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A METHOD FOR THE PREPARATION OF LUBRICATING OIL FROM MICROALGAE BIOMASS

METODA WYTWARZANIA OLEJU SMAROWEGO Z BIOMASY MIKROALG

Key words:

lubricating oil, algal oil, microalgae biomass, viscosity-temperature characteristics, FTIR spectrophotometry

Słowa kluczowe:

olej smarowy, olej z alg, biomasa mikroalg, charakterystyka lepkościowo-temperaturowa, spektrofotometria FTIR

Abstract

The paper presents a method for the preparation of lubricating oil from the biomass of single-cell green algae *Chlorella sp.* The microalgae were grown in a synthetic nutrient medium under laboratory conditions. The biomass, which was obtained from the culture, was subjected to the process of dehydration, freeze-drying, and solvent extraction, in order to separate lipids that may be a feedstock for eco-friendly lubricants. The chemical structure of obtained

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bioproducts (biomass and algal oil) was investigated by means of Fourier Transform Infrared Spectrophotometry. Moreover, rheological characteristics (kinematic viscosity at 40 and 100°C, dynamic viscosity at 0–100°C) of the algal oil were determined. The results of the laboratory tests show that the oil has the chemical structure and viscosity-temperature properties similar to the rapeseed oil. This creates a potential opportunity to replace used vegetable lubricants or additives by algal oil in many technical areas.

INTRODUCTION

The rapid development of industry in the world is associated with an increasing demand for energy, but it raises a number of risks, especially related to pollution and environmental degradation [L. 1, 2]. About 90% of global energy demand is covered by coal, oil, and natural gas, but their resources are limited, and over the next few decades will be completely exhausted [L. 2]. Conventional energy sources are replaced with clean renewable energy, including biofuels of the second and third generation, which are derived from materials not competing with food production [L. 3]. Second-generation biofuels are mainly made from lignocellulosic biomass or woody crops, agricultural residues or waste. Third-generation biofuels, referred to as "fuels of the future", are obtained from the oil produced from algae, which are able to accumulate large amounts of lipids in their cells (such as *Chlorella sp.*) [L. 2, 4-6].

Technologies that use the cultivation of algae are becoming increasingly popular in the world, including the Polish market [L. 7]. The annual commercial production of *Chlorella* biomass from major producers such as Germany, Japan, and Taiwan was estimated for 2010 to be 2000 tons, valued at an average 36 USD/kg [L. 8]. Microalgae have a high photosynthetic efficiency, a short cycle of growth, as well as a high content of lipids, proteins and polysaccharides, which makes them one of the most promising sources of biomass that can be used in many industries, including the energy industry [L. 7, 9–11].

In order to discharge the biological material from the microalgae cellular structures outside the cells, various cell-wall disruption methods are used, i.e. [L. 12–15]:

- Liquid homogenization,
- Sonification (ultrasound at a frequency of 15-20 kHz),
- Freeze/thaw cycles,
- Enzymatic digestion,
- Supercritical Fluid Extraction,
- Detergent-based lysis,
- Osmotic stress, and
- Mechanical disruption (e.g., ball mill, mortar and pestle, French press).

In practice, many other chemical, physical and biological methods (such as high-pressure homogenization, thermal shock, decompression, application of chelating agents, solvents or antibiotics) and their combinations with solvent extraction are carried out [L. 8, 12]. Depending on the selected option, the process can be a one-stage or two-stage.

Some currently used ecological lubricants are based on pure, unmodified vegetable oils. These include triacylglycerols (TAGs), i.e. esters derived from glycerol and three fatty acids, whose structure is shown in **Fig. 1**. As a result of the transesterification of TAGs with methanol, fatty acid methyl esters (FAME) are formed and they are used as biofuels and biocomponents of diesel fuel. So far in Europe for these purposes, rapeseed and sunflower oil has been mainly used. This caused the necessity of search for oil plants, which are outside the area of interest of the food industry. In contrast to traditional crops, the cultivation of microalgae helps solve the problem of the quantity and availability of raw materials and protect their resources.

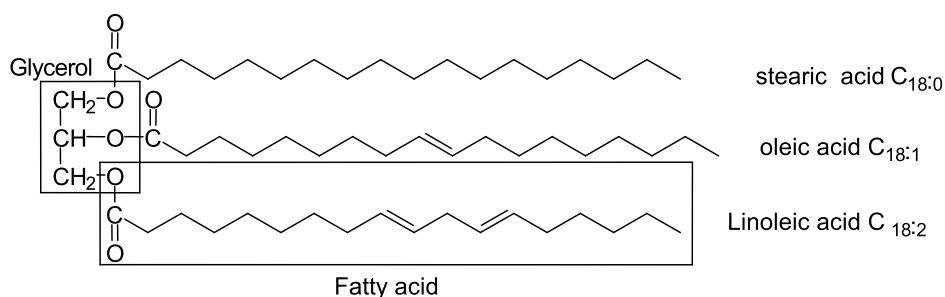


Fig. 1. Exemplary chemical structure of triacylglycerol

Rys. 1. Przykładowa struktura chemiczna triacyloglicerolu

Fatty acids present in natural vegetable oils vary in chain length, the number of double bonds as well as the possible presence of other substituents and functional groups. Natural triglycerides are readily biodegradable, and they are very effective lubricants (characterized by a relatively high viscosity). However, their thermal, oxidative, and hydrolytic stability is unsatisfactory. Therefore, unmodified vegetable oils are usually used under low temperature conditions.

A potential substitute for environmentally friendly lubricants (mainly those based on oils used in the production of food) or their components could be algal oil, especially when a suitable control process of the algae culture can increase the production of the desired metabolites. The results of previous studies [L. 16, 17] indicate that the lubricating properties of algal oil in steel-steel tribological system are only slightly inferior to those of rapeseed oil, and in

coating(WC/C)-coating(WC/C) tribological systems, they are significantly better in both antiwear and antifricition properties. The formulation of original processes related to algal oil will open wide its perspectives for its use in industry.

The aim of this study was to develop a method for the separation and recovery of lipids from the microalgae biomass for the preparation of environmentally friendly lubricating oils. Achieving this objective required the following: the cultivation of microalgae, the separation of the algae biomass from culture medium, the drying of wet biomass, the extraction of lipids from the biomass, and the investigation of the chemical composition and viscosity-temperature properties of the extracted bioproduct.

EXPERIMENTAL

Microalgae biomass production

A cultivation of unicellular algae was conducted in laboratory reactors with a capacity of approximately 3 dm³, illuminated by artificial light (white LED light). The culture medium was tap water supplied with synthetic nutrient medium BG-11 [L. 18]. The culture was inoculated with green algae *Chlorella sp.* from Culture Collection of Baltic Algae (University of Gdansk, Institute of Oceanography). The experiment was carried out in 30-day cycles under laboratory conditions ensuring the effective growth of the algae biomass [L. 19]. The conditions were as follows:

- Temperature: 26±2°C,
- Mixing: 260 rpm,
- Light intensity: 700 lux, and
- Photoperiod: 16/8 h (light/darkness).

Drying the algae biomass

Separation of the microalgae cells from culture medium consist of the following processes: sedimentation, centrifugation, and lyophilisation. During the sedimentation process, the algal suspension was initially separated from the water solution. The obtained biomass was subjected to centrifugal force using the *Eppendorf Centrifuge 5430*–8000 rpm, 10 min. In a next step, the wet sample was frozen with liquid nitrogen, and then subjected to freeze-drying at –80°C under vacuum (<10 Pa) using *Lobconco FreeZone 2.5 plus*.

Cell disruption and extraction of lipids

The disruption of the microalgae cells and the solvent extraction of lipids were carried out in a single stage process according to the Bligh and Dyer method

[L. 20]. Samples of freeze-dried biomass (40 mg each) were placed in test tubes, and they were then filled with distilled water and mixture of methanol and chloroform (in a volume ratio of 3:4). The prepared samples were shaken (*Eppendorf Thermomixer C*) for 20 s and then refilled with another portion of chloroform and distilled water. This mixture was centrifuged (*Eppendorf Centrifuge 5430*) – 5000 rpm for 10 min. The lipid fraction was collected into a laboratory vessel and the residual solid was subjected to the next extraction process. The chloroform fraction was evaporated using a rotary evaporator.

Viscosity-temperature characteristics

The viscosity-temperature characteristics of the algal oil were determined using a rotational rheometer *Anton Paar Physica MCR 101* equipped with a diffusion air bearing, oil free *Jun-Air* compressor, and air dryer unit. The tests were carried out using a measuring cone-plate system at a constant shear rate of 100 s^{-1} and temperature of 0-100°C.

The kinematic viscosity at 40°C and 100°C was determined according to the standard PN-EN ISO 3104:2004 *Determination of kinematic viscosity and calculation of dynamic viscosity* using *Herzog HVU 481* automated capillary viscometer. Based on the obtained results, the viscosity index was determined according to the standard PN-ISO 2909:2009 *Calculation of viscosity index from kinematic viscosity*.

The results were compared to the characteristics of commercially available oils: refined rapeseed oil and poly- α -olefin synthetic oil (PAO-6).

Chemical composition of algal oil

To analyse the chemical composition of obtained products (biomass and algal oil) Fourier Transform Infrared Spectrophotometry (FTIR), in the wave number range of 4000-400 cm^{-1} (resolution 4 cm^{-1} , 30 scans for background and sample), was applied. The spectral investigations were performed by means of *Jasco FT/IR-6200* spectrometer. To examine the algal oil, an Attenuated Total Reflectance (ATR) sampling technique with ZnSe crystal was used. To study the dried biomass, transmission sampling by means of KBr pellet (279.1 mg KBr, and 2.3 mg of sample) was used. The resulting spectrum of the biomass was automatically obtained in the deconvolution algorithm. The distinct absorption bands in all recorded spectra were assigned to specific molecular groups based on published studies [L. 21–24].

RESULTS

The cultivation of microalgae *Chlorella sp.* in the laboratory required suitable conditions, limiting the effective growth of the biomass, i.e. temperature,

culture medium rich in nutrients (synthetic medium), and the access of carbon dioxide and light (within the respective cycles). The efficiency of microalgae growth under the experimental conditions, as determined by weight, was 2.5–3.0 mg d.w./dm³.

From the resulting suspension, after prior sedimentation and centrifugation, the wet biomass was separated. It was then dried by evaporation of the ice crystals in the vacuum chamber of the freeze dryer. The use of a lyophilisation process ensured that a high quality product was obtained by minimizing the potential for undesirable reactions (e.g., oxidation), and microbial activity. The dried algal biomass (powder) was in the form of lumps (**Fig. 2**).



Fig. 2. Freeze-dried biomass of microalgae *Chlorella* sp.
Rys. 2. Zliofilizowana biomasa mikroalg *Chlorella* sp.

Important indicators for the control of the microalgae culture and the evaluation of the biomass quality are the relative contents of lipids, proteins, and polysaccharides. In this study, the most important was to achieve the highest possible productivity of lipids. So far, the determination of these parameters is needed to be respectively measured individually using conventional methods, which are complicated, expensive, and time-consuming. Currently, for this purpose, a FTIR spectrophotometry is also used [**L. 25**]. The advantages of this technique, in addition to a low cost and time-savings, are the low quantity demand on the sample for analysis, high repeatability, and lack of a need to use organic solvents.

Infrared spectrophotometry was used at this stage of research for the qualitative analysis of the freeze-dried biomass. The transmission spectrum includes three main absorption bands: 3000–2800 cm⁻¹ and a peak in 1738 cm⁻¹ (lipids, fatty acids), 1720–1450 cm⁻¹ (proteins) and 1200–950 cm⁻¹ (polysaccharides). **Fig. 3** shows the FTIR spectrum in the range of 2000–400 cm⁻¹, previously subjected to mathematical processing (deconvolution), aimed at increasing its resolution. Interpretation of the characteristic spectral bands is shown in **Tab. 1**.

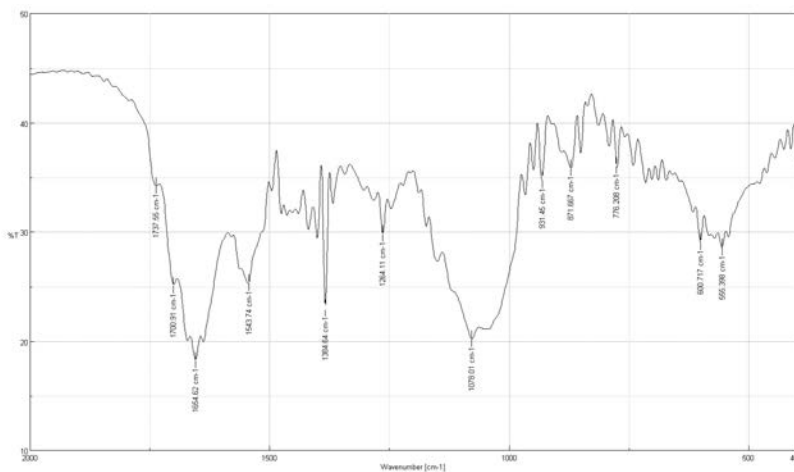


Fig. 3. FTIR spectrum of *Chlorella sp.* biomass (transmission technique)

Rys. 3. Widmo FTIR biomasy *Chlorella sp.* zarejestrowane techniką transmisyjną

Table 1. Interpretation of spectral frequencies in Fig. 2

Tabela 1. Interpretacja pasm spektralnych widma przedstawionego na Rys. 2

Frequency [cm ⁻¹]	Nature of vibration	Identification
1738	$\nu(\text{C}=\text{O})$ stretching of ester groups	lipids
1655	$\nu(\text{C}=\text{O})$ stretching of amides I	proteins
1544	$\delta(\text{N-H})$ bending of amides II	
1457	$\delta_{\text{as}}(\text{CH}_2)$, $\delta_{\text{as}}(\text{CH}_3)$ bending of methyl	
1385	$\delta_{\text{as}}(\text{CH}_2)$, $\delta_{\text{as}}(\text{CH}_3)$ bending of methyl groups $\nu_{\text{s}}(\text{C}-\text{O})$ stretching of COO^- groups	proteins / lipids
1264	$\nu_{\text{as}}(>\text{P}=\text{O})$ stretching of phosphoryl groups	Phosphorylated proteins
1200-950	$\nu(\text{C}-\text{O}-\text{C})$ stretching of glycosidic linkages	polysaccharides

Lipids were isolated from the dried biomass in a one-step process (homogenization of the dried biological substrate and lipid extraction) using organic solvents (chloroform, methanol) and water. As a result of this experiment, the miscible system was separating into two layers: a chloroform layer containing most of the lipids and an aqueous methanol layer containing more polar compounds [L. 26]. After evaporation of the chloroform and other residual solvents, the chemical composition of the obtained bioproduct (algal oil) was identified based on a FTIR spectrum recorded using the ATR technique (Fig. 4). The productivity of lipids was $26 \pm 2\%$ by weight (in relation to the freeze-dried biomass).

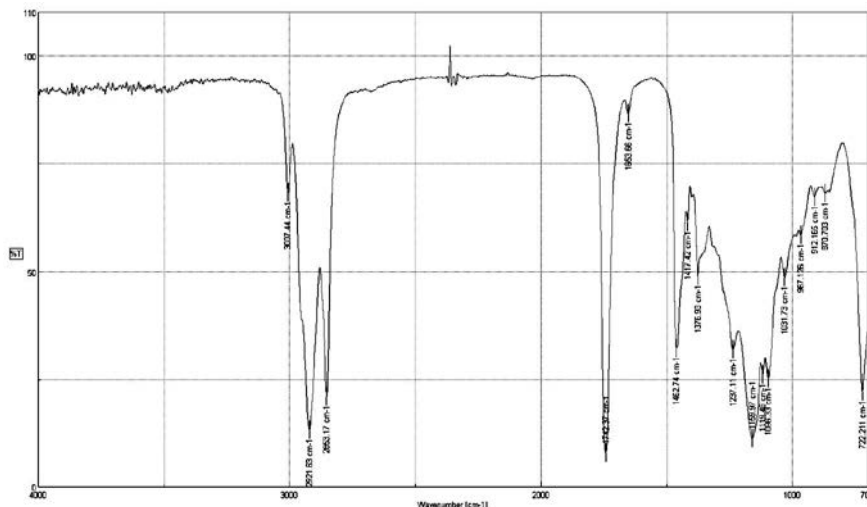


Fig. 4. The FTIR spectrum of algal oil obtained from *Chlorella sp.* biomass (ATR technique)
 Rys. 4. Widmo FTIR oleju otrzymanego z biomasy mikroalg *Chlorella sp.* zarejestrowane techniką ATR

The FTIR spectrum of the obtained lipids is significantly different from the spectrum of the biomass from which they were extracted. There has been a large increase in the signals coming from the TAGs. Detailed interpretation of the main spectral bands (**Tab. 2**) indicates that it is a vegetable oil with the typical structure shown in **Fig. 1**. In this spectrum, there are intense peaks derived from long-chain aliphatic hydrocarbons, both saturated (2922 cm^{-1} , 2853 cm^{-1} , 1463 cm^{-1} , 1377 cm^{-1} , 722 cm^{-1}) and unsaturated (3007 cm^{-1} and $1000\text{--}700\text{ cm}^{-1}$), as well as esters (1742 cm^{-1} , $1250\text{--}1050\text{ cm}^{-1}$).

Table 2. Interpretation of spectral frequencies in Fig. 4

Tabela 2. Interpretacja pasm spektralnych widma przedstawionego na Rys. 4

Frequency [cm^{-1}]	Nature of vibration	Identification
3007	$\nu(\text{cis} = \text{C-H})$ stretching of methylene groups	alkenes
2922	$\nu_{\text{as}}(=\text{CH}_2)$ stretching of methylene groups	alkanes
2853	$\nu_{\text{s}}(=\text{CH}_2)$ stretching of methylene groups	
1742	$\nu(\text{C=O})$ stretching of carbonyl groups	saturated aliphatic esters
1463	$\delta_{\text{s}}(\text{CH}_2)$ bending of methylene groups $\delta_{\text{as}}(\text{CH}_3)$ bending of methyl groups	alkanes
1377	$\delta_{\text{s}}(\text{CH}_3)$ bending of methyl groups	
1238	$\nu(\text{C-O})$ stretching	esters
1160		
1119		
1096		
722	$-(\text{CH}_2)_n-$ rocking of long-chain methylene groups <i>cis</i> $-\text{HC}=\text{CH}-$ bending of methylene groups	long chain alkanes alkenes

This allows one to conclude that, by using the proposed method, it is possible to receive oil from the microalgae biomass, and the chemical composition of this algal oil is typical for other vegetable oils. The authors plan to use FTIR spectrophotometry for quantitative analysis (use amide I band $\sim 1655\text{ cm}^{-1}$ as an internal reference peak for the calculation of the relative content of lipids in algae biomass) in future studies [L. 25].

The obtained algal oil was then subjected to rheological testing. The viscosity of vegetable oils depends substantially on the length of the chains of fatty acids of which they are composed. However, a change in their viscosity with temperature is not as significant as in the case of mineral oils. In addition, vegetable oils have a high viscosity index, even two-times higher than mineral oils. In the experimental work, viscosity-temperature characteristics of the algal oil were determined and compared with those of commercial vegetable oil (rapeseed oil), and synthetic oil (PAO-6). **Table 3** presents the kinematic viscosities at 40°C and 100°C and viscosity indexes of these oils.

Table 3. Comparison of kinematic viscosity of algal oil and commercial oils

Tabela 3. Porównanie lepkości kinematycznej oleju z alg i olejów komercyjnych

Parameter	Value		
	PAO-6	rapeseed oil	algal oil
kinematic viscosity at 40°C , mm^2/s	31.6	35.2	37.5
kinematic viscosity at 100°C , mm^2/s	6.0	8.5	8.3
viscosity index	139	232	206

The kinematic viscosity of algal oil at 40°C was the highest ($37.5\text{ mm}^2/\text{s}$) of all tested oils. Its value was similar to rapeseed oil ($35.2\text{ mm}^2/\text{s}$). The kinematic viscosity of algal oil at 100°C was slightly lower than that of rapeseed oil (respectively, $8.3\text{ mm}^2/\text{s}$ and $8.5\text{ mm}^2/\text{s}$), but higher than that of synthetic oil ($6.0\text{ mm}^2/\text{s}$) tested under the same conditions. Similarly, in the case of the viscosity index, algal oil was only slightly worse than rapeseed oil (VI respectively 206 and 232) and better than PAO-6.

Dynamic viscosity is also an important utility parameter of fuels and lubricants, because it determines their resistance to shearing flows and their tribological properties. The analysis of obtained results shows that the dynamic viscosity of the tested oils decreases with increasing temperature over the measuring range. The range of the dynamic viscosities at $0\text{--}100^\circ\text{C}$ was $0.249\text{--}0.00978\text{ Pa}\cdot\text{s}$ for rapeseed oil, $0.186\text{--}0.00408\text{ Pa}\cdot\text{s}$ – for algal oil and $0.186\text{--}0.00250\text{ Pa}\cdot\text{s}$ – for synthetic oil (**Fig. 5**). Therefore, it can be concluded that this parameter in the case of algal oil reached lower values than rapeseed oil, but slightly higher than the synthetic oil.

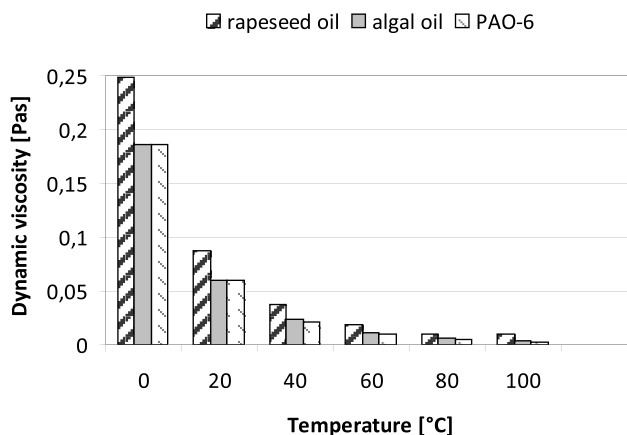


Fig. 5. Comparison of viscosity-temperature characteristics of algal oil and commercial oils

Rys. 5. Porównanie charakterystyk lepkościowo-temperaturowych oleju z alg oraz olejów komercyjnych

Based on the results of all laboratory tests, it can be concluded that the product consists of triacylglycerols, which were obtained in a single stage process of cell disruption and solvent extraction of lipids. Its chemical composition and viscosity-temperature characteristics are similar to commercial vegetable oils. In order to confirm the possibility of replacing the vegetable oils with the oil derived from the microalgae *Chlorella sp.*, it is necessary to conduct further studies on the effective growth of algae biomass and accumulation of high-value lipids in the microalgae cells as well as investigate the properties of algal oil produced under different conditions in terms of the standard requirements for the lubricants.

CONCLUSIONS

The developed method allows the production of lubricating oil from the biomass of microalgae *Chlorella sp.* under laboratory conditions. The chemical composition of the obtained algal oil is typical of vegetable oils, and it includes mainly lipids (TAGs). Based on the results of the study, it has been found that the potential of microalgae in the preparation of lubricants is enormous, and in the next few years, it may be used at commercial scale.

The obtained results show the possibility of using algal oil as a substitute for commercial vegetable oils in technical applications, for example, to produce environmentally friendly lubricants dedicated to the lubrication of non-conventional frictional couples (e.g., machine elements with low friction coatings). This concept requires a more detailed analysis, but it can be a starting point for comprehensive research in this area. Further work will focus

on increasing the algae biomass production yield through the selection of the optimum parameters of cultivation. An increase in the content of triacylglycerols can be achieved by one of the most promising methods consisting in nutrient stress (e.g., nitrogen and/or phosphorous starvation). Moreover, a large biodiversity of microalgae and the changes that occur in genetic and metabolic engineering open up the possibility of producing a much wider range of new bioproducts.

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Streszczenie

W artykule przedstawiono metodę otrzymania oleju smarowego z biomasy mikroalg *Chlorella sp.* Przeprowadzono laboratoryjną hodowlę mikroalg na bazie pożywki syntetycznej. Uzyskana z hodowli biomasa została poddana procesowi odwodnienia, liofilizacji oraz ekstrakcji rozpuszczalnikowej w celu pozyskania lipidów, które mogą być substratem przy komponowaniu ekologicznych środków smarowych. Uzyskane bioprodukty (biomasa i olej z alg) zostały poddane badaniom spektrofotometrycznym w podczerwieni z transformacją Fouriera oraz badaniom reologicznym. Wyniki przeprowadzonych badań laboratoryjnych wskazują, iż otrzymany olej z alg ma strukturę chemiczną i właściwości lepkościowo-temperaturowe (lepkość kinematyczna w temperaturze 40 i 100°C, lepkość dynamiczna w temperaturze 0–100°C) zbliżone do oleju rzepakowego. Stwarza to potencjalną możliwość zastąpienia stosowanych dotychczas roślinnych olejów smarowych lub dodatków uszlachetniających przez olej z alg w wielu obszarach technicznych.