 5000 chromatograph (Chromatec, Russia), column dimensions: 105 × 0.25, carrier gas was nitrogen, inlet temperature 250 °C, the column temperature 140 °C. The chromatographic analysis was performed according to the standards GOST R ISO 5508-2010 and GOST 31663 2012.

2.7. Determination of the Yields of the Different Lipid Fractions

The yield of the NUCF and UCF fractions were determined by gravimetric method. The fractions were dried at 40 °C by vacuum evaporation in pre-weighed dried flasks, and then dried to constant weight at 55 °C in SNOL 200/200 oven. The yields were then calculated as follow:

$$\text{YF } (\%) = \frac{\text{m}_{fraction} \times 100}{\text{m}_{total\ lipids}} \quad (3)$$

where YF is the yield of the lipid fraction (NUCF or UCF); $\text{m}_{fraction}$ is the mass of the lipid fraction in grams, $\text{m}_{total\ lipids}$ is the mass of the algal oil in grams.

The yields of recovery of fatty acids (EPA or DHA) were determined based on the equation:

$$\text{RY } (\%) = \frac{\%\text{FA}_{fraction} \times \%\text{YF}}{\%\text{FA}_{total\ lipids}} \quad (4)$$

where RY is the recovery yield of fatty acids (EPA or DHA) after urea-complexation; $\%\text{FA}_{fraction}$ is the percentage of fatty acids (EPA or DHA) contained in the lipid fraction (UCF or NUCF); and $\%\text{FA}_{total\ lipids}$ is the percentage of the fatty acid in the algal oil before its purification.

2.8. Re-Esterification of the NUCF to Glycerides

The re-esterification of the free fatty acids contained in the NUCF was carried out according to the method developed by D. B. de Rezende et al. [59]. This process involves

the addition of glycerol (4% excess) to the free fatty acids fraction and heating at a high temperature (235 °C) for 80 min. To our knowledge, this was the first time that this re-esterification method was used on algal lipids for obtaining edible oil concentrates.

2.9. Determination of the Quality of the Lipid Samples

The acidity and peroxide number of the samples were determined according to the standards GOST 31933-2012. The organoleptic characteristics of the NUCF oil were assessed according to the standards GOST 1129-2013.

2.10. Statistical Analysis

All the experiments, including the extraction of lipids, gas chromatography analyses of fatty acids, purification, re-esterification, and quality control procedures, were carried out in triplicate. Data in this paper are presented as mean ± standard deviation.

3. Results

On an indicative basis, data on the microalgal growth and the biochemical composition of the biomass are presented in Figures 3 and 4, respectively. The methods of determination of protein, carbohydrates and pigments are described in our previous study [25].

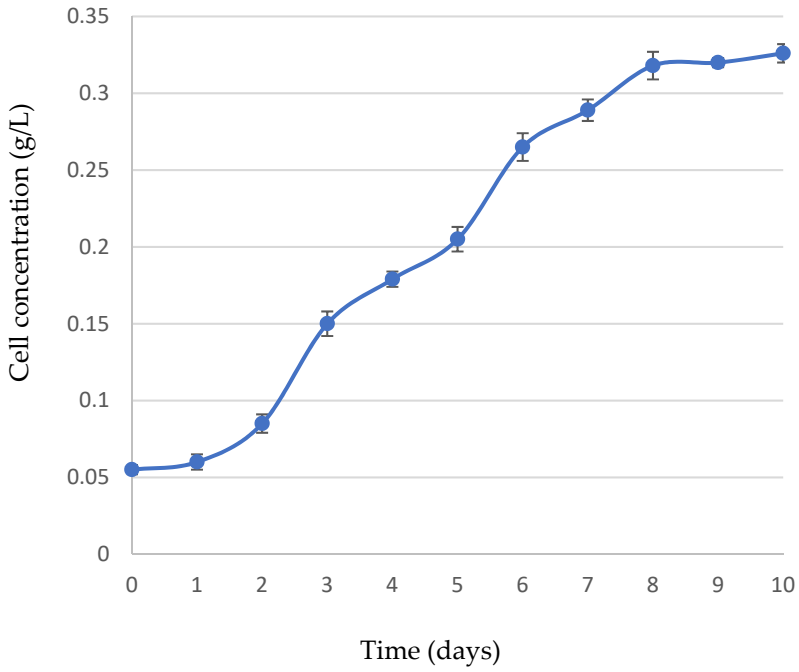

Figure 3. Growth curve of microalga *Chlorella sorokiniana*.

Figure 3 shows that after a lag phase of about 1 day, microalgae cells grow exponentially till reaching a plateau at day 8. However, the stationary phase cannot be concluded due to the short duration of the experiment. Under the mentioned cultivation conditions, the biomass of *Chlorella sorokiniana* contained a majority of protein (42% by dry weight (DW) followed by carbohydrates (33%, including 46% monosaccharides and 54% oligo- and polysaccharides), lipids (22% DW), and pigments (3% DW) represented by chlorophylls (90%) and carotenoids (10%). These data illustrate once again that *Chlorella* biomass is a valuable raw material that could find multiple industrial applications. The value of this microalga does not only lay in its high biomass productivity and considerable content of valuable compounds but also in the quality of these compounds.

After the extraction and purification of lipids, the fatty acid content of the different fractions has been evaluated. The fatty acid profiles and the recovery yields of the different samples are presented in Table 1.

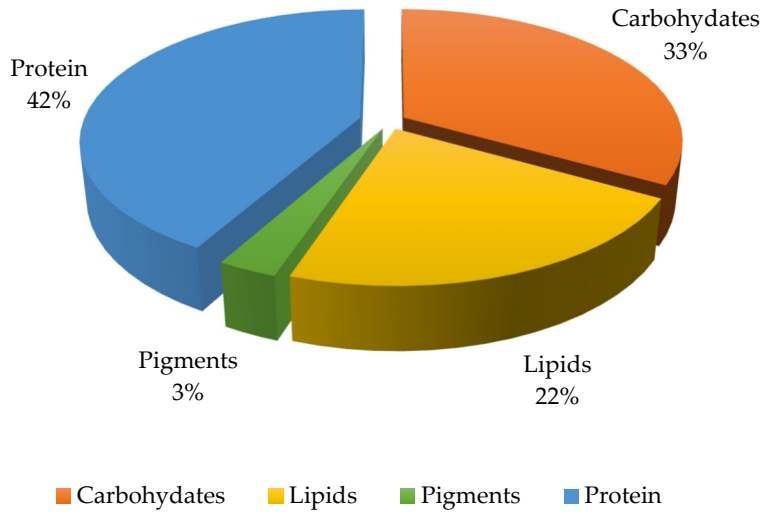

Figure 4. Biochemical composition of the dry biomass of *Chlorella sorokiniana*.

Table 1. Fatty acid profiles and recovery yields of DHA and EPA in the different lipid fractions.

Fatty Acids	Total Lipids (wt %)	NUCF (wt %)	UCF (wt %)
C 11:0	0.448 ± 0.05	0.224 ± 0.03	4.146 ± 0.01
C 12:0	7.988 ± 0.06	0.478 ± 0.04	10.433 ± 0.03
C 14:0	1.347 ± 0.05	0.575 ± 0.07	2.949 ± 0.02
C 14:1	0.489 ± 0.03	0.118 ± 0.03	1.014 ± 0.01
C 15:1	0.179 ± 0.04	0.259 ± 0.04	1.363 ± 0.02
C 16:0	7.882 ± 0.09	1.210 ± 0.04	8.405 ± 0.05
C 18:0	0.810 ± 0.08	0.126 ± 0.02	16.449 ± 0.05
C 18:1 n9 cis	3.643 ± 0.03	8.126 ± 0.10	11.449 ± 0.08
C 18-2n6-cis	1.843 ± 0.07	0.513 ± 0.03	1.272 ± 0.02
C 20:3n3c	1.038 ± 0.06	5.453 ± 0.06	0.022 ± 0.01
EPA: C 20:5n3c	1.563 ± 0.08	**16.529** ± 0.12	1.097 ± 0.06
C 22:2c	4.950 ± 0.05	2.991 ± 0.02	5.458 ± 0.02
C 23:0	4.416 ± 0.06	1.021 ± 0.01	7.719 ± 0.03
DHA: C22:6n3c	4.960 ± 0.06	**35.080** ± 0.13	5.687 ± 0.02
SFA	27.510 ± 0.12	7.520 ± 0.07	54.465 ± 0.26
MUFA	11.547 ± 0.11	11.542 ± 0.06	19.091 ± 0.15
PUFA	60.996 ± 0.17	81.002 ± 0.38	27.376 ± 0.09
Omega 3	50.982 ± 0.12	72.997 ± 0.22	17.008 ± 0.12
Omega 6	4.715 ± 0.03	39.663 ± 0.17	9.681 ± 0.04
Omega 9	6.599 ± 0.02	9.848 ± 0.05	15.028 ± 0.14
Yields (wt %)	22.030 ± 1.05 [1]	8.220 ± 0.31 [2]	23.330 ± 0.60 [2]
Recovery yields (wt %)			
DHA	/	59.09 ± 0.19 [3]	27.27 ± 0.21 [3]
EPA	/	87.14 ± 0.80 [3]	16.42 ± 0.26 [3]

Key: EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; Total Lipids = the totality of the extracted lipids; NUCF = non-urea-complexed fraction; UCF = urea-complexed fraction; wt% = weight percent. [1] The yield of total lipids by dry weight of biomass; [2] the yields of the lipid fractions were determined according to Equation (3); [3] yields of DHA and EPA have been determined according to Equation (4). Note: the text in bold underlines the increase in the percentage of EPA and DHA in the NUCF fraction as a result of Urea-condensation. This shows that this method is efficient and that the goals of this experiment were reached.

The use of urea complexation allows the obtaining of two lipid fractions that could be destined for different purposes. The total lipids were first fractionated and saponified by KOH and the free fatty acids contained in the saponifiable fraction were separated by acid hydrolysis. After determining the yield of the extraction, an ethanolic solution of urea was added to the free fatty acids and left to crystallize at 4 °C. The differences between the NUCF and UCF fatty acid profiles are due to the formation mechanism of urea crystals

with fatty acids. It has been established in previous studies that urea tends to preferably form complexes with fatty acids with a higher degree of saturation [56–58].

The yield of the UCF by dry weight was almost 3-fold higher than the one of NUCF. This fraction contains higher concentrations of monounsaturated fatty acids (MUFA), 19.091%, and saturated fatty acids, 54.46%, and thus could find applications in the biofuel or food industries to produce algae-based oils as these would be more stable to oxidation and resist higher temperatures [24]. According to Bermúdez et al., urea complexation is recommended as an economic method for purification of oils that would allow the longer preservation of FFA in their crystal form [47]. However, further studies are required for providing concrete evidence on the ecological and economical efficiencies of this purification method applied to microalgae lipids.

Results also show that the NUCF is mainly composed of long-chain PUFA, the majority of which are represented by EPA (16.52%) and DHA (35.08%). These results are comparable to the results obtained in a previous study [63]. The recovery yields of DHA and EPA in the NUCF were 59.09% and 87.14%, respectively, making this oil of great potential for the nutraceutical industry.

It is known that free fatty acids are more prone to oxidation than neutral lipids and are linked to increased oil acidity and rancidity [65]. For this reason, the level of free fatty acid is measured to assess the quality and commercial value of oils and fats. In addition, it is possible to re-esterify free fatty acids in oils with glycerol. The glyceride formation commonly involves the use of catalysts such as Zinc and anhydrous niobium phosphate (NbOPO4). These substances have been shown to produce undesirable compounds such as soaps and Zn carboxylates. For this reason, a simple and effective method of re-esterification was implemented using glycerol and high temperature [66]. The quality of the lipid samples has been estimated based on physicochemical and organoleptic characteristics (Table 2). All analyses were performed in triplicate.

Table 2. Physicochemical and organoleptic characteristics of NUCF lipids before and after re-esterification.

Quality Characteristics		NUCF	Re-Esterified NUCF
Physicochemical characteristics	Moisture, %	0.11 ± 0.02	0.18 ± 0.02
	Acidity, mg KOH/g	2.24 ± 0.02	0.28 ± 0.04
	Peroxide number, mmol act. oxygen/kg	9.53 ± 0.05	3.25 ± 0.06
Organoleptic characteristics	Aspect	Yellow extract, solid at room temperature.	Slightly yellow, without any visible turbidity. Fluid oil at room temperature
	Fragrance	Strong acrid smell	Slight fish smell without foreign smells
	Taste	Bitter	Slight fish taste

Note: NUCF = The non-urea complexed fraction.

According to the guidelines of WHO/FAO regarding the quality of edible oils, the maximum allowable limits of moisture, acid value, and peroxide value are 0.2%, 0.6 mg of potassium hydroxide/g oil and 10 milliequivalents oxygen/kg, accordingly [67]. Results show that the NUCF extract is not safe for consumption as this fraction has a high acidity value. After the re-esterification of the free fatty acids, the acidity and peroxide values dropped considerably, which is in accordance with the data found in the literature [66]. The physicochemical and organoleptic characteristics of the obtained oil were within the limits of WHO/FAO standards for edible oils and GOST 1129-2013. The obtained DHA–EPA-rich algal oil could therefore potentially be considered as an alternative to fish oils. This concentrate would present similar health benefits as fish oil without the need for consuming large amounts of it, thus decreasing the calorie intake. These results also point out to the possibility of using this non-toxic method for the re-esterification of free fatty acids of microalgal origin for obtaining a final product suitable for food.

4. Conclusions

The study proposes a method for obtaining algal oils from *Chlorella sorokiniana* with different characteristics that could be destined for various industries. Urea complexation is a well-established method of lipid purification and could be recommended for microalgae oil processing. Results show that the NUCF contained 81% of PUFA with a DHA and EPA recovery yield of 59.09% and 87.14%, respectively. This fraction could be suitable for producing long-chain omega-3 PUFA dietary supplements. The UCF was composed of 19.091% MUFA and 54.46% saturated fatty acids, making it suitable for obtaining cooking oils as well as for biofuels.

After the re-esterification of NUCF with glycerol, the physicochemical and organoleptic characteristics of the resulting oil concentrate were within the norms recommended by the WHO/FAO standards for edible oils and GOST 1129-2013. This product could be interesting not only for its nutritional value, but also in terms of consumer appeal. The biorefinery concept proposed in this article could not only allow for the simultaneous production of edible lipids and biofuels, but also be combined to methods of cascade extraction of other valuable compounds. This could potentially increase the generated profits while adhering to concepts of circular economy.

This study is planned to be pursued. The economic feasibility of the described technology will be further studied and eventually improved. The authors intend to have a more detailed disclosure of socio-economic and environmental aspects in their future research.

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