

Optimisation of microalgae lipid extraction for sustainable omega-3 production: Integrating microwave-assisted cell disruption, wet biomass processing, and ethanol as a green solvent

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Received: 20.06.2026

Revised: 05.08.2026

Accepted: 01.09.2026

Online: 15.09.2026

Abstract Microalgae-derived lipids offer a sustainable alternative to conventional omega-3 sources, yet industrial-scale production remains constrained by energy-intensive downstream processing and reliance on toxic solvents. This study systematically optimised three critical downstream steps: cell disruption, biomass drying, and lipid extraction, for four microalgae species cultivated in Sri Lanka: *Chlorella vulgaris*, *Nannochloropsis oculata*, *Chaetoceros calcitrans*, and *Thalassiosira pseudonana*. Three cell disruption methods (microwave, autoclave, and osmotic shock) were compared, followed by evaluation of wet versus oven-dried biomass and assessment of ethanol as a green solvent alternative to the conventional Bligh & Dyer method. Microwave-assisted disruption yielded the highest lipid recovery across all species (10.3–22.5% DW), significantly outperforming autoclave treatment ($p < 0.05$). Lipid yields from wet and dry biomass were not significantly different ($p > 0.05$) for any species, indicating that the energy-intensive drying step can be eliminated without compromising yield. Ethanol extraction yielded lower total lipid recovery (55.3–83.5% relative to Bligh & Dyer) but selectively enriched polyunsaturated fatty acids, with total unsaturated fatty acid content reaching 74.4% in *T. pseudonana*. *Nannochloropsis oculata* exhibited the highest lipid content (22.5% DW) and a favourable fatty acid profile, making it the most promising candidate for omega-3 production. These findings demonstrate that combining microwave-assisted disruption with wet biomass processing and ethanol extraction offers a cost-effective, energy-efficient, and food-safe protocol for microalgae lipid recovery, with strong potential for industrial adoption in tropical developing nations.

Keywords: Microalgae lipid extraction, Green solvent, Microwave-assisted cell disruption, Omega-3 fatty acids

1. INTRODUCTION

Microalgae are a diverse group of unicellular, photosynthetic microorganisms spanning marine, freshwater, and terrestrial ecosystems. Over the past decade, they have garnered immense biotechnological interest due to their rich biochemical repertoire, comprising proteins, lipids, sterols, pigments (carotenoids and chlorophyll), vitamins, and antioxidants, which underpins their application in biofuels, wastewater bioremediation, carbon sequestration, and the production of high-value nutraceuticals and pharmaceuticals (Napiórkowska-Krzebietke, 2017; Khan et al.,

2018; Araujo et al., 2022). In the context of the global circular bioeconomy, microalgae are increasingly recognized not merely as feedstock, but as versatile cell factories capable of addressing both food security and environmental sustainability (Kumar et al., 2024).

Among their biochemical assets, microalgae-derived lipids have emerged as a sustainable and promising alternative source of omega-3 polyunsaturated fatty acids (PUFAs) for the food and nutraceutical industries. Unlike traditional fish oil sources, which face declining wild catches, bioaccumulation of marine pollutants, and seasonal variability, microalgae offer several distinct



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advantages: they act as the primary producers of PUFAs in marine food webs, can be cultivated under strictly controlled aquaculture conditions with minimal ecological footprint, do not compete for arable land or freshwater, exhibit high areal productivity, and often contain substantial oil fractions (Ryckebosch et al., 2012; Figueiredo et al., 2019). With the global omega-3 market projected to exceed USD 8 billion by 2030, microalgae represent a viable, scalable alternative to meet this surging demand while circumventing the ecological limits of fishery-dependent supply chains (Coleman et al., 2023).

However, the industrial scalability of microalgal lipid production is severely hampered by bottlenecks in downstream processing, which encompasses harvesting, dewatering, cell disruption, and lipid extraction. Harvesting and dewatering alone can account for 20–30% of total production costs, while the drying step is notoriously energy-intensive, consuming up to 60–80% of the total energy input in a conventional biorefinery (Yao et al., 2022). Physical disruption methods such as bead beating and high-pressure homogenization, though effective, require substantial capital investment and high operational energy. Conversely, chemical or thermal approaches, including autoclaving, risk degrading thermolabile PUFAs. Critically, conventional biomass drying, especially at elevated temperatures, accelerates the auto-oxidation of PUFAs, severely compromising the nutritional quality and oxidative stability of the final lipid extract. While novel techniques like accelerated solvent extraction (ASE) and supercritical CO₂ (SC-CO₂) offer high efficiency, they remain economically prohibitive for large-scale industrial deployment in developing nations. On the solvent front, the gold-standard Bligh & Dyer and Folch methods rely on chloroform and methanol, solvents that are toxic, environmentally hazardous, and incompatible with food-grade or nutraceutical certification. Although emerging green technologies (e.g., ultrasound-assisted extraction, enzyme-assisted extraction, and deep eutectic solvents) have been proposed to mitigate these issues, they are largely confined to laboratory settings due to high reagent costs, complex recovery steps, and limited validation on diverse microalgal strains (Chemat et al., 2011).

Consequently, extensive research has been directed towards optimizing these steps globally, exploring synergistic combinations of mild cell disruption, wet biomass processing, and food-grade solvents. However, studies that address the specific challenges of tropical regions, particularly Sri Lanka, remain critically scarce. Sri Lanka's tropical climate offers favourable year-round conditions for microalgae cultivation; however, locally maintained strains (e.g., *Nannochloropsis oculata*, *Chaetoceros calcitrans*) exhibit unique cell wall architectures (e.g., algaenan layers in eustigmatophytes, silica frustules in diatoms) and lipid profiles that differ substantially from the model strains predominantly studied in temperate regions. These distinct biophysical properties necessitate indigenous optimization rather than direct transplantation of protocols developed elsewhere. Furthermore, the economic constraints of a developing economy demand solutions that prioritize cost-effective, energy-efficient, and low-tech equipment over sophisticated, capital-intensive instrumentation.

In light of this critical knowledge gap and local industrial need, the present study was designed to systematically optimise a microalgae lipid extraction protocol tailored for future Sri Lankan industrial adoption. Specifically, this paper presents the initial stage of this optimisation, targeting the three most energy- and cost-intensive downstream processing steps. The specific objectives were: (i) to evaluate and compare the efficiency of three readily accessible cell disruption methods (microwave irradiation, autoclave treatment, and osmotic shock) across four locally cultivated species (*Chlorella vulgaris*, *Nannochloropsis oculata*, *Chaetoceros calcitrans*, and *Thalassiosira pseudonana*); (ii) to investigate the effect of biomass moisture content by comparing lipid yields and fatty acid profiles obtained from wet versus oven-dried biomass, thereby assessing the feasibility of bypassing the energy-intensive drying step; and (iii) to assess the viability of ethanol as a green, food-grade solvent alternative to the toxic chloroform/methanol system used in the conventional Bligh & Dyer method, while evaluating the trade-offs between total lipid recovery and preservation of nutritionally valuable PUFAs.

2. MATERIALS AND METHODS

2.1. Microalgae Cultivation and Harvesting

Pure cultures of two green algae species, *Chlorella vulgaris* and *Nannochloropsis oculata*, and two diatom species, *Chaetoceros calcitrans* and *Thalassiosira pseudonana*, were obtained from commercial shrimp hatcheries. All cultures were confirmed for axenicity by plating on agarized F/2 medium and checking for bacterial/fungal growth under a light microscope (Olympus CX23, Japan).

Microalgae were cultivated in sterile modified F/2 medium (Guillard and Ryther, 1962) prepared with filtered (via commercial hatchery facilities by sand, activated carbon and UV filters) and autoclaved seawater (121 °C, 15 min, 15 psi). The modified F/2 medium composition was as follows: NaNO₃ (100 g L⁻¹), NaH₂PO₄·H₂O (20 g L⁻¹), Na₂SiO₃·9H₂O (80 g L⁻¹ for diatoms only), f/2 trace metal solution (1 mL L⁻¹), and f/2 vitamin solution (0.375 mL L⁻¹). Cultures were maintained under continuous illumination (100–120 μmol photons m⁻² s⁻¹) provided by cool-white fluorescent lamps (Green, 8W) with continuous aeration (0.8 vvm) using an aquarium air pump (RS Electrical RS-608, China). The temperature was maintained at 25 ± 2 °C using a temperature-controlled cultivation room. The pH of the medium was adjusted to 8.0–8.2 using 1 M HCl or 1 M NaOH prior to inoculation.

Cell growth was monitored daily by measuring optical density at 680 nm (OD₆₈₀) using a UV-Vis spectrophotometer (Thermo Genesys 10S UV-Vis, USA). Biomass was harvested at the late exponential growth phase (typically 7–10 days post-inoculation, when OD₆₈₀ reached a plateau) through centrifugation at 6,000 × g for 3 min at 24 °C (Sigma Laborzentrifugen GmbH 6-16KHS, Germany) and washed three times with deionized water to minimize salt and residual medium components. The resulting pellet was either used directly as fresh wet biomass or oven-dried (YIHDER DK-600DT, Taiwan) at 60 ± 2 °C for 24–48 h or until a constant weight was achieved (± 0.001 g). Moisture content of the wet biomass was determined gravimetrically by drying a subsample (1 g) at 105 °C for 24 h (AOAC Method 930.15). Dried biomass was ground to a fine powder using a mortar and pestle, passed through a fine sieve and stored in airtight amber glass vials in refrigerated conditions until further use to prevent lipid oxidation.

2.2. Experimental Design

The study was conducted as three sequential experiments to systematically optimize the microalgae lipid extraction protocol:

- Experiment 1: Screening of cell disruption methods: comparing microwave, autoclave, and osmotic shock treatments using dried biomass to identify the most effective method.
- Experiment 2: Effect of biomass moisture: comparing lipid yields and fatty acid profiles from wet versus oven-dried biomass using the optimal disruption method identified in Experiment 1.
- Experiment 3: Green solvent assessment: comparing conventional Bligh & Dyer extraction with ethanol-based extraction using the optimal disruption method.

All experiments were performed in triplicate (n = 3 technical replicates) with two culture batches, yielding a total of six observations per treatment (n = 6). Results are expressed as mean ± standard deviation (SD).

2.3. Experiment 1 – Effect of Cell Disruption Methods

In the first experiment, the most effective cell disruption method was investigated by comparing three methods that are readily available, cost-effective, and commonly reported in the literature. Homogenized dried microalgae powder (100 ± 1 mg) of *C. vulgaris*, *N. oculata*, *C. calcitrans*, and *T. pseudonana* was used for this experiment.

a) Microwave method

Cell disruption by the microwave method was performed as described by Pohndorf et al. (2016) with slight modifications. Briefly, 100 mg of dried microalgae powder was placed in a glass Petri dish (90 mm diameter) and exposed to microwave irradiation at 100 °C for 2 min with an operational frequency of 2450 MHz using a commercial microwave oven (LG, MC3283FMPG, 900 W, India). The sample temperature was verified immediately after treatment to ensure consistent

heating (± 2 °C). Samples were then cooled to room temperature in a desiccator prior to lipid extraction.

b) Autoclave method

The autoclave cell disruption method was carried out as described by Rakesh et al. (2015) with minor modifications. Briefly, 100 mg of dried microalgae powder was suspended in 0.8 mL of deionized water in a 15 mL screw-cap glass tube. The mixture was autoclaved at 121 °C and 0.6 MPa (approximately 15 psi) for 15 min using a vertical autoclave (Biobase Vertical Autoclave BKQ-B50L, China). Samples were allowed to cool to room temperature before lipid extraction.

c) Osmotic shock method

For the osmotic shock treatment, 100 mg of dried microalgae biomass was placed in a 15 mL polypropylene centrifuge tube, and 0.25 mL of 10% (w/v) NaCl solution was added to achieve a final salt concentration of approximately 25% (w/v). The mixture was vortex-mixed for 2 min and then incubated in the dark for 48 h at room temperature (25 ± 2 °C), following the protocol described by Ramola et al. (2019).

d) Lipid extraction following cell disruption

Following each cell disruption treatment, lipid extraction was performed using the standard Bligh and Dyer method as described below under Section 2.6.1.

2.4. Experiment 2 - Effect of Biomass Moisture on Lipid Yield and Fatty Acid Composition

The drying step in microalgae downstream processing is energy-intensive and economically unfavorable. Moreover, lipid oxidation, especially of polyunsaturated fatty acids (PUFAs), is inevitable to some degree upon heat exposure (Yao et al., 2022). Thus, in this experiment, the effect of biomass moisture content on lipid yield and fatty acid composition was investigated using freshly harvested wet biomass and oven-dried biomass of *C. vulgaris*, *N. oculata*, and *C. calcitrans*.

Since the highest lipid yield was obtained using the microwave cell disruption method in Experiment 1, it was adopted for Experiment 2 and Experiment 3 (as described in below Section 2.5). Both wet (approximately 70–85% moisture content,

determined gravimetrically) and dry biomass underwent cell lysis by microwave irradiation prior to lipid extraction using the standard Bligh and Dyer method (Section 2.6.1.). For wet biomass, the sample weight was adjusted to ensure equivalent dry cell weight (100 mg dry weight equivalent) across all treatments. The dry weight equivalent of wet biomass was calculated as: $DCW = \text{wet weight} \times (1 - \text{moisture content})$. The total lipid yield was quantified gravimetrically, followed by fatty acid methyl ester (FAME) derivatization using the BF_3 method (Vázquez and Akoh, 2012) and gas chromatography-flame ionization detection (GC-FID) for fatty acid profiling (Section 2.7.2).

2.5. Experiment 3 - Assessment of Ethanol as a Green Solvent in Lipid Extraction

Conventional lipid extraction methods rely heavily on toxic and non-eco-friendly solvents (e.g., chloroform, methanol), while modern techniques (accelerated solvent extraction, supercritical CO_2) are often energy-inefficient and economically unviable for industrial scale-up (Ryckebosch et al., 2012). In light of this, the third experiment was designed to modify the standard Bligh & Dyer lipid extraction protocol by replacing the solvent system with a green alternative.

Dried microalgae powder (100 mg) of *C. vulgaris*, *N. oculata*, *C. calcitrans*, and *T. pseudonana* was subjected to microwave cell lysis (Section 2.4.1). Lipid extraction was then performed using both the standard Bligh and Dyer method (Section 2.6.1) and an ethanol extraction method adapted from Figueiredo et al. (2019) with modifications (Section 2.6.2). Total lipid content was quantified by gravimetric method, followed by fatty acid methyl ester preparation via the BF_3 method (Vázquez and Akoh, 2012) for fatty acid profile analysis through gas chromatography-mass spectrometry (GC-MS) (Section 2.7.3).

2.6. Analysis of total lipid content

2.6.1. Bligh and Dyer method

In this study, the Bligh and Dyer method (Bligh and Dyer, 1959) with slight modifications was used as the standard lipid extraction method for all three experiments.

Briefly, pre-weighed (W) cell-lysed microalgae samples (approximately 100 mg dry weight

equivalent) were placed in 15 mL centrifuge tubes with screw caps. Chloroform and methanol were added in a ratio of 1:2 (v/v) (total volume 3 mL). The mixture was vortex-mixed for 5 min using a vortex mixer (DAIHAN Lab Tech LVM-202, Korea). Subsequently, an additional 1 mL of chloroform was added, followed by another vortexing step (5 min). The solution was then centrifuged (Gemmy PLC-025, Taiwan) at $13,080 \times g$ for 10 min, after which the supernatants were carefully transferred into a fresh centrifuge tube. To facilitate phase separation, 1.8 mL of deionized water was added to the supernatant to achieve a final ratio of chloroform:methanol:water of 2:2:1.8 (v/v/v). The remaining microalgae pellet was re-extracted twice following the same procedure to ensure complete lipid recovery.

Subsequently, combined supernatants of the same sample were centrifuged at $13,080 \times g$ for 10 min to further facilitate phase separation. The organic phase (lower phase), containing the extracted lipids, was separated from the aqueous phase (upper phase) using a glass Pasteur pipette and transferred to a pre-weighed aluminium cup (W_0). The solvent in the organic phase was then evaporated under a fume hood at room temperature for approximately 2 h. Upon complete solvent evaporation, the lipid residue in the aluminium cup was further dried in a hot air oven at 40°C for 10 min to remove any residual moisture present. Finally, the weight of the aluminium cup containing the extracted lipid was recorded (W_1), and total lipid yield was reported gravimetrically and expressed as a percentage of dry cell weight (% dry weight, %DW) using the following equation:

$$\text{Lipid yield (\%)} = \frac{(W_1 - W_0)}{W} \times 100$$

where W = initial dry weight of the microalgae sample (mg), W_0 = weight of empty aluminium cup (mg), and W_1 = weight of aluminium cup + extracted lipid (mg).

2.6.2. Ethanol extraction method

The ethanol lipid extraction was adapted from Figueiredo et al. (2019) with several adjustments. Initially, dried and pre-weighed (W) microalgae samples (100 mg), disrupted using the microwave method (Section 2.3a), were placed in 15 mL

centrifuge tubes. Two millilitres (2 mL) of absolute ethanol ($\geq 99.8\%$ purity) was added to the samples, followed by vortexing for 5 min for homogenization. Afterwards, homogenized samples were incubated at 30°C for 30 min in an incubator. The supernatants from each sample were carefully decanted into a pre-weighed (W_0) aluminium cup. This extraction cycle was repeated three times or until the solvent became colourless (indicating complete extraction). Pooled supernatants were then evaporated under a fume hood at room temperature for 2–3 h or until complete dryness. Following solvent evaporation, the crude extract was dried in an oven at 40°C for 10 min to remove any residual moisture present. Upon drying, the weight of the aluminium cup containing the total crude extract was recorded (W_1).

Subsequently, the total crude extract underwent a purification process using liquid-liquid partition to remove non-lipid components (pigments, proteins, and carbohydrates). Firstly, the total crude extracts were re-dissolved in a chloroform:methanol:water (2:2:1.8, v/v/v) mixture. The mixture was vortex-mixed for 1 min, followed by phase separation by centrifugation at $13,080 \times g$ for 10 min. The lower organic phase was collected into a new, clean, pre-weighed aluminium cup (W_2), while the aqueous phase was re-extracted with fresh chloroform (2 mL) five times to recover any residual lipids. The combined organic phases were collected and evaporated under a fume hood at room temperature. After solvent evaporation, the purified lipid was dried in a hot air oven at 40°C for 10 min. After drying, the weight of the aluminium cup containing the purified lipid was recorded (W_3).

Finally, the percentage crude extract yield and percentage purified lipid yield were calculated based on the dry weight of the sample, using the following equations:

$$\text{Crude extract yield (\%)} = \frac{(W_1 - W_0)}{W} \times 100$$

$$\text{Purified lipid yield (\%)} = \frac{(W_3 - W_2)}{W} \times 100$$

where W = initial dry weight of the microalgae sample (mg), W_0 = empty aluminium cup weight for

crude extract (mg), W_1 = weight of aluminium cup + crude extract (mg), W_2 = empty aluminium cup weight for purified lipid (mg), and W_3 = weight of aluminium cup + purified lipid (mg).

2.7. Analysis of fatty acid composition

2.7.1. Fatty Acid Methyl Ester (FAME) Preparation

Fatty acid methyl esters (FAMES) were prepared according to the BF_3 method described by Vázquez and Akoh (2012) with slight modifications. Lipid samples (approximately 70 mg) were weighed into clean, dried, screw-cap glass vials. Then, 2 mL of 0.5 M sodium hydroxide in methanol was added. The vial was tightly capped, vortex-mixed for 1 min, and then incubated at 100 °C for 5 min using a water bath. After cooling to room temperature, 2 mL of 14% boron trifluoride (BF_3) in methanol solution was added, and the sample was vortex-mixed for 1 min. The vortexed samples were then incubated at 100 °C for 5 min to complete the methylation reaction. Following incubation, the FAMES were extracted by adding 2 mL of hexane and 2 mL of saturated NaCl solution, followed by vortex-mixing for 2 min and centrifugation at $1453 \times g$ for 5 min. The top organic layer (hexane phase) was carefully separated using a glass Pasteur pipette and treated with anhydrous sodium sulphate (approximately 0.5 g) to remove any residual water. The hexane extract was then filtered through a 0.22 μm PTFE syringe filter into a 2 mL amber GC vial and stored at -20 °C until analysis.

2.7.2. Gas Chromatography-Flame Ionization Detection (GC-FID) Conditions

For samples from Experiment 2, the FAMES obtained were analysed by gas chromatography coupled with a flame ionization detector (GC-FID) using an Agilent 7890B system equipped with a CP-Sil 88 capillary column for FAME analysis (100 m $\times$ 0.25 mm i.d. $\times$ 0.2 μm film thickness) (Agilent J & W, CA, USA). The initial column temperature was set at 100 °C, held for 4 min, then progressively increased to 180 °C at 8 °C min^{-1} , and subsequently to 230 °C at 1 °C min^{-1} , and held at 230 °C for 5 min. The injector temperature was maintained at 260 °C with an injection volume of 1 μL in splitless mode (splitless time: 1 min). The carrier gas was ultra-high purity nitrogen (purity $\geq 99.9995\%$) at a

constant flow rate of 1.0 mL min^{-1} . FAMES were identified by comparing their retention times with those of authentic standards (Supelco 37 Component FAME Mix, Sigma-Aldrich). Individual fatty acids were quantified using relative percentage normalization (peak area % of total identified fatty acids).

2.7.3. Gas Chromatography-Mass Spectrometry (GC-MS) Conditions

For samples from Experiment 3, fatty acid profiles were analysed using gas chromatography-mass spectrometry (GC-MS) for more definitive identification of individual fatty acid species. The GC-MS analysis was performed on an Agilent 7890B gas chromatograph coupled with an Agilent 5977A mass selective detector (MSD) and 7890B Auto sampler, equipped with DB-FATWAX UI (30 m $\times$ 0.250 mm $\times$ 0.25 μm) column. The initial column temperature was set at 40 °C, held for 1 min, then progressively increased to 200 °C at 5 °C min^{-1} , and subsequently to 240 °C at 10 °C min^{-1} , and held at 240 °C for 14 min. The injector temperature means was maintained at 250 °C with an injection volume of 1 μL in splitless mode (splitless time: 1 min). The carrier gas was helium (purity $\geq 99.999\%$) at a constant flow rate of 1.0 mL min^{-1} .

The mass spectrometer was operated in electron ionization (EI) mode, with a source temperature of 230 °C and quadrupole temperature of 150 °C. The scan range was 50–550 m/z for full-scan acquisition. FAMES were identified by comparing mass spectra with the NIST 14, W9N11 mass spectral libraries and by matching retention times with authentic standards. Relative percentages of individual fatty acids were determined from total ion chromatogram (TIC) peak areas.

2.8. Statistical analysis

All experimental data are presented as mean $\pm$ standard deviation (SD). Statistical analysis was conducted using SPSS Statistics version 27.0. A 5% significance level ($p < 0.05$) was used for all statistical tests.

- For Experiment 1 (cell disruption methods): Two-way analysis of variance (ANOVA)

followed by post-hoc Bonferroni test was used to determine the effect of species and cell disruption method on lipid yield.

- For Experiment 2 (wet vs. dry biomass): Independent sample t-tests were used to compare lipid yields and individual fatty acid percentages between wet and dry biomass for each species.
- For Experiment 3 (extraction methods): Independent sample t-tests were used to compare lipid yields and individual fatty acid percentages between the Bligh & Dyer and ethanol extraction methods.

3. RESULTS AND DISCUSSION

3.1. Experiment 1 - Effect of cell disruption methods

Efficient cell disruption is a critical determinant of lipid extraction efficiency in microalgae, as intracellular lipids are protected by complex,

species-specific cell wall architectures that vary considerably across taxonomic groups (Lee et al., 2010; Kapoore et al., 2018). In this study, the total lipid yield obtained after each disruption method was used as a proxy for cell disruption efficiency, allowing direct comparison of three readily available and cost-effective techniques: microwave irradiation, autoclave treatment, and osmotic shock.

The microwave treatment consistently yielded the highest lipid recovery across all four species, followed by osmotic shock, while autoclave treatment generally produced the lowest yields (Fig 1). Specifically, microwave-assisted disruption yielded $19.4 \pm 0.4\%$, $24.2 \pm 0.4\%$, $14.5 \pm 0.3\%$, and $19.2 \pm 0.4\%$ DW for *C. vulgaris*, *N. oculata*, *C. calcitrans*, and *T. pseudonana*, respectively. These values were 1.1–1.4-fold higher than those obtained via osmotic shock and 1.2–1.5-fold higher than autoclave treatment, depending on species.

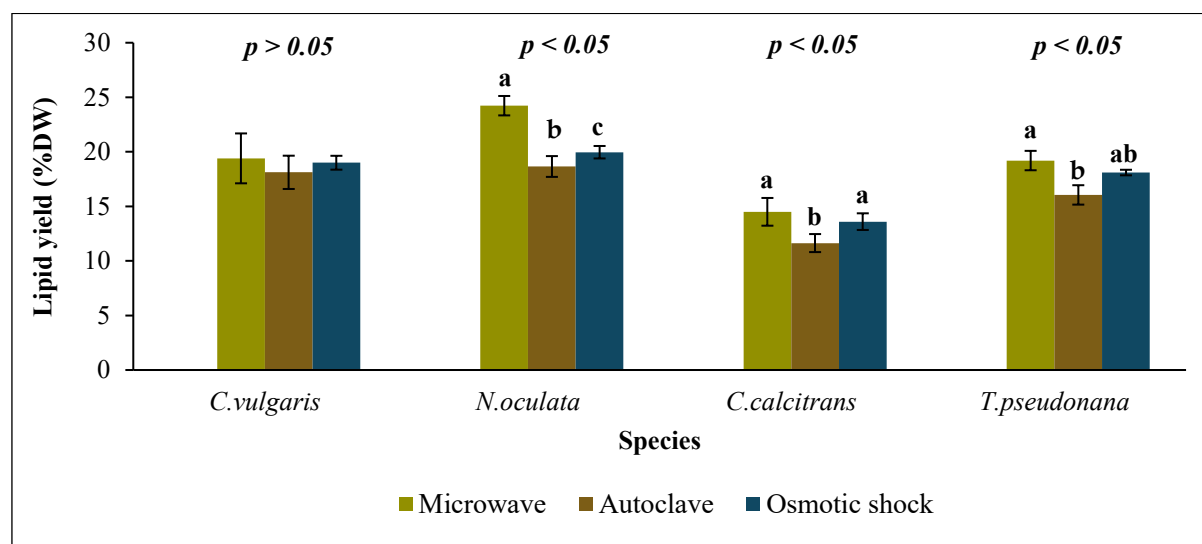


Fig 1. Effect of cell disruption method on total lipid yield of *C. vulgaris*, *N. oculata*, *C. calcitrans* and *T. pseudonana*

The superior performance of microwave irradiation can be attributed to its unique mechanism of action. Microwave energy generates rapid, localized heating through dipolar polarization and ionic conduction, creating thermal gradients and internal pressure build-up within the cell. This leads to explosive cell rupture and efficient release of

intracellular lipids with minimal thermal degradation, owing to the short exposure time (2 min) (Kapoore et al., 2018). Furthermore, microwave treatment disrupts hydrogen bonding between water molecules and cell wall components, further weakening the cell envelope and enhancing solvent penetration (Pohndorf et al., 2016). In

contrast, autoclave treatment relies on prolonged high-pressure steam (121 °C, 15 min), which may cause cell wall shrinkage, protein denaturation, and partial lipid degradation, thereby reducing extraction efficiency (Lee et al., 2010). Osmotic shock, while effective for some species, induces cell rupture through extreme water movement, a mechanism that is less effective for species with rigid or structurally complex cell walls (Frenz et al., 1989).

A two-way ANOVA revealed a significant interaction between species and cell disruption method on total lipid content ($F(6, 12) = 3.82, p < 0.05$), indicating that the effect of cell disruption method differed among species. Pairwise comparisons for *C. vulgaris* did not show any significant difference ($p > 0.05$) among the three methods, suggesting that its cell wall is relatively fragile and amenable to disruption by multiple mechanisms. This is consistent with reports that some *Chlorella* strains lack the rigid sporopollenin layer found in more resistant species (Burezyk and Hesse, 1981). In contrast, *N. oculata* exhibited significantly higher lipid yield ($p < 0.05$) with microwave treatment compared to both autoclave and osmotic shock, reflecting the recalcitrance of its algaenan-containing cell wall (Ryckebosch et al., 2014). For the two diatom species, *C. calcitrans* and *T. pseudonana*, both microwave and osmotic shock methods yielded significantly higher lipid recovery ($p < 0.05$) than autoclave treatment.

The differential response of diatoms can be explained by their unique silica frustule architecture. The frustule of *Chaetoceros* species possesses spines (setae) and pores, while *Thalassiosira* species have overlapping valves with numerous areolae (pores) (Round et al., 1990; Armbrust, 2009). These pores facilitate nutrient exchange and increase structural permeability, allowing partial leakage or diffusion of intracellular lipids even without strong mechanical disruption. Osmotic shock, which creates an osmotic gradient that draws water out of cells and collapses membranes, may exploit these pores to enhance lipid release (Martin-Jézéquel et al., 2000). This explains why osmotic shock performed comparably to microwave treatment in diatoms but not in *N. oculata*, whose cell wall lacks such porosity.

Given its consistently high efficiency across all four species, simplicity, short processing time, and low equipment cost, microwave treatment was

selected as the standard cell lysis method for Experiments 2 and 3. This choice aligns with the study's overarching goal of developing a cost-effective, energy-efficient, and scalable protocol suitable for local industrial adoption.

3.2. Experiment 2 – Effect of Biomass Moisture on Lipid Yield and Fatty Acid Composition

The drying step in microalgae downstream processing is widely recognized as energy-intensive and economically burdensome, often accounting for 60–80% of total energy input in conventional biorefineries (Yao et al., 2022). Moreover, thermal drying can induce lipid oxidation, particularly of PUFAs, compromising the nutritional quality of the final extract. Therefore, this experiment evaluated whether wet biomass could serve as a viable alternative to dried biomass without sacrificing lipid yield or fatty acid quality.

The results demonstrated that lipid yield was generally higher from dry biomass for *C. vulgaris* and *N. oculata*, whereas wet biomass yielded higher lipid recovery for *C. calcitrans* (Fig 2). However, these differences were not statistically significant ($p > 0.05$) for any species, indicating that both wet and dry biomass are equally viable for lipid extraction under the tested conditions. Specifically, lipid yields from wet vs. dry biomass were $14.1 \pm 2.1\%$ vs. $18.2 \pm 2.9\%$ for *C. vulgaris*, $19.9 \pm 0.7\%$ vs. $31.0 \pm 5.2\%$ for *N. oculata*, and $15.5 \pm 4.2\%$ vs. $12.9 \pm 0.6\%$ for *C. calcitrans*.

The slightly higher yields from dry biomass in green algae may be attributed to the absence of water, which allows solvents to penetrate the cell matrix more readily and dissolve lipids more efficiently (Arabian, 2022). Water can act as a barrier to solvent diffusion and may also promote emulsion formation during phase separation, reducing recovery. Conversely, the higher yield from wet *C. calcitrans* biomass suggests that drying may have induced structural changes or partial lipid degradation in this diatom species, possibly due to the susceptibility of its silica frustule to thermal stress or the oxidation of labile PUFAs during oven drying.

The absence of significant differences between wet and dry biomass has important industrial implications. Eliminating the drying step would

substantially reduce energy consumption, processing time, and capital costs, making the overall process more economically viable and environmentally sustainable. This finding is particularly relevant for tropical regions like Sri Lanka, where solar drying could be exploited but

would still require land area, time, and labor, whereas direct wet processing could simplify the workflow.

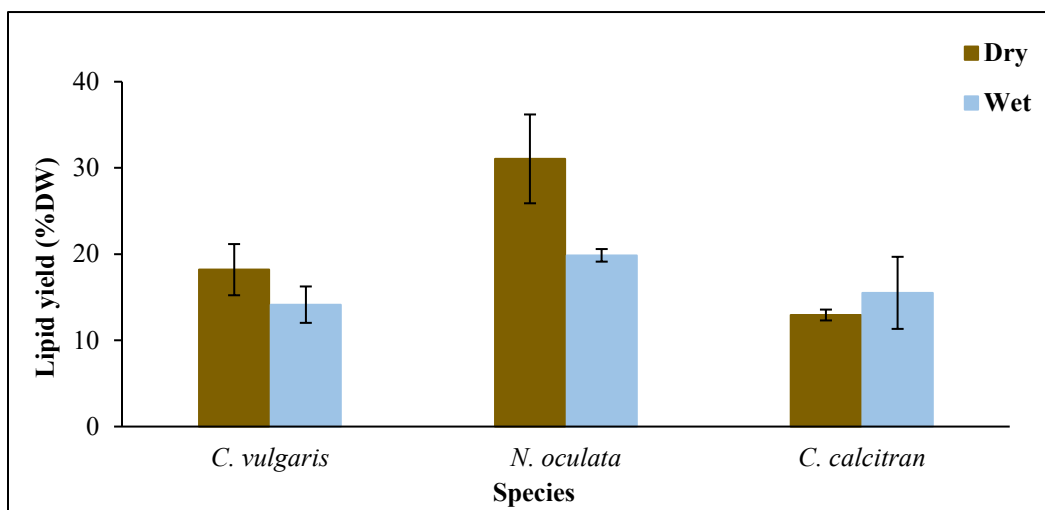


Fig 2. Effect of biomass moisture on total lipid yield of *C. vulgaris*, *N. oculata* and *C. calcitrans*

The fatty acid composition analysis revealed notable differences between wet and dry biomass (Fig 3). Wet biomass extraction favoured polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) in *C. calcitrans*, MUFAs in *C. vulgaris*, and saturated fatty acids (SFAs) in *N. oculata*. In contrast, dry biomass yielded higher percentages of SFAs in *C. calcitrans*, SFAs and PUFAs in *C. vulgaris*, and PUFAs and MUFAs in *N. oculata*. The highest PUFA percentage was recorded in *C. calcitrans* lipids extracted from wet biomass, underscoring the potential of wet processing for preserving nutritionally valuable PUFAs.

The shift toward higher SFA percentages in dry biomass for some species is consistent with the known susceptibility of PUFAs to thermal oxidation during drying. Oxidation of PUFAs leads to the formation of hydroperoxides, aldehydes, and other secondary products, effectively reducing the measurable PUFA content. In contrast, wet biomass processing minimizes heat exposure, thereby preserving PUFA integrity. This is particularly important for omega-3-rich

species destined for nutraceutical applications, where fatty acid quality is paramount.

In this experiment, microwave method was selected as the cell disruption method for subsequent wet biomass extractions. Ideally, a preliminary trial using three cell disruption methods would also have been conducted with wet biomass to determine the most suitable method. However, the primary objective of this experiment was to compare the fatty acid profile of the lipids extracted from dry and wet biomass, the same cell disruption method (microwave treatment) was used to maintain the consistency among the samples.

Collectively, these results suggest that wet biomass processing is not only feasible but may offer advantages in terms of PUFA preservation, while dry biomass remains a valid option when stable, long-term storage is required. The choice between wet and dry biomass should therefore be guided by the intended application, downstream processing constraints, and the target fatty acid profile.

3.3. Experiment 3 - Assessment of ethanol as a green solvent in lipid extraction

The increasing demand for omega-3 fatty acids in functional foods and nutraceuticals has intensified the search for extraction solvents that are safe for food applications, environmentally benign, and economically viable. Ethanol is a promising candidate: it is a food-grade solvent, classified as Generally Recognized as Safe (GRAS) by the U.S. FDA, and is permitted under European Union regulations for food ingredient production (Directive 2009/32/EC). This experiment therefore evaluated ethanol as a green alternative to the chloroform/methanol system used in the Bligh & Dyer method.

The results demonstrated that ethanol extraction yielded significantly lower lipid recovery ($p < 0.05$) than the Bligh and Dyer method across all four species (Fig 4). Specifically, lipid yields from ethanol extraction were $16.1 \pm 0.4\%$ vs. $19.4 \pm 0.3\%$ for *C. vulgaris*, $19.9 \pm 0.2\%$ vs. $24.2 \pm 0.1\%$ for *N. oculata*, $8.6 \pm 0.9\%$ vs. $14.5 \pm 0.1\%$ for *C. calcitrans*, and $12.6 \pm 0.6\%$ vs. $19.2 \pm 0.1\%$ for *T. pseudonana*. The corresponding lipid recovery percentages relative to the Bligh and Dyer method were 83.5%, 82.1%, 55.3%, and 66.4%, respectively, indicating that ethanol extraction was more efficient for green algae than for diatoms.

The lower lipid yield from ethanol extraction can be explained by the solvent's polarity. The Bligh and Dyer method employs a solvent mixture containing both polar (methanol) and non-polar (chloroform) components, enabling the extraction of both polar membrane lipids and non-polar neutral lipids. Polar lipids are often bound to proteins in cell membranes; polar solvents disrupt these interactions and release the lipids, which then dissolve in the non-polar phase. Non-polar solvents, in turn, dissolve neutral lipids stored in the cytoplasm (Ryckebosch et al., 2012). Ethanol, being a single polar solvent, is less effective at extracting non-polar neutral lipids, resulting in lower total lipid recovery.

These findings are consistent with previous studies. Dos Santos et al. (2015) and Couto et al. (2022) reported lower lipid yields from ethanol extraction in *C. vulgaris* compared to Bligh and Dyer, while Figueiredo et al. (2019) observed similar trends in *Nannochloropsis oceanica*. However, to the best of our knowledge, no previous studies have reported lipid recovery data for *C. calcitrans* and *T. pseudonana* using ethanol

extraction, making this study the first to provide such comparative data for these diatom species.

The notably lower recovery from diatoms (55.3% and 66.4%) compared to green algae (83.5% and 82.1%) suggests that the silica frustule and the distinct lipid composition of diatoms may hinder ethanol penetration or extraction efficiency. Diatoms are known to contain higher proportions of polar lipids, particularly glycolipids and phospholipids, which may interact strongly with the silica matrix and require more polar solvent systems for efficient extraction. Additionally, the presence of the rigid frustule may limit solvent access to intracellular lipid bodies, further reducing extraction efficiency in the absence of a non-polar solvent component.

Despite the lower total lipid yield, the fatty acid profile of ethanol-extracted lipids revealed important advantages. The percentage of total unsaturated fatty acids (UFAs) was 51.2%, 68.0%, 62.3%, and 74.4% for *C. vulgaris*, *N. oculata*, *C. calcitrans*, and *T. pseudonana*, respectively. For the green algae *C. vulgaris* and *N. oculata*, the SFA, MUFA, and PUFA percentages were comparable to those obtained with the Bligh and Dyer method (Fig 5). However, for the diatoms *C. calcitrans* and *T. pseudonana*, ethanol extraction resulted in higher MUFA and PUFA percentages and lower SFA percentages compared to Bligh and Dyer.

These results suggest that ethanol extraction selectively enriches unsaturated fatty acids, particularly PUFAs, while reducing the proportion of SFAs. This selectivity may arise from the preferential solubility of polar PUFA-containing lipids (e.g., glycolipids, phospholipids) in ethanol, while non-polar SFAs (e.g., triacylglycerols) are less efficiently extracted. From a nutraceutical perspective, this is a favourable trade-off: although total lipid yield is lower, the resulting extract is enriched in the health-promoting omega-3 PUFAs.

The dominant PUFAs in ethanol extracts were linoleic acid (C18:2) and α -linolenic acid (C18:3) in *C. vulgaris*, α -linolenic acid (C18:3) in *N. oculata*, linoelaidic acid (C18:2) and eicosapentaenoic acid (EPA, C20:5) in *C. calcitrans*, and linoelaidic acid (C18:2), α -linolenic acid (C18:3), and γ -linolenic acid (C20:2) in *T. pseudonana*. The presence of EPA and α -linolenic acid in diatoms is particularly noteworthy, as they are high-value omega-3 fatty acid with established cardiovascular and anti-inflammatory benefits.

Ethanol is a cheap, food-grade, and environmentally benign solvent, making it an attractive alternative for industrial applications where solvent safety and regulatory compliance are critical. However, the lower lipid yields obtained in this study suggest that further optimization is needed to enhance extraction efficiency. Possible strategies include coupling ethanol extraction with

ultrasound-assisted or enzyme-assisted pre-treatment, which have been shown to improve cell disruption and lipid release (Chemat et al., 2011; Figueiredo et al., 2019). Additionally, sequential extraction with ethanol followed by a non-polar solvent could recover both polar and non-polar lipids while minimizing toxic solvent use.

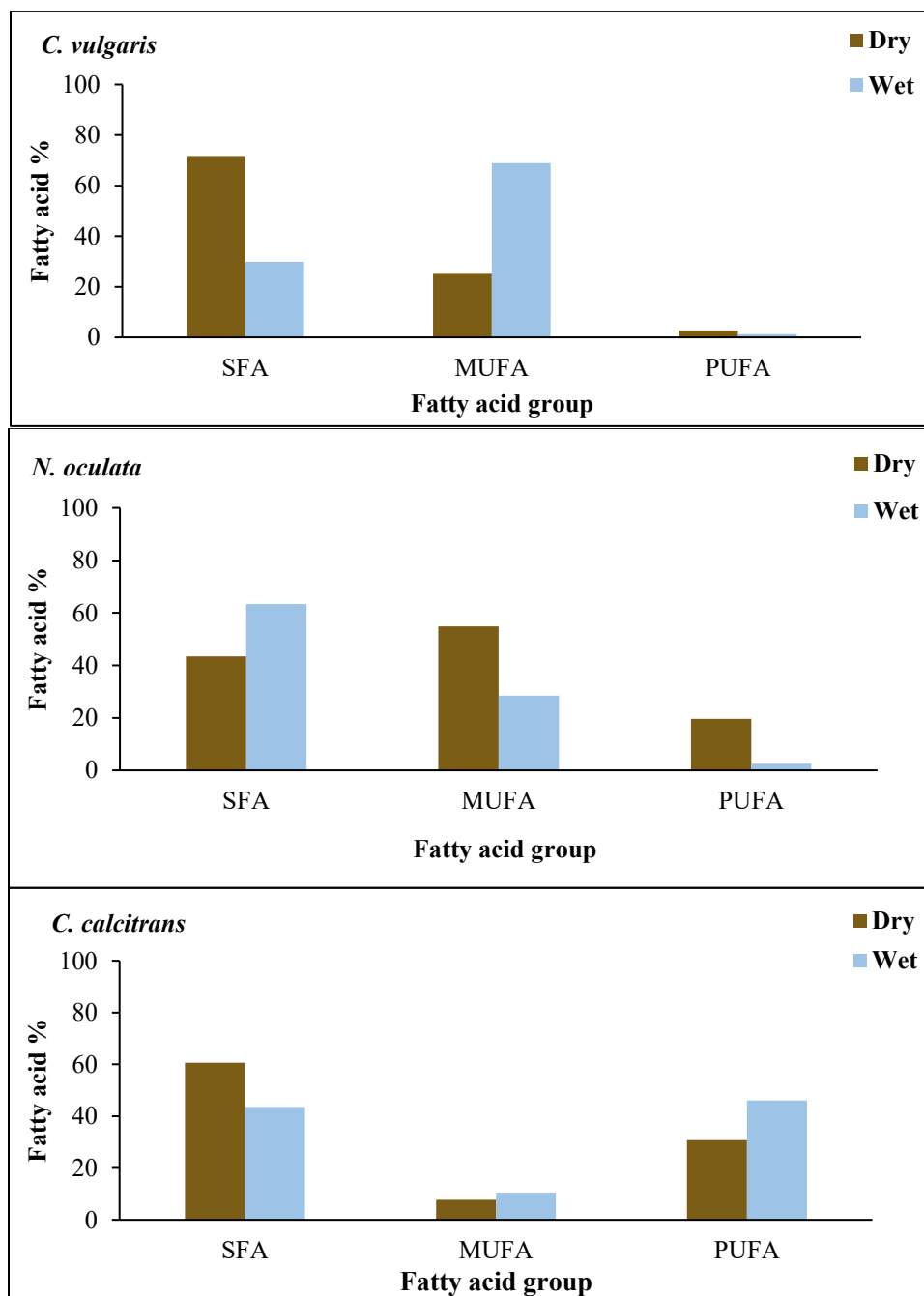


Fig 3. Effect of biomass moisture on fatty acid percentages of *C. vulgaris*, *N. oculata* and *C. calcitrans*

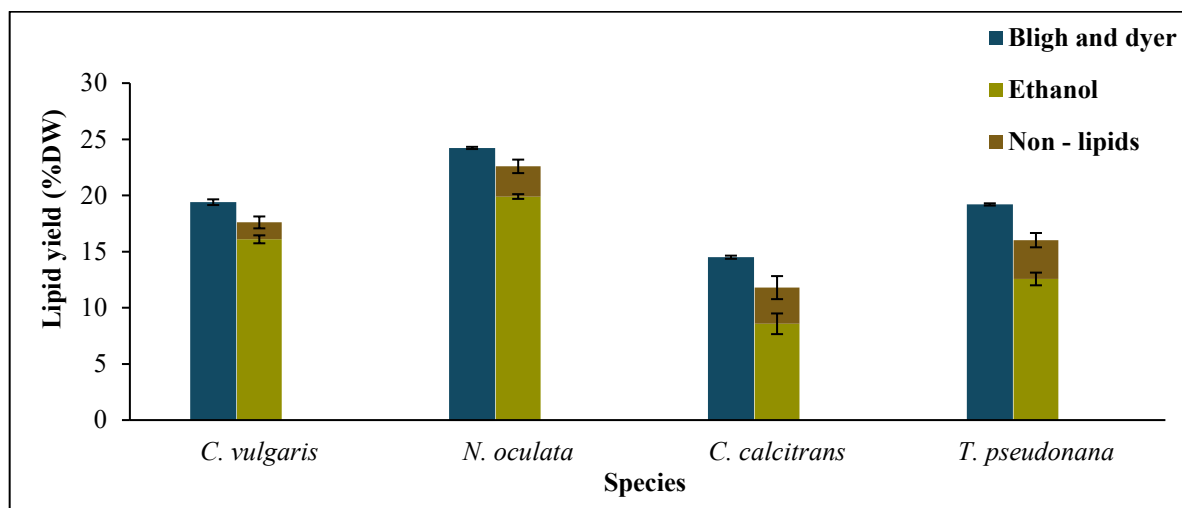


Fig 4. Effect of lipid extraction method on lipid yield of *C. vulgaris*, *N. oculata*, *C. calcitrans* and *T. pseudonana*

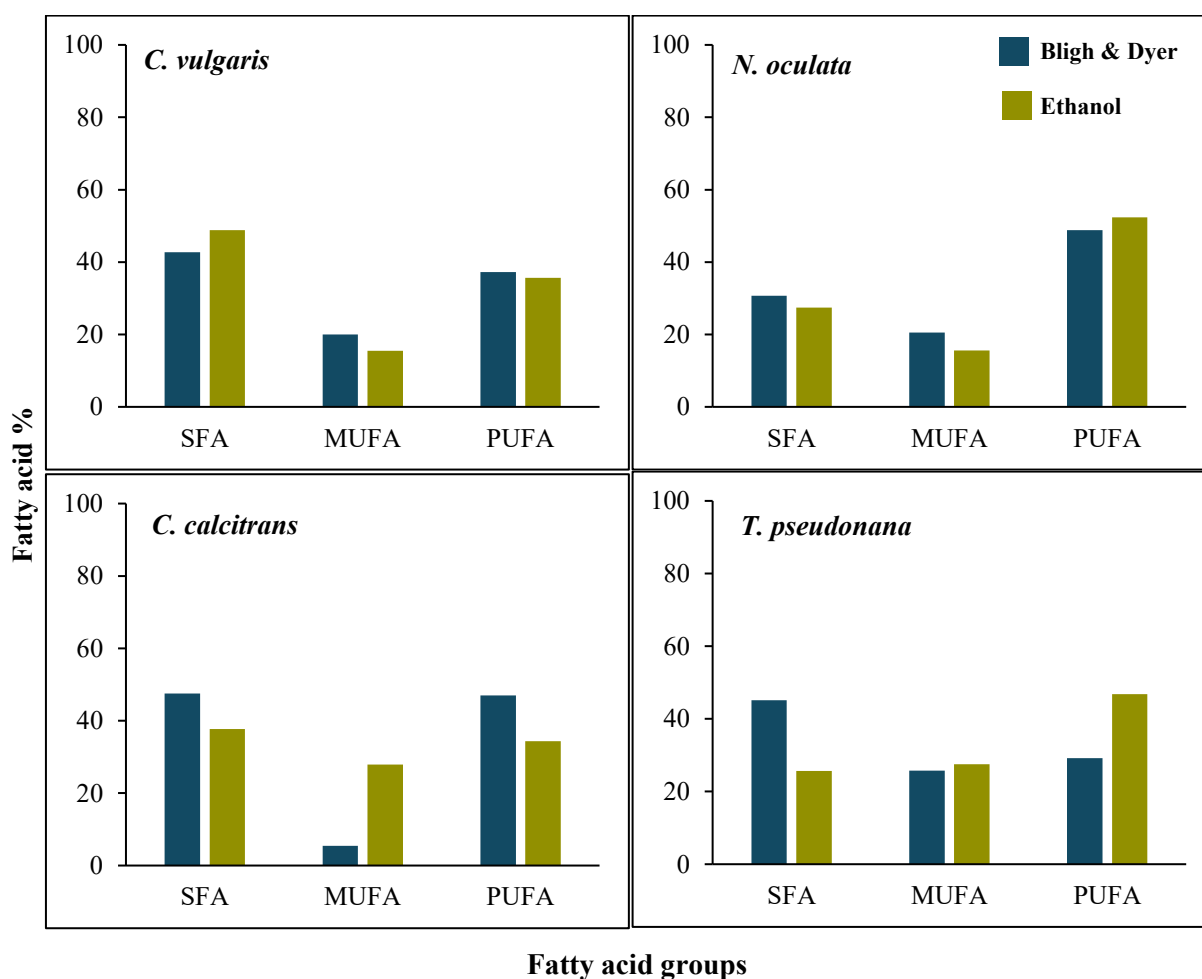


Fig 5. Effect of lipid extraction method on fatty acid percentage of *C. vulgaris*, *N. oculata*, *C. calcitrans* and *T. pseudonana*

This study provides a systematic evaluation of three critical steps in microalgae lipid downstream processing: cell disruption, biomass drying, and solvent selection. The key findings can be summarized as follows:

Microwave-assisted cell disruption is the most efficient and versatile method across all four species, offering high lipid recovery, short processing time, and low equipment cost.

Wet biomass processing yields lipid recovery comparable to dry biomass, with the added benefit of preserving PUFA integrity and eliminating the energy-intensive drying step.

Ethanol extraction, while yielding lower total lipid recovery, selectively enriches unsaturated fatty acids and offers a food-grade, environmentally friendly alternative to chloroform/methanol, particularly for green algae.

From an industrial perspective, these findings support the development of a simplified, cost-effective, and sustainable lipid extraction protocol. The combination of microwave-assisted disruption, wet biomass processing, and ethanol extraction could reduce energy consumption, minimize toxic solvent use, and improve the nutritional quality of the final lipid extract. This is particularly relevant for developing countries like Sri Lanka, where capital investment and operational costs are major barriers to industrial adoption.

However, several limitations should be acknowledged. First, the study was conducted at laboratory scale; scale-up to pilot or industrial scale may introduce challenges related to heat transfer, mixing efficiency, and solvent recovery. Second, the fatty acid profiling was based on relative percentage normalization rather than absolute quantification, which limits direct comparison of absolute fatty acid yields. Further, in both experiments 2 and 3, a single composite sample was used for fatty acid profile analysis. However, the use of multiple replicate samples would have been ideal to account for potential instrumental variability. Third, the oxidative stability of the extracted lipids was not assessed; future studies should evaluate peroxide value, anisidine value, and tocopherol content to fully assess lipid quality. Fourth, the economic feasibility of the proposed protocol was not evaluated; techno-economic analysis and life-cycle assessment are recommended for future work.

Despite these limitations, this study represents a significant advancement in microalgae lipid extraction optimization, particularly for tropical strains that remain underexplored in the literature. The findings provide a solid foundation for further optimization and scale-up, contributing to the development of a sustainable microalgae biorefinery in Sri Lanka and beyond.

4. CONCLUSIONS

This study successfully optimized key steps in the microalgae lipid extraction protocol, demonstrating that microwave-assisted cell disruption, wet biomass processing, and ethanol extraction collectively offer a sustainable, cost-effective, and food-safe approach for lipid recovery from locally cultivated microalgae. Among the four species tested, *N. oculata* exhibited the highest lipid content (22.5% DW) and favourable PUFA profile, making it the most promising candidate for omega-3 production. The comparable performance of wet and dry biomass suggests that eliminating the drying step could substantially reduce processing costs and energy consumption. While ethanol extraction yielded lower total lipid recovery (55–84%), it selectively enriched PUFAs and aligns with food-grade regulatory requirements, warranting further optimization through emerging green technologies. Future work should focus on scale-up, oxidative stability assessment, and techno-economic analysis to fully realize the industrial potential of this optimized protocol.

ACKNOWLEDGEMENTS

The authors are grateful to Taprobane Sea Foods Private Limited, Sri Lanka for providing the microalgae sample.

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