

Fatty acid profile of *Chlorella sorokiniana* InaCC M38 grown in tofu wastewater for biofuel potential

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Abstract. *Susanty D, Safitri LN, Hutagaol RP, Oksari AA, Nurlela. 2025. Fatty acid profile of Chlorella sorokiniana InaCC M38 grown in tofu wastewater for biofuel potential. Nusantara Bioscience 17: 226-234.* *Chlorella sorokiniana* Shihira & Krauss, 1965 is a microalgae that can grow in tofu liquid waste medium by utilizing the nutrients in the waste in the form of carbon (C), nitrogen (N), phosphorus (P), and potassium (K). This research aims to determine the lipid content and fatty acid compounds contained in *C. sorokiniana* cultured in 25, 30, and 35% Tofu Liquid Waste (TLW) medium using two extraction methods. In this study, the growth of *C. sorokiniana* on TLW medium was determined by calculating the cell density of *C. sorokiniana* using a hemocytometer, and the biomass was collected by centrifuging. *Chlorella sorokiniana* was cultivated for seven days. Lipid extraction was performed using maceration and a combined ultrasonic-assisted extraction (UAE)–maceration method. Biomass and lipid yields, as well as fatty acid methyl esters (FAME) profiles, were analyzed using GC–MS. *Chlorella sorokiniana* grows well on 30% TLW medium with a dry biomass weight of 0.45 g/L on day 7; extraction using the combined method extracted more fatty acid compounds than the maceration method. Lipid yield using the extraction by combined extraction method had more excellent results (4.61%) than the maceration method (3.82%). The fatty acid composition of *C. sorokiniana* extracted by combination extraction consists of 11 types of Saturated Fatty Acids (SFA), two types of Monounsaturated Fatty Acids (MUFA), and two types of Polyunsaturated Fatty Acids (PUFA). The Saturated fatty acid most commonly contained in *C. sorokiniana* was palmitic acid, which could be applied as biodiesel. *Chlorella sorokiniana* cultured in 30% TLW has a higher percentage of SFA (56.58%) with less PUFA, making it more potential for biodiesel.

Keywords: *Chlorella sorokiniana*, fatty acid, maceration, medium, ultrasound-assisted extraction

INTRODUCTION

Lipids from microalgae are commercial compounds in various industries, such as nutritional products, pharmaceuticals, and biodiesel (Sun et al. 2018). The utilization of lipids from microalgae is related to their fatty acid composition. Several studies state that omega-3 and omega-6 fatty acids are essential for pharmaceutical and nutritional needs (Perdana et al. 2021), while palmitic and oleic acids are the primary fatty acids for biodiesel (Sun et al. 2018). The development of microalgae as a lipid source must consider culture costs. Waste medium is a solution to reduce costs for microalgae production.

The use of municipal wastewater for the cultivation of *Acutodesmus obliquus* CN01 and *Desmodesmus maximus* CN06 showed high lipid productivity in *D. maximus* CN06 (3.43 mg/L.day), which mainly consisted of hexadecanoic acid (C16:0) and oleic acid (C18:1), making it suitable for biodiesel (Purba et al. 2022). *Scenedesmus obliquus* cultured in Chinese city wastewater yielded a lipid yield of 0.367 gL⁻¹, indicating its feasibility for biodiesel production (Han et al. 2021). *Chlorella vulgaris* Beij. grown in wastewater with higher light intensity show nitrogen and phosphorus consumption of 75%, with oleic acid as the highest fatty acid, so it has the potential to be a

source of biodiesel (Nzayisenga et al. 2020). *Monoraphidium braunii* was cultured in the waste medium, produced high lipid productivity of 5.26 mg L⁻¹ day⁻¹ (El-Sheekh et al. 2023).

One of the microalgae that is known to be able to grow in waste medium is *Chlorella sorokiniana* Shihira & Krauss, 1965. The growth of *C. sorokiniana* has been studied in real wastewater (RWW) (Rani and Ojha 2021), unsterilized domestic wastewater (Bulynina et al. 2023), cassava wastewater (Padri et al. 2025), cooking cocoon wastewater (Xue et al. 2021), wastewater from the industrial processing of instant coffee (WIC) (Melo et al. 2022), chicken broiler waste medium (Susanty and Oksari 2020), and tofu liquid waste (Mursandi et al. 2022). Tofu liquid waste (TLW) contains carbon, nitrogen, and phosphorus, so that it can be used as a source of nutrition for the microalgae *C. sorokiniana*. The growth of *C. sorokiniana* at TLW medium concentrations of 15, 20, 25, and 30% shows that 30% TLW medium provides the best growth (Mursandi et al. 2022) but has yet to show optimal results. Further research needs to be conducted to determine the optimum medium concentration by testing the growth of *C. sorokiniana* on 25, 30, and 35% TLW medium.

Growth medium can influence fatty acids in microalgae. *Chlorella sorokiniana* cultured on PE-001

medium showed the presence of palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid (Qiu et al. 2017). *Chlorella sorokiniana* cultured in Tris-acetate-phosphate medium (TAP) with ascorbic acid and Iron III Chloride (FeCl_3) showed the C16 and C18 fatty acids, but in different amounts (Ammar et al. 2021). *Chlorella sorokiniana* cultured mixotrophically using DWW medium yielded a total lipid of 0.433 ± 0.060 g/g DW (Hamidian and Zamani 2022). The use of TLW as a culture medium for *C. sorokiniana* has been studied. However, the fatty acids have not been studied based on the concentration of the medium used.

The extraction method used also influences the fatty acids extracted from microalgae biomass. This research was carried out in two ways: by maceration and a combination of ultrasound-assisted extraction (UAE) and maceration. *Chlorella sorokiniana* has strong cell walls with a complex matrix composed of carbohydrates, polypeptides, and glycoproteins (Kim et al. 2024), so the process of destroying microalgae cell walls is necessary. The UAE method provides a cavitation wave effect that helps the process of destroying the microalgae matrix, making the extraction process more effective (Shevelyuhina et al. 2022). Combining UAE and maceration extraction needs to be compared with maceration to determine the difference in the yield of extracted lipids and fatty acids profile. Research regarding the identification of fatty acid compounds of *C. sorokiniana* cultured from TLW medium needs to be carried out to determine the fatty acid composition of *C. sorokiniana* cultured at different TLW medium concentrations and different extraction methods. The fatty acid profile of *C. sorokiniana* cultured in various concentrations of TLW medium and extraction using a combination of UAE and maceration is a novelty of this research. This study aims to examine lipid productivity and fatty acid profiles at TLW concentrations and extraction.

MATERIALS AND METHODS

Study area

Chlorella sorokiniana inaCC M38 is an isolate purchased from the inaCC BRIN collection. Tofu liquid waste used as culture medium was obtained from tofu factories in the Ciriung area, Bogor District, West Java, Indonesia. In this study, the cultivation of *C. sorokiniana* was carried out on tofu liquid waste medium with three concentration variations, namely 25, 30, and 25%. *Chlorella sorokiniana* biomass was extracted using two extraction methods, namely maceration and combined maceration with UAE, to compare lipids and fatty acids.

Nutrient analysis of tofu liquid waste

In this study, the tofu liquid waste was used as a culture medium for *C. sorokiniana*. Macronutrients in the form of carbon (C), nitrogen (N), phosphorus (P), and potassium (K) contained in tofu liquid waste are tested first based on SNI 19-7030-2004.

Preparation of tofu liquid waste medium

The material used as a medium for *C. sorokiniana* is tofu liquid waste that is taken from tofu pressing. Tofu liquid waste was taken by homogenizing the waste and measuring the pH before being used as a culture medium. Variations in TLW medium concentration were 25, 30 and 35%. The medium was added to NaOH 2N solution until it reached a neutral pH of 7 (Mursandi et al. 2022).

Cultivation of the microalgae *Chlorella sorokiniana*

Cultivation of the microalgae *C. sorokiniana* was carried out in TLW medium with concentrations of 25, 30, and 35%, and sterilization was carried out using an autoclave at a temperature of 121°C , pressure of 1 atm for 15 minutes. *Chlorella sorokiniana* was cultured in sterile LCT medium with an initial density of $\pm 10^5$ cells/mL. Cultivation was carried out at 30°C and light intensity from two TL lamps (2,300 lux) (Mursandi et al. 2022). The remaining waste after cultivation is disposed of in a collection of organic waste for which the laboratory has prepared a collection tank.

Calculation of *Chlorella sorokiniana* cell abundance

Chlorella sorokiniana cells were taken with a pipette and then observed under a microscope with a magnification of 40×10 . The abundance of *C. sorokiniana* cells was counted with three repetitions. The abundance of *C. sorokiniana* cells was calculated using an improved Neubauer hemocytometer. Cell abundance was determined by counting microalgae cells in 5 small boxes (Mursandi et al. 2022).

$$\text{Cell density (cells/mL)} \times N = \text{Total number of cells} \times 10^4$$

Chlorella sorokiniana biomass collection

Harvesting or collection of *C. sorokiniana* microalgae biomass was carried out on day 7 (during optimum growth). *Chlorella sorokiniana* biomass was harvested by separating the medium from the microalgae using a centrifuge at a speed of 3,600 rpm for 3 minutes. The biomass was dried at room temperature for 24 hours until dry biomass was obtained (Mursandi et al. 2022).

Lipid extraction of *Chlorella sorokiniana*

Chlorella sorokiniana biomass was extracted using a combined method of Ultrasonic-Assisted Extraction (UAE) and maceration. The sample was added with n-hexane solvent (Sivaramakrishnan and Incharoensakdi 2019) in a ratio of 1:10 (w/v). The sample was put into an ultrasonic device for an extraction process for 90 minutes at a frequency of 42 KHz at room temperature. After the sample was sonicated, it was continued with maceration for 24 hours and stirred using a shaker. After the extraction process was complete, filtering was carried out using filter paper to separate the extraction results from the resulting dregs. The solvent was removed using a rotary evaporator until no more solvent drips. The remaining solvent is evaporated using a water bath.

$$\text{Lipid content (\%)} = (\text{lipid weight}) / (\text{sample weight}) \times 100\%$$

Esterification process of *Chlorella sorokiniana*

The esterification process begins by placing the sample into a boiling flask and mixing it with 5 mL of 1% sulfuric acid dissolved in methanol. The mixture was then heated using a reflux condenser for 1 hour. After the mixture had cooled, 20 mL of distilled water and 5 mL of n-hexane were added to the solution. The methyl ester is then taken by evaporating the hexane fraction of the mixture.

Identification of fatty acid methyl ester (FAME) of *Chlorella sorokiniana*

The fatty acid compounds in *C. sorokiniana* extract were identified using Gas Chromatography-Mass Spectrometry (GC-MS), carried out on the optimal yield of *C. sorokiniana* microalgae oil extract by injecting one μL of sample solution into the GC-MS. The mobile phase used is ultra high purity (UHP) helium, while the stationary phase used is an RTX 5 column, which is non-polar, containing 5% diphenyl and 95% dimethylpolysiloxane.

Data analysis

Cell growth analysis was done by making a growth curve between cultivation time and cell density. The dry biomass of *C. sorokiniana* was compared among three LCT medium concentration treatments. Data analysis of the influence of TLW concentration on biomass by one-way ANOVA statistical test and Duncan Multiple Range Test (DMRT) test using IBM SPSS version 23. The fatty acids identified from the GC-MS results were compared using the extraction method and the culture medium concentration.

RESULTS AND DISCUSSION

Tofu liquid waste nutrition

Tofu liquid waste is a by-product of tofu production, which is still rich in organic substances. The use of this waste has been researched as plant fertilizer because of the presence of elements N, P, and K. The right amount of N, P, and K will promote growth, but if it is excessive, it will have a negative impact, so appropriate concentrations are needed (Kurniawan et al. 2024). Tofu Liquid Waste (TLW) can be used as a culture medium because it contains good nutrients for the growth of *C. sorokiniana* (Mursandi 2022). Table 1 shows that the availability of nutrients (carbon, nitrogen, phosphorus, and potassium) in TLW is relatively high. *C. sorokiniana* can reduce 78.82% nitrate and 88.17% phosphate in dairy wastewater (DWW) (Hamidian and Zamani 2022).

Tofu liquid waste is a by-product of tofu production, which is still nutrient-rich. The availability of nutrients in the medium influences the rate of microalgae productivity because microalgae utilize nutrients as a source for their body's metabolism (Ziganshina et al. 2022). Nitrogen plays a role in the formation of amino acid compounds, lipids, and chlorophyll (Negi et al. 2015; Toumi and Politaeva 2021). Potassium plays a role in the process of photosynthesis and affects cell density (He et al. 2020).

The growth and biomass of *Chlorella sorokiniana*

TLW contains carbon and nitrogen in high concentrations (Table 1). TLW dilution was conducted to see the limits of *C. sorokiniana*'s growth ability. At the three TLW dilution concentrations (25, 30, and 35%), *C. sorokiniana* could adapt and grow well immediately. This condition is indicated by the cell density, which directly increases on the first day of cultivation. However, at 25% TLW, the cell division ability of *C. sorokiniana* began to decrease on the fourth day, caused by the amount of nutrients being more limited than in the other two concentrations. The 30% TLW provides good growth with more cell density than 25 and 35% TLW (Figure 1). Nutrient availability at 30% TLW provides more appropriate growth with the highest cell density on day 7 (4.89×10^5 cells/mL) with a dry biomass of 0.45 g/L.

TLW generally contains nitrogen as ammonium (Nuryoto et al. 2025). The growth of *C. sorokiniana* at 35% TLW was lower than at 30% TLW. The presence of high levels of ammonium can be toxic to cells. Free ammonium can induce reactive oxygen species (ROS formation) in cells, inhibiting cell growth (Shen et al. 2020). Cell density correlates with the biomass produced. The high cell density of *C. sorokiniana* at 30% TLW provides a high biomass (Table 2).

Lipid yield and fatty acid profile of *Chlorella sorokiniana*

The highest lipid yield was obtained from *C. sorokiniana*, cultured at 30% TLW, and extracted using a combined extraction method (Figure 2). Extraction by sonication utilizes ultrasonic waves so that the solvent exposed to these waves experiences an increase in density; the molecules approach each other and become denser. Increasingly larger cavitation bubbles often accompany this process. The collision between the microalgae cell wall and the solvent medium caused by ultrasonic vibrations opens the pores in the cell wall. As a result, the components contained in microalgae can more easily dissolve into the solvent through a diffusion process (Shevelyuhina et al. 2022). Microalgae cells have thick and rigid cell walls, so the combination of UAE and maceration gives optimal results for lipids. There are also more types of fatty acid compounds extracted using the combined UAE and maceration extraction method compared to only the maceration method (Figure 3). The process of breaking down microalgae cell walls with the help of sonication produces a more diverse fatty acid composition (twelve types of saturated fatty acids and four types of unsaturated fatty acids) than the maceration method (5 types of saturated fatty acids and two types of unsaturated fatty acids). These results are almost the same as previous research, which identified 19 different types of fatty acids (Djamaludin and Chamidah 2021).

The extract obtained from the combined extraction method contains higher lipid yield percentages than the maceration method. Microalgae cells have thick and rigid cell walls. Hence, the combination of UAE and maceration gives a higher lipid yield percentage than the maceration method. The research results showed that extraction,

starting with breaking down the cell walls using ultrasound, produced an extract with higher amounts of fatty acids. *Chlorella* has the characteristics of a strong cell wall (Widyaningrum and Prianto 2021), so special treatment is required to break down the cell wall, enhancing the diffusion of molecules inside the cell with an extraction solvent.

Extraction using the combined method yielded a greater variety of extracted fatty acids compared to maceration (Figure 3). Monounsaturated fatty acids were extracted using the combined extraction method. The percentage of saturated fatty acids was higher in the combined extraction method compared to the maceration method (Figure 4). The extraction method can affect the profile of the extracted fatty acids (Pérez-Barradas et al. 2023). The percentage of saturated fatty acids was higher in the combined extraction method with myristic acid. The palmitic acid extracted using the maceration method was higher than that extracted using the combined method. Maceration extraction showed a higher content of unsaturated fatty acids, potentially resulting in a lower cetane number for biodiesel use.

Identification of fatty acid compounds in *C. sorokiniana* cultured at different TLW medium concentrations was chosen based on the optimal results of the extraction method, namely, using a combination of UAE extraction and maceration. *Chlorella sorokiniana* cultured at 30% TLW produces more diverse types of saturated fatty acids than those cultured at 25 and 35% TLW (Figure 5). The dominant fatty acids in *C. sorokiniana* cultured at all variations in TLW concentrations were C16:0 and C18:2. These results are

comparable to other studies on fatty acids in *Chlorella* (Table 3).

The 9,12-octadecadienoic acid (C18:2) is identified in *C. sorokiniana* that was cultured in TLW 25 and 35%. Another form of C18:2 identified in *C. sorokiniana* is 10,13-octadecadienoic acid, which is also found in *C. vulgaris* (Morowvat and Ghasemi 2016). *Chlorella sorokiniana* cultured at 25 and 35% TLW showed the presence of 7,10-Hexadecadienoic acid. This fatty acid was identified in *Chlorella* (Fernandes and Cordeiro 2020). The stearic acid (C18:0) and palmitoleic acid (C16:1) were only identified in *C. sorokiniana* cultured in 30% TLW medium.

Table 1. Nutrition contained in tofu liquid waste

Component	Content (mg/L)
Organic Carbon (C)	1,136
Nitrogen (N)	5,879
Phosphorus (P)	74.4
Potassium (K)	437.1

Table 2. Biomass (dry weight) of *C. sorokiniana* at various concentrations of TLW medium

Concentration of TLW medium	Biomass of <i>C. sorokiniana</i> (g/L)
25%	0.36 ^a ±0.07
30%	0.45 ^b ±0.05
35%	0.43 ^{ab} ±0.07

Note: There is no statistical difference between numbers separated by the same letter at $p < 0.05$ using DMRT

Table 3. Comparison of the major fatty acids in *Chlorella*

Species	Medium	Major Fatty Acid	Reference
<i>Chlorella vulgaris</i>	BG11	Palmitic, oleic, and linoleic acids	(Moradi-Kheibari et al. 2022)
<i>Chlorella vulgaris</i>	wastewater of the dairy industry with 25% dilution	Palmitic acid	(Khalaji 2022)
<i>Chlorella vulgaris</i>	Bold Bassal Medium with MnCl	Palmitic acid (53.59%)	(Saber et al. 2024)
<i>Chlorella sorokiniana</i>	BG11 with secondary treated effluents	α -linolenic acid (17.652%) and Palmitic acid (8.321%)	(Asadi et al. 2020)
<i>Chlorella sorokiniana</i> KNUA114	BG11 (35°C)	Palmitic Acid (43.25%), C18:2 ω 6 (17.87%)	(Yun et al. 2020)
<i>Chlorella sorokiniana</i> inaCC M38	TLW (30%)	10,13-octadecadienoic acid (C18:2), Palmitic Acid	This study (Figure 5)

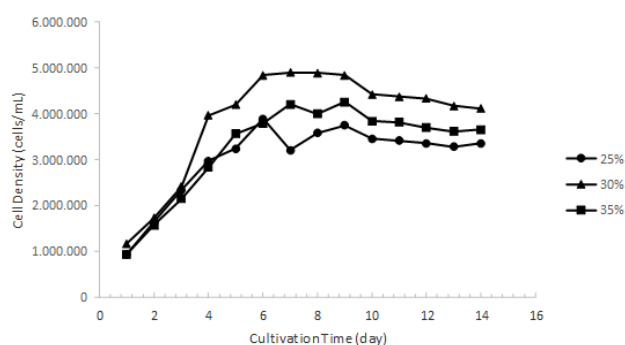


Figure 1. Cell density of *C. sorokiniana* in TLW medium

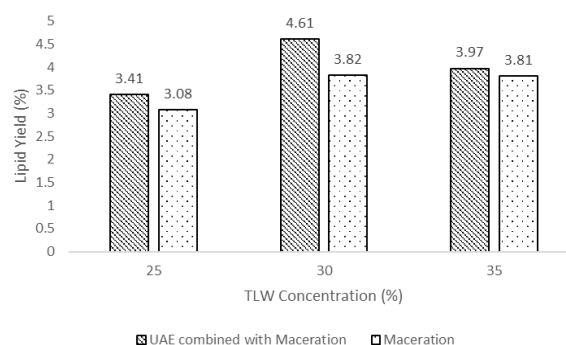


Figure 2. Lipid yield of *Chlorella sorokiniana* at different extraction methods and variation of TLW concentration

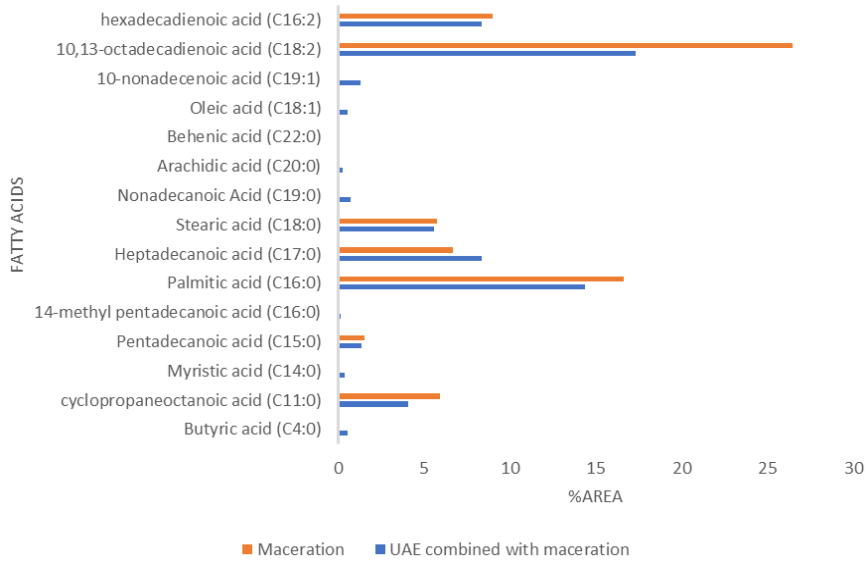


Figure 3. Fatty acids of *C. sorokiniana* based on extraction methods

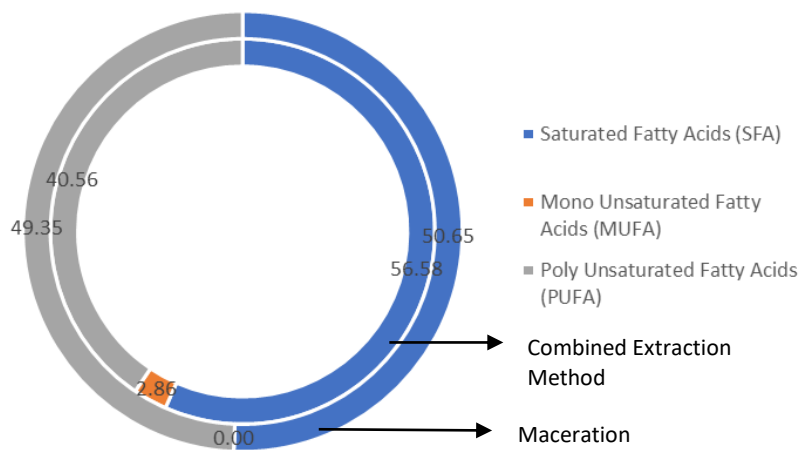


Figure 4. Comparison of SFA/MUFA/PUFA in *Chlorella sorokiniana* extract based on the extraction method

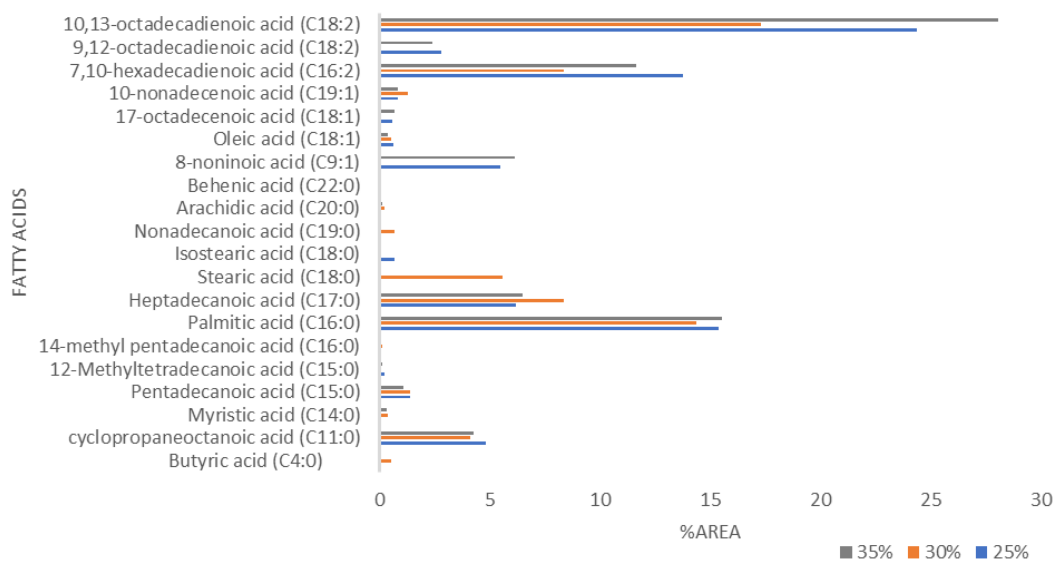


Figure 5. *C. sorokiniana* fatty acids cultured at variations in TLW medium concentration by UAE combination extraction method with maceration

Cyclopropaneoctanoic acid (C11:0) is found in *C. sorokiniana* cultured in all three TLW medium concentrations. This fatty acid is one of the minor fatty acids in microalgae under specific cultivation conditions. Previous studies examining several microalgae species detected cyclopropaneoctanoic acid in *Micractinium thermotolerans* VKM A1-332 (Krivina et al. 2024). Cyclopropaneoctanoic acid is a fatty acid with a cyclic chain. Reactions during extraction and esterification may cause the presence of cyclic fatty acids. In this study, both maceration methods showed the presence of these fatty acids (Figure 3).

Heptadecanoic acid (C17:0), oleic acid (C18:1), and 10,13-octadecadienoic acid (C18:2) were also identified in *C. sorokiniana* cultured at all three variations of TLW medium concentrations extracted by the combination method. Heptadecanoic acid (C17:0) is a saturated fatty acid with odd chains. These odd fatty acids in algae oil do not show any adverse effects. Heptadecanoic acid (C17:0) was found in considerable amounts (29.1%) in mixotrophically cultured *Messastrum gracile* SVMIICT7 (Kuravi and Mohan 2021).

Oleic acid (C18:1) is a monounsaturated fatty acid (MUFA) abundant in microalgae, including *C. sorokiniana*. Other studies on *C. sorokiniana* have shown a varied oleic acid content based on nitrogen availability and light intensity (Papapanagiotou et al. 2024). Oleic acid studies on *C. sorokiniana* immobilized in calcium-alginate beads showed a higher oleic acid content compared to free cell cultures (Alfaro-Sayes et al. 2023). Nitrogen-deficient medium encourages *C. sorokiniana* to increase the synthesis of monounsaturated fatty acids (MUFA), one of which is oleic acid (Papapanagiotou et al. 2024). According to this study's results, *C. sorokiniana* cultured on 25%

TLW medium has oleic acid with a higher percentage area than TLW 30 and 35%. Stress conditions with lower amounts of nitrogen indicate higher amounts of oleic acid in microalgae; research on *Scenedesmus* sp. SVMIICT1 showed high levels of oleic acid in dual stress (nitrate depletion and salinity stress) and mixotropic conditions (Kona et al. 2022).

The fatty acids that were only identified in *C. sorokiniana* extracts cultured in 30% TLW medium were butyric acid (C4:0), 14-methyl pentadecanoic acid (C16:0), stearic acid (C18:0), and nonadecanoic acid (C19:0). Stearic acid (C18:0) and hexadecadienoic acid (C16:1) have a reasonably high %area and are only identified in *C. sorokiniana* extracts cultured on a 30% TLW medium. Stearic acid is one of the fatty acids that has the potential to be biodiesel (Chen and Chang 2016). Other studies on the fatty acids in *Chlorella* show that the species of *C. sorokiniana* generally has higher saturated fatty acids than *C. vulgaris* (Yun et al. 2020).

Polyunsaturated Fatty Acids (PUFA) are more commonly identified in *C. sorokiniana* extracts cultured on 25 and 35% TLW medium (Figure 5), namely in the form of 7,10-hexadecadienoic acid (C16:2), 9,12-octadecadienoic acid (C18:2), and 10,13-octadecadienoic acid (C18:2). In general, microalgae contain 9,12-octadecadienoic acid (C18:2); however, 10,13-octadecadienoic acid is also found in *Chlorella vulgaris* and *Nostoc muscorum* (Morowvat and Ghasemi 2016). *Chlorella sorokiniana* inaCC M38 cultured on TLW medium has the highest fatty acid composition of C16:0 and C18:2. This is in line with other studies of *C. sorokiniana* that were cultured on a lab scale in phototropic and mixotropic conditions (Barouh et al. 2024).

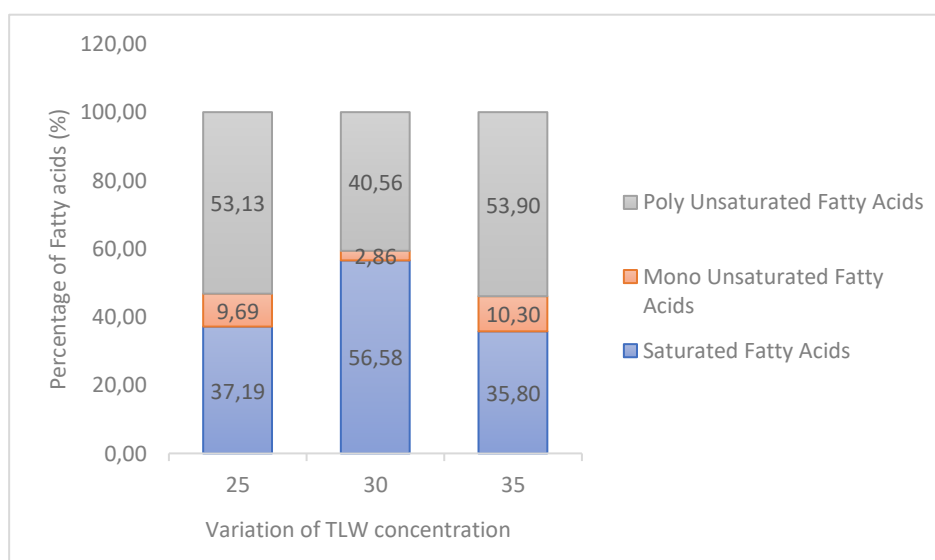


Figure 6. Comparison of SFA/MUFA/PUFA in *Chlorella sorokiniana* extract based on variation of TLW concentration using combined extraction method

Several biodiesel quality parameters, such as flash point, oxidation stability, and cetane number, are influenced by fatty acid composition. A higher PUFA composition with shorter carbon chains can lower the cetane number (Mujtaba et al. 2021; Omwoyo and Mengo 2023). Saturated fatty acids provide better biodiesel quality with a higher cetane number (Mekonnen et al. 2024). Higher oleic and linoleic acids provide a lower cetane number than oils with higher palmitic content (Sumatri et al. 2023). *Chlorella vulgaris* KP2, with a composition of 49.26% SFA, 38.73% MUFA, and 10.12% PUFA of the total fatty acids, has higher oxidation stability and cetane number (58.73) (Andeden et al. 2021). Research on *Nannochloropsis oculata* by giving stress to the growth medium produces C16-C20 fatty acids with SFA and MUFA composition reaching 88% so that it has the potential as a raw material for biodiesel (El-Sayed et al. 2022). The high quality of biodiesel from *Chlorella muelleri* is due to the high composition of C14-C18 fatty acids (81%) and oleic acid (28%) (Andrew et al. 2022). The results are comparable to the species *Coelastrella* sp. and *Verrucodesmus verrucosus*, which contain 80-90% C16-C18 fatty acids (Rodríguez-Palacio et al. 2022).

The nutrients in the culture medium affect the metabolism and fatty acids produced by microalgae. This study showed that 30% TLW medium provided a higher SFA composition percentage than 25 and 35% TLW media (Figure 6). The 50% F/2 medium used to culture *Chlorella vulgaris*, *Oocystis submarina*, and *Monoraphidium* showed a higher saturated fatty acid profile and better biodiesel quality (Hawrot-Paw et al. 2021). Nutrients in the medium that are limited to a certain amount have a better effect on the properties of biodiesel produced from microalgae because they tend to produce higher saturated fatty acids. In this study, *C. sorokiniana* cultured in 30% TLW media showed better potential as a biodiesel feedstock based on its fatty acid profile.

The lipid productivity of *C. sorokiniana* InaCC M38 cultured in TLW medium at various concentrations of 25, 30, and 35% demonstrates the strain's specific response to the availability of organic nutrients in the TLW medium. While these findings provide valuable information regarding the potential of tofu wastewater as a low-cost microalgae growth medium, they may not be fully generalizable to all *C. sorokiniana* strains. Further research involving multiple strains is recommended to validate the universality of the lipid accumulation mechanism under similar environmental stressors.

The utilization of tofu wastewater in this study focused on providing nutrients for *Chlorella sorokiniana* InaCC M38. This wastewater utilization positively contributes to environmental sustainability by reducing the risk of water pollution. Reusing tofu wastewater reduces reliance on synthetic media, minimizes nutrient input costs, and minimizes environmental burdens. Future studies are needed, including a life cycle assessment (LCA), to evaluate the overall energy balance and carbon footprint of cultivating *C. sorokiniana* InaCC M38 in tofu wastewater, ensuring the process is both environmentally and economically sustainable.

Based on this study, it can be concluded that *Chlorella sorokiniana* InaCC M38 grows better on a TLW medium of 30%. Biomass production on the 7th day was 0.45 g/L. Extraction by the combination method, with cell wall breaking first using UAE, further increases the yield of extracted lipids. The extract has more fatty acids (4.61% DW) compared to single extraction by maceration (3.82% DW). This study provides the basis for better extraction methods in extracting compounds in *C. sorokiniana*. The presence of fatty acids in *C. sorokiniana* cultured on TLW 30% medium indicates more Saturated Fatty acids (56.58%) with palmitic acid as a major SFA that can show its potential as a biodiesel material. However, further experimental optimization is needed to improve lipid production of *C. sorokiniana*, to scale up testing, life-cycle assessment, and field cultivation validation. Overall, *C. sorokiniana* grown in tofu wastewater represents a promising, low-cost strategy for integrating biofuel production with waste management systems.

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