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Extraction and purification of omega-3-rich galactolipids from *Nannochloropsis oceanica* and *Medicago sativa* using green solvents

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ABSTRACT

Large-scale purification of omega-3-rich galactolipids is of growing interest but relies on hazardous solvents. This study presents a scalable and low-toxicity approach using ethanol-based extraction from *Medicago sativa* and *Nannochloropsis oceanica*. Pigments were removed using activated carbon, and non-lipid compounds were eliminated via ethyl acetate partitioning. Fractionation was performed on silica gel using *n*-butyl acetate, ethyl acetate, and acetone. Ethyl acetate fraction yielded 0.57 g (93.6 % α -linolenic acid) and 0.91 g (55.9 % eicosapentaenoic acid) of monogalactosyldiacylglycerols per 100 g of dry biomass of *M. sativa* and *N. oceanica*, respectively. Acetone fractions were further purified by anion exchange chromatography using ethanol, yielding 0.41 g (79.5 % α -linolenic acid) and 0.75 g (29.6 % eicosapentaenoic acid) of digalactosyldiacylglycerols per 100 g of dry *M. sativa* and *N. oceanica*, respectively. The high purity of galactolipid fractions was demonstrated by high-performance liquid chromatography and high-performance thin-layer chromatography. To the best of our knowledge, this is the first complete purification process for galactolipids that relies exclusively on green solvents and is suitable for preparative scale.

1. Introduction

Galactolipids are polar lipids found in plants and microalgae chloroplasts. The two major galactolipids are monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG) (Hölzl and Dörmann, 2019). In plants and microalgae containing high proportions of omega-3 (n-3) such as *Medicago sativa* and *Nannochloropsis oceanica*, galactolipids are packed with exceptionally high amounts of α -linolenic acid (ALA, 18:3 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3), respectively (Da Costa et al., 2016; Sahaka et al., 2020). These essential fatty acids play a crucial role in human health (Saidaiah et al., 2024). However, the bioavailability of n-3 fatty acids carried by galactolipids is underexplored (Kagan et al., 2015) and *in vivo* studies are still needed to fully understand this aspect. Such experiments require large quantities of purified galactolipids, which are commercially available only at high cost and from limited sources. For instance, in their study, Kagan et al. (2015) administered a total of 36 g of n-3 fatty acids to 20 rats over 7 days, comparing *Nannochloropsis* galactolipid-rich oil with krill oil. Consequently, high-throughput extraction and purification methods are

required for performing such *in vivo* studies on n-3-rich galactolipids.

Conventional extraction usually relies on large volumes of organic solvents such as chloroform, methanol, or hexane. These solvents are highly hazardous and their use in large volumes raises serious safety and environmental concerns (Prat et al., 2015). The CHEM21 (Chemical Manufacturing Methods for the 21st Century) is a European consortium promoting sustainable biological and chemical methodologies. Its solvent selection guide has become a highly cited reference, which provides a solid basis for selecting green solvents in the context of greener chemical processes, based on health, safety, and environmental scores. It encourages the use of less toxic, more sustainable, and renewable alternatives such as ethanol (Prat et al., 2015). Ethanol has been extensively studied for lipid extraction (Sousa et al., 2023), and it could be of great interest for galactolipid extraction because of its high polarity (Servaes et al., 2015). Although satisfactory yield can be obtained for some microalgae and terrestrial plants, most studies on *Nannochloropsis* showed that the overall lipid extraction yield obtained with ethanol without a cell disruption method is poor (Figueiredo et al., 2019; Melo et al., 2021). This is explained by the resistant cell wall of

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Nannochloropsis, hindering the penetration of the solvent into the microalgal cells. Therefore, ethanol extraction should be assisted by processes that promote solvent diffusion inside the cell to achieve high lipid yield (Zhao et al., 2022; Shivakumar et al., 2024). Ultrasound-assisted extraction and pressurized-liquid extraction (PLE) are efficient processes to improve cell permeability, solvent diffusion and/or mass transfer during ethanol extraction of *Nannochloropsis* lipids (Blanco-Llamero and Señoráns, 2021; Figueiredo et al., 2019). However, there is currently no systematic comparison of ethanol-based extraction methods for *N. oceanica* and *M. sativa*, nor any comparison against a conventional reference method – typically a Bligh and Dyer-type method – which is unambiguously recognized as the most efficient approach for total lipid extraction.

Yet, ethanol has a low selectivity as it extracts many other components such as proteins and carbohydrates along with lipids (Ryckeboesch et al., 2014). Therefore, when ethanol is used as the extraction solvent, it must be paired with another solvent of low to medium polarity in a two-phase partitioning system to isolate lipids from non-lipid compounds before proceeding with purification. Ethyl acetate is a selective, bio-based, and low-toxicity solvent meeting these requirements. Breil et al. (2017) used a mixture of ethyl acetate, ethanol, and water to extract lipids from *Yarrowia lipolytica* and concluded that the selectivity of this solvent system was comparable to that of the universally adopted Folch method. Therefore, ethyl acetate could be used in a two-phase partitioning step applied to ethanolic extracts as a greener alternative to the chloroform-methanol mixtures generally used.

Conventional fractionation and purification methods for galactolipids include preparative high-performance liquid chromatography (HPLC) or thin layer chromatography (TLC), solid-phase extraction (SPE), and gravity column chromatography, used either individually or in combination. Among these methods, gravity column chromatography allows for the purification of high amounts of galactolipids in a short time, offering high resolution with minimal equipment requirements (Rathinavel et al., 2025). As in conventional extraction methods, it usually requires hazardous solvents, including but not limited to chloroform, methanol, or hexane (Gallant and Leblanc, 1991; Amara et al., 2010; Lee et al., 2020), and studies on the fractionation of lipids using green solvents remain scarce. Although Sánchez et al. (2023) separated neutral and polar lipids from *N. oceanica* by silica gel chromatography using *n*-butyl acetate and ethanol, there is no study on further separation of different polar lipid classes using green solvents.

Additionally, *M. sativa* and *N. oceanica* are rich in pigments such as chlorophylls and carotenoids (Altuner, 2021; Lubian et al., 2000) which means that galactolipid fractions purified from natural sources are also likely to contain high amounts of pigments (Van Der Veen and Olcott, 1964). Although a few studies have reported the use of activated carbon for galactolipid purification (Yamaguchi et al., 2012; Hazahari et al., 2018), its potential remains underexplored and it has never been used exclusively with green solvents.

To date, no study has described the adaptation of conventional purification processes into a fully green solvent-based method for the preparation of gram-scale quantities of pure galactolipids, including pigment removal. This work is the first to systematically compare ethanol-based maceration, ultrasound, and PLE extraction protocols coupled with ethyl acetate partitioning for *M. sativa* and *N. oceanica*, and to evaluate them against chloroform-methanol extraction. Activated carbon was also explored for pigment removal without using chlorinated solvents, offering a simple and efficient alternative to more hazardous or complex procedures. Therefore, this work aimed to obtain n-3 rich MGDG and DGDG fractions, with high yield and purity, from *M. sativa* and *N. oceanica* using low-toxicity, economical, and widely available solvents such as ethanol, ethyl acetate, *n*-butyl acetate, and acetone. These solvents are classified as “recommended” according to the CHEM21 solvent selection guide, in contrast to chloroform and hexane, which are ranked as “hazardous” or “highly hazardous”. Notably, this is the first study to fully substitute chloroform and methanol with greener

alternatives in the entire fractionation process, while achieving comparable purity and yield. Finally, the method was successfully applied at a preparative scale, demonstrating its relevance for producing n-3-rich galactolipids suitable for further *in vivo* studies.

2. Materials and methods

Galactolipids from *M. sativa* and *N. oceanica* were obtained by extracting total lipids from the biomass, followed by fractionation into lipid classes. These two steps were first carried out at the analytical scale, using either conventional or green solvents. The two methods were compared in terms of total lipid yield, fatty acid profile, galactolipid yield, and purity to assess the suitability of green solvents for galactolipid purification (Fig. 1A). Subsequently, the elution scheme was refined to improve purity, while a pigment removal and a DGDG purification steps were established. Ultimately, these steps were implemented at a preparative scale (Fig. 1B). The galactolipid fractions obtained were analyzed in terms of yield, purity, fatty acid profile, and pigment content.

2.1. Materials

Fresh *M. sativa* leaves and stems were harvested in July 2023 in Pays de la Loire (France). They were immediately rinsed with tap water, blanched in boiling water for 3 min before being freeze-dried, ground, sieved at Ø1.25 mm, and then stored in an airtight container at -20°C . *N. oceanica* freeze-dried powder was purchased from Provion Industries NV (Hemiksem, Belgium) and stored at -20°C .

Chloroform (HPLC, 99.9 % stabilized with ethanol), methanol (HPLC, 99.9 %), 2-propanol (HPLC, 99.9 %) acetone (HPLC, 99.9 %), ethanol (CP, dehydrated, 99 %) and ethyl acetate (AR, 99.8 %), were purchased from Biosolve (Dieuze, France). Toluene (Reag. Ph. Eur., 99.8 %), cyclohexane (RS, 99.8 %), glacial acetic acid (AR, 99.8 %), hydrochloric acid (37 % solution), and ammonia solution (AR, 30 %) were purchased from Carlo Erba Reagents (Val de Reuil, France). *n*-Butyl acetate (99 %) was purchased from Chimie-Plus Laboratories (Saint-Paul-de-Varax, France). Methanol solution of 14 % boron-trifluoride, as well as potassium hydroxide (≥ 90 %), primuline, 1-naphthol, and activated carbon (DARCO®, 100 mesh particle size powder) were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany). Sulfuric acid (95–98 %) and sodium chloride were purchased from Panreac Appli-chem S.A. (Barcelona, Spain). Silica gel cartridges (Sep-Pak Silica Classic, 690 mg silica) were purchased from Waters Corporation (Milford, USA). Silica gel powder (EcoChrom MP Silica, 63–100 μm , 60 Å pore size) was purchased from MP Biomedicals (Illkirch, France). DEAE-C (Diethylaminoethyl-cellulose, ref: SC-211213) was purchased from Santa Cruz Biotechnology (Dallas, USA). Anhydrous sodium sulfate, HPTLC plates (silica gel 60 F₂₅₄, 20 × 10 cm), and FAMES standard (C4–C24 mix) were purchased from Merck (Darmstadt, Germany). Heptadecanoic acid standard was purchased from Fluka (Honeywell Research Chemicals, Seelze, Germany). MGDG, DGDG, and sulfoquinovosyldiacylglycerol (SQDG) standards were purchased from Avanti Polar Lipids (Alabaster, USA). Diatomaceous earth (Dionex ASE Prep DE) was purchased from Thermo Fisher Scientific (Sunnyvale, USA). Filter paper (Whatman Grade 1, Cat No 1005-070) was purchased from Cytiva (Marlborough, USA).

For solvent mixtures, ratios are expressed as volume-to-volume (v/v).

2.2. Sample handling

After all extraction and purification steps described below, lipid extracts and fractions were dried and stored in the same manner unless otherwise specified. Samples were initially dried by rotary evaporation at 40°C under vacuum, followed by complete drying under a stream of nitrogen at room temperature. Dried samples were weighed and

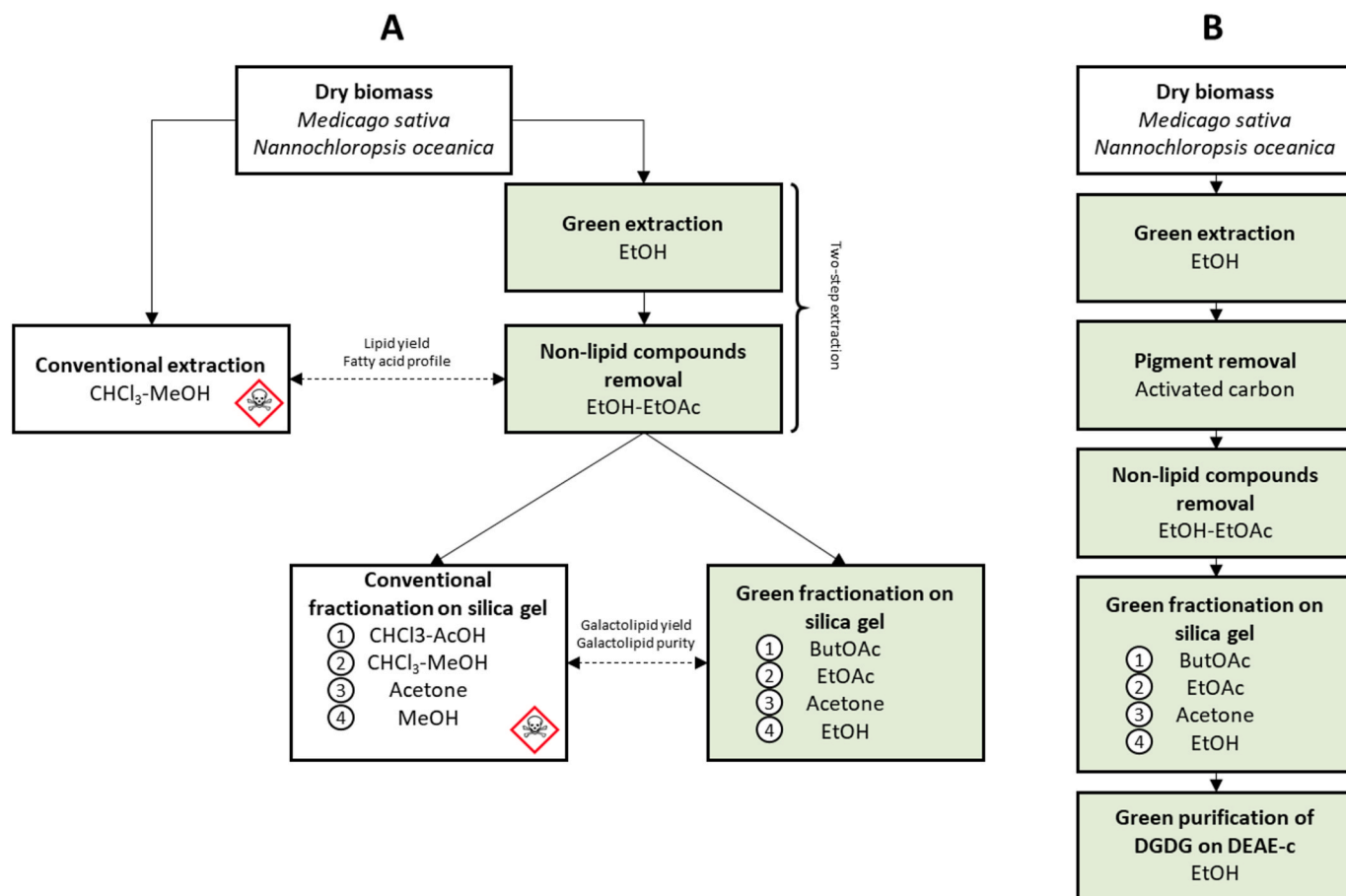


Fig. 1. Scheme of the A) green extraction and fractionation of galactolipids from *Nannochloropsis oceanica* and *Medicago sativa* in comparison with conventional methods and B) final green extraction, fractionation and purification method. CHCl₃: chloroform, MeOH: methanol, EtOAc: ethyl acetate, ButOAc: *n*-butyl acetate, EtOH: ethanol, AcOH: acetic acid, DEAE-C: diethylaminoethyl cellulose.

gravimetric yield was expressed per 100 g of dry biomass (DW) initially used for extraction. Samples were then dissolved in chloroform and stored at - 80 °C after flushing the headspace with nitrogen.

2.3. Conventional extraction and fractionation methods

Reference extraction yields were determined using chloroform-methanol extraction methods. After a preliminary study, the Bligh and Dyer method (chloroform-methanol 1:2) (Bligh and Dyer, 1959) was chosen for *M. sativa*, and the modified Bligh and Dyer method (Ryckebosch et al. (2014)) was chosen for *N. oceanica* (chloroform-methanol 1:1), as they provided higher extraction yields compared with the Folch method (chloroform-methanol 2:1).

Bligh and Dyer extraction of *M. sativa*: *M. sativa* powder (1 g) was pre-wetted with 6 mL of Milli-Q water before adding 22.5 mL of chloroform-methanol (1:2). The mixture was stirred for 3 h at room temperature and then centrifuged (1000 g for 10 min at 20 °C). The supernatant was collected while the pellets were re-extracted twice for 30 min and 15 min respectively, with 20 mL of chloroform-methanol-water (1:2:0.8), followed by centrifugation. The three supernatants were combined in a separatory funnel before adding 18 mL of chloroform and 18 mL of 0.9 % sodium chloride solution. The sample was mixed and allowed to settle overnight at 7 °C before the organic phase was collected and filtered through anhydrous sodium sulfate. The organic phase was subsequently dried, weighed, and stored as described above.

Ryckebosch extraction of *N. oceanica*: *N. oceanica* powder (300 mg) was mixed with 12 mL of methanol and vortexed for 30 s.

Subsequently, 6 mL of chloroform and 1.2 mL of 0.9 % sodium chloride solution were added, and the mixture was vortexed again for 30 s. This step was repeated with an additional 6 mL of chloroform and 6 mL of 0.9 % sodium chloride solution followed by vortexing for another 30 s. After centrifugation, the aqueous phase was discarded, and the organic phase was filtered through anhydrous sodium sulfate. The pellets were re-extracted twice, each time using 6 mL of chloroform, 6 mL of methanol, and 3.6 mL of 0.9 % sodium chloride solution, followed by vortexing, centrifugation, removal of the aqueous phase, and filtration of the organic phase through anhydrous sodium sulfate as described above. The organic phase was subsequently dried, weighed, and stored as described above.

2.4. Green extraction methods

For both *M. sativa* and *N. oceanica*, three extraction methods were compared: maceration in ethanol and ultrasound-assisted ethanol extraction were adapted from the analytical scale method described by Figueiredo et al. (2019). Pressurized ethanol extraction was adapted from previously described works that identified optimal lipid extraction conditions for *Nannochloropsis* sp. (Jiménez Callejón et al., 2022).

Maceration in ethanol: 10 mL of ethanol was added to 1 g of *M. sativa* or *N. oceanica* and stirred for 8 min at room temperature. The mixture was filtered on a sintered glass funnel No. 4 (pore sizes 10–16 µm) with cellulose filter paper and the residual biomass was re-extracted seven times using the same procedure. The eight filtered extracts were combined, dried, and stored as described above.

Ultrasound-assisted ethanol extraction: 10 mL of ethanol was

added to 1 g of *M. sativa* or *N. oceanica*. The mixture was placed on an ice bath and sonicated with an S-4000 Ultrasonic Processor (Misonix, Newtown, USA) equipped with a microtip probe set at 70 % amplitude and a frequency of 20 kHz for six cycles of 20 s pulses followed by 1 min pauses to avoid excessive heating (2 min sonication, total duration of 8 min). The crude extract was filtered on a sintered glass funnel No. 4 with cellulose filter paper and the residual biomass was re-extracted seven times with the same procedure. The eight filtered extracts were combined, dried, and stored as described above.

Pressurized ethanol extraction was performed in a Dionex ASE 350 Accelerated Solvent Extraction System (Thermo Scientific, Sunnyvale, USA) with 22-mL extraction cells. *M. sativa* or *N. oceanica* powder was mixed with diatomaceous earth in a 1:1 mass ratio and poured into the extraction cell (approximately 6 g of mixture per cell). The extraction was performed at 125 °C and 10 MPa over three static extraction cycles of 5 min each with a 100 % cell volume flush. The extracts were combined, dried, and stored as described above.

The extracts obtained with these green methods are referred to as “crude extracts” as they contain lipids and co-extracted non-lipid compounds.

2.5. Removal of non-lipid compounds by two-phase partitioning using ethyl acetate

The lipid part of the crude extracts was isolated by two-phase partitioning using ethyl acetate, ethanol, and water. An amount of dry crude extract equivalent to 1 g of initial biomass was redissolved in 2.8 mL of ethanol and 11.2 mL of ethyl acetate. Then 11.2 mL of 0.9 % sodium chloride solution was added. The mixture formed a biphasic system that was thoroughly mixed and the two phases were then separated either by centrifugation (small volume tubes) or overnight settling in a separatory funnel (large volumes for preparative scale) at 7 °C. The organic phase was then collected and filtered through anhydrous sodium sulfate before drying, weighing, and storing as described above.

2.6. Pigment removal using activated carbon

Pigments were removed from crude extracts before lipid isolation. Activated carbon (31.5, 62.5, 125, 187.5, 250, or 375 mg for *M. sativa*; 40, 80, 120, 160, 250, or 375 mg for *N. oceanica*) was added to 8 mL of ethanol containing 50 mg of lipids. Samples were gently mixed for 45 min at room temperature and filtered through a sintered glass funnel No. 4 with cellulose filter paper. The clarified crude extract was then used as is, or after dilution for pigment analysis.

2.7. Fractionation of total lipid extracts into lipid classes using silica gel and conventional solvents

Lipid extracts were fractionated by SPE using 690 mg silica gel cartridges previously washed with 10 mL of methanol and conditioned with 10 mL of chloroform. A volume equivalent to 40 mg of lipid extract was loaded on the cartridge and four lipid fractions were successively eluted according to the elution scheme described in Table 1. The collected

Table 1

Elution scheme of total lipid extracts using a silica gel cartridge and conventional solvents.

Solvent	Volume (mL/g silica gel)	Collected fraction
Chloroform-acetic acid (99:1)	14.5	NL
Chloroform-methanol (95:5)	14.5	MGDG
Acetone	58	DGDG + SQDG
Methanol	14.5	PL

NL: neutral lipids, MGDG: monogalactosyldiacylglycerols, DGDG: digalactosyldiacylglycerols, SQDG: sulfoquinovosyldiacylglycerols, PL: phospholipids.

fractions were dried, weighed, and stored as described above.

2.8. Fractionation of total lipid extracts into lipid classes using silica gel and green solvents

At the analytical scale, green fractionation of lipid extracts was performed using 690 mg silica gel cartridges washed with 10 mL of ethanol, then conditioned with 10 mL of *n*-butyl acetate and loaded with 40 mg of lipid extract. Five fractions were successively eluted according to the elution scheme described in Table 2 *n*-Butyl acetate volume was optimized (7.3 and 9.0 mL/g for *M. sativa* and *N. oceanica*, respectively) to ensure complete elution of neutral lipids in the *n*-butyl acetate fraction with limited loss of MGDG. In addition, an initial 3.6 mL/g of ethyl acetate was used to remove residual *n*-butyl acetate from the cartridge, preventing its collection in the MGDG fraction. This was necessary due to the high boiling point of *n*-butyl acetate (125 °C) which makes it difficult to remove by evaporation. The collected fractions were then dried, weighed, and stored as described above.

At the preparative scale, a glass column (32 cm × 3.4 cm i.d.) was packed with 25 g of silica gel slurry in ethanol. The column was conditioned with 400 mL of *n*-butyl acetate and loaded with 1800 mg of lipid extract. The collected fractions were dried, weighed, and stored as described above.

2.9. DGDG purification by anion exchange chromatography on DEAE-C using green solvents

DEAE-C (15 g) was poured into 1 M hydrochloric acid and gently mixed for 5 min. Then it was rinsed with Milli-Q water on a filter funnel until the filtrate was neutral. The same procedure was repeated using 0.1 M potassium hydroxide instead of hydrochloric acid, followed by rinsing until neutrality. The DEAE-C was then conditioned with 3 vol of glacial acetic acid for 12 h, then poured into a glass column (32 × 3.4 cm i.d.) and thoroughly washed with 1 L of ethanol. The DGDG + SQDG fraction previously collected from silica gel fractionation (acetone fraction) was concentrated until its volume reached 50 mL, and was then loaded on the DEAE-C. Anionic SQDG was retained by the DEAE-C, while DGDG was eluted using 1100 mL of ethanol.

2.10. Analysis of pigment content by UV-VIS spectrophotometry

Since chlorophylls and carotenoids (including xanthophylls) are the main pigments in *M. sativa* (Altuner, 2021) and *N. oceanica* (Lubian et al., 2000), their concentration was determined in total lipids and galactolipid fractions. Lipids were dried and dissolved in ethanol, then analyzed with a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Chlorophyll and carotenoid concentrations were determined using equations according to Lichtenthaler and Buschmann (2001):

$$c_a (\mu\text{g}\cdot\text{mL}^{-1}) = 13.36A_{664} - 5.19A_{648} \quad (1)$$

$$c_b (\mu\text{g}\cdot\text{mL}^{-1}) = 27.43A_{648} - 8.12A_{664} \quad (2)$$

Table 2

Elution scheme of total lipid extracts using a silica gel cartridge and green solvents.

Solvent	Volume (mL/g silica gel)	Collected fraction
<i>n</i> -Butyl acetate	7.3–9	NL
Ethyl acetate	3.6	Residual <i>n</i> -butyl acetate
Ethyl acetate	18	MGDG
Acetone	58	DGDG + SQDG
Ethanol	58	PL

$$c_c (\mu\text{g}\cdot\text{mL}^{-1}) = (1000A_{470} - 2.13c_a - 97.64c_b) / 209 \quad (3)$$

with c_a : chlorophyll *a* concentration, c_b : chlorophyll *b* concentration, c_c : carotenoids concentration, A : absorbance at the specified wavelength.

The pigment concentration was then normalized to the lipid concentration in the sample and expressed as $\mu\text{g}/\text{mg}$ of lipids or as % of lipid mass.

The pigment removal rate was calculated using the following equation:

$$\text{Pigment removal rate (\%)} = \frac{P_{nd} - P_d}{P_{nd}} \times 100 \quad (4)$$

where P_{nd} : total pigment content (chlorophylls + carotenoids) in non-depigmented extract, P_d : total pigment content in depigmented extract. All pigment contents are expressed as micrograms (μg) of pigments per milligram (mg) of total lipids in the extract.

2.11. Qualitative analysis of polar lipids by high-performance thin layer chromatography (HPTLC)

Polar lipids were analyzed using HPTLC as described by Laillet et al. (2024). HPTLC plates were washed in 2-propanol and activated at 120 °C for 30 min. Samples were deposited with an Automatic TLC Sampler 4 (CAMAG, Muttentz, Switzerland). The mobile phase was chloroform-methanol-30 % ammonia (65:35:5). The plates were developed to a distance of 85 mm in an Automatic Developing Chamber 2 (CAMAG, Muttentz, Switzerland) after saturation with the mobile phase and humidity setting to 33 %. Non-specific derivatization was achieved using a primuline solution in acetone-water (80:20) followed by fluorescence visualization at 366 nm in a TLC Visualizer 3 (CAMAG, Muttentz, Switzerland). Specific derivatization of glycolipids was performed using a 1-naphthol solution in sulfuric acid-ethanol-water (1:8:1), followed by heating at 120 °C for 3 min and white light visualization.

2.12. Quantification of MGDG and DGDG by HPLC coupled with evaporative light scattering detector (HPLC-ELSD)

Galactolipids were quantified using an HPLC Ultimate 3000 RS System (Dionex, Voisin Le Bretonneux, France) coupled with an ELSD Sedex 85 (Sedere S.A., Alfortville, France). The column was an Uptisphere CS Evolution (150 mm $\times$ 4.6 mm internal diameter, 2.6 μm particle size, 85 Å pore size) (Interchim, Montluçon, France). The mobile phase consisted of chloroform (solvent A) and methanol-chloroform-30 % ammonia (92:7:1) (solvent B). Samples were eluted at a constant flow rate of 1.5 mL/min, in gradient mode starting with 100 % solvent A, decreased to 80 % after 3 min and 0 % after 12 min, followed by a 3-min hold. Column temperature and ELSD temperatures were set at 30 °C and 40 °C, respectively. The nebulizing gas (air) pressure was set at 3.5 bars and the photomultiplier sensitivity was set to a gain value of 6. Galactolipids were quantified using a calibration curve obtained using MGDG and DGDG standards. Data acquisition and chromatogram integration were performed using Chromeleon 7.2.10 ES (Thermo Fisher Scientific, Villebon sur Yvette, France).

2.13. Analysis of fatty acid content by gas chromatography coupled with flame ionization detector (GC-FID)

Fatty acid methyl esters (FAMES) were prepared from lipid extracts and fractions using a methanol solution of 14 % boron trifluoride. One milligram of lipids was mixed with 100 μg of heptadecanoic acid (used as an internal standard) and dried under a stream of nitrogen. Then, 1 mL of toluene, and 1 mL of boron trifluoride solution were added. Samples were heated at 100 °C for 45 min and then cooled at room temperature before adding 1 mL of cyclohexane and 0.5 mL of Milli-Q water. The samples were vortexed, and centrifuged (1000 g for 5 min

at 20 °C). The supernatant was analyzed by GC equipped with an FID (Clarus GC 690, PerkinElmer Scientific, Villepinte, France) and a fused-silica capillary column coated with cyanopropylphenyl and dimethylpolysiloxane (DB 225 MS, 30 m $\times$ 0.32 mm i.d. $\times$ 0.25 μm film thickness). The carrier gas (dihydrogen) was set at 2 mL/min constant flow. Two microliters of FAME solution were injected at 250 °C in splitless mode. The initial column temperature was held at 50 °C for 1 min. Then it was increased at a rate of 15 °C/min to 180 °C and held for 1 min. Finally, it was increased at a rate of 5 °C/min to 220 °C and held for 10 min. The FID temperature was set at 250 °C. FAMES were identified by comparison with the FAME standard. Data acquisition and chromatogram integration were performed with Chromeleon 7.2.10 ES (Thermo Fisher Scientific, Villebon sur Yvette, France).

2.14. Calculation of lipid yield and recovery

Lipid yield was defined as the amount of extracted or purified lipids relative to initial dry biomass and was systematically expressed in g per 100 g of dry biomass. It was calculated using the following equation:

$$\text{Yield (g / 100 g)} = \frac{\text{Mass of extracted or purified lipids (g)}}{\text{Initial biomass (g)}} \times 100 \quad (5)$$

Galactolipid recovery was defined as the amount of a given galactolipid (either MGDG or DGDG) recovered after a specific step, relative to its total amount in the initial lipid extract. It was calculated using the following equation:

$$\text{Recovery} = \frac{\text{Mass of recovered galactolipid (g)}}{\text{Total mass of galactolipid in the initial lipid extract (g)}} \times 100 \quad (6)$$

2.15. Statistics

All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation. Statistical significance was assessed using ANOVA followed by Tukey's multiple comparison test, with a significance level set at $p < 0.05$. Statistical analysis was performed using R 4.2.2.

3. Results and discussion

This section presents a stepwise evaluation of the green extraction, fractionation, and purification processes. First, the efficiency of the green extraction methods is assessed at the analytical scale by comparing lipid yields and fatty acid profiles with those obtained using the reference method. Next, the performance of the green fractionation process is evaluated based on the lipid composition of the resulting fractions, in comparison with the reference approach. The subsequent parts describe the purification process for DGDG and the implementation of a pigment removal method, both using ethanol as the sole solvent. Finally, the step-by-step recovery of the target compounds at the preparative scale is evaluated, and the composition of the galactolipid-rich fractions obtained is also described.

3.1. Comparison of conventional and green extraction methods

The total lipid extraction yield obtained with green extraction methods was compared with that of a reference method. For *M. sativa*, the reference method (Bligh and Dyer) yielded 5.8 g/100 g DW, a value close to the total lipid yield of 6.4 g/100 g reported by Van Der Veen and Olcott (1964). Simple maceration in ethanol yielded 5.0 g/100 g (86 % recovery of total lipids), but ultrasound-assisted extraction and PLE resulted in a similar yield (6.1 and 5.7 g/100 g, respectively) compared to the reference method (Fig. 2A). For *N. oceanica*, the reference method (modified Bligh and Dyer) yielded 20.4 g/100 g, in line with the 22.7

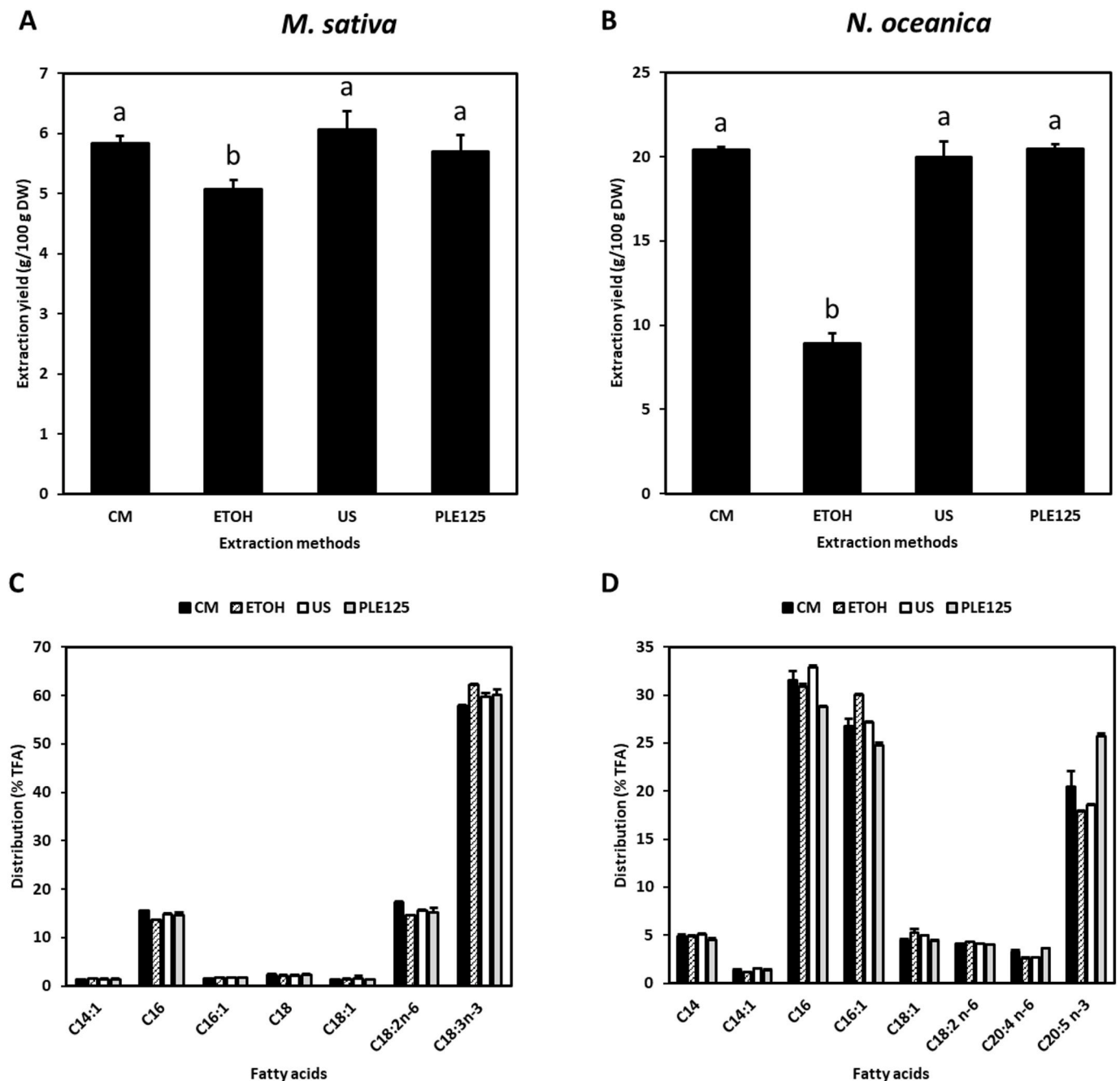


Fig. 2. Extraction yield of total lipids and fatty acid composition of lipid extracts from *M. sativa* (A, C) and from *N. oceanica* (B, D) obtained using different methods. DW: dry biomass weight, CM: chloroform-methanol extraction, ETOH: extraction by maceration in ethanol, US: ultrasound-assisted ethanol extraction, PLE125: pressurized ethanol extraction at 125 °C, TFA: total fatty acids. Different letters indicate significant differences ($p < 0.05$). Only fatty acids representing more than 1 % of the total fatty acids are shown.

g/100 g reported by Uthaiah et al. (2025). Maceration in ethanol led to significantly lower lipid yield (8.9 g/100 g, corresponding to a 43 % recovery of total lipids), but PLE and ultrasound-assisted extraction yielded similar lipid amounts (20.0 and 20.5 g/100 g, respectively) to the reference method (Fig. 2B).

The fatty acid profile of the lipid extracts was analyzed by GC-FID. For *M. sativa*, the reference extract contained 57.8 ± 0.2 % of ALA, as well as C18:2n-6 and C16:0 as secondary fatty acids. The fatty acid profiles of the green extracts were similar to that of the reference extract (Fig. 2C). For *N. oceanica*, the reference extract contained 30.8 ± 1.0 % of C16:0, 26.7 ± 0.8 % of C16:1, and 20.4 ± 1.7 % of EPA. Maceration in ethanol and ultrasound-assisted extraction yielded significantly less EPA

than the reference method (17.8 % and 18.5 %, respectively). On the other hand, the PLE extract contained significantly more EPA (25.8 ± 0.2 %) as well as less C16:0 and C16:1 than all the other extracts (Fig. 2D).

Although extraction of total lipids is typically performed using hazardous solvents, a simple and mild ethanol extraction (maceration method) achieved high lipid recovery for *M. sativa*. For *N. oceanica*, maceration resulted in unsatisfactory yield (43 % recovery of total lipids), as previously reported by Figueiredo et al. (2019). To address this limitation, two green extraction methods known to achieve cell wall disruption or enhance solvent penetration into the cell were evaluated: ultrasound-assisted extraction, and PLE (125 °C), both using ethanol

(Zhou et al., 2022; Sousa et al., 2023). Both techniques yielded lipid amounts comparable to those obtained with the reference extraction method for both species. Moreover, the fatty acid composition of the green extracts was comparable to that of the reference extract for *M. sativa*, whereas PLE improved the extraction of EPA from *N. oceanica*. The latter was unexpected, as chloroform-methanol extraction is generally considered to provide complete lipid recovery from microalgae (Ryckebosch et al., 2012). Findings from the present study are consistent with those of Mitra and Mishra (2019) and Castejón and Señoráns (2019), who reported comparable lipid yield with higher EPA content using alternative solvent systems (hexane-methanol 3:2 and hexane with PLE at 120 °C, respectively) to extract *Nannochloropsis* lipids. These findings suggest that the chloroform-methanol system may not extract all lipids from *Nannochloropsis* as previously assumed, whereas some alternative solvent systems may exhibit higher selectivity

for lipids that are rich in n-3 fatty acids.

Given the results of extraction yields and fatty acid composition of the different methods, maceration in ethanol and PLE at 125 °C were selected for the extraction of *M. sativa* and *N. oceanica* lipids, respectively. All lipid extracts referred to as “green” in the following experiments were obtained with these methods.

3.2. Fractionation of total lipid extracts into lipid classes

The fractionation was first performed at the analytical scale on non-depigmented total lipid extracts. The lipid composition of total lipids and galactolipid fractions, whether from conventional solvents or green solvents, was analyzed by HPTLC (Fig. 3A and B). Based on a comparison with commercial standards, the intense bands with retention factors (R_F) of 0.18 and 0.70 were attributed to DGDG and MGDG, respectively.

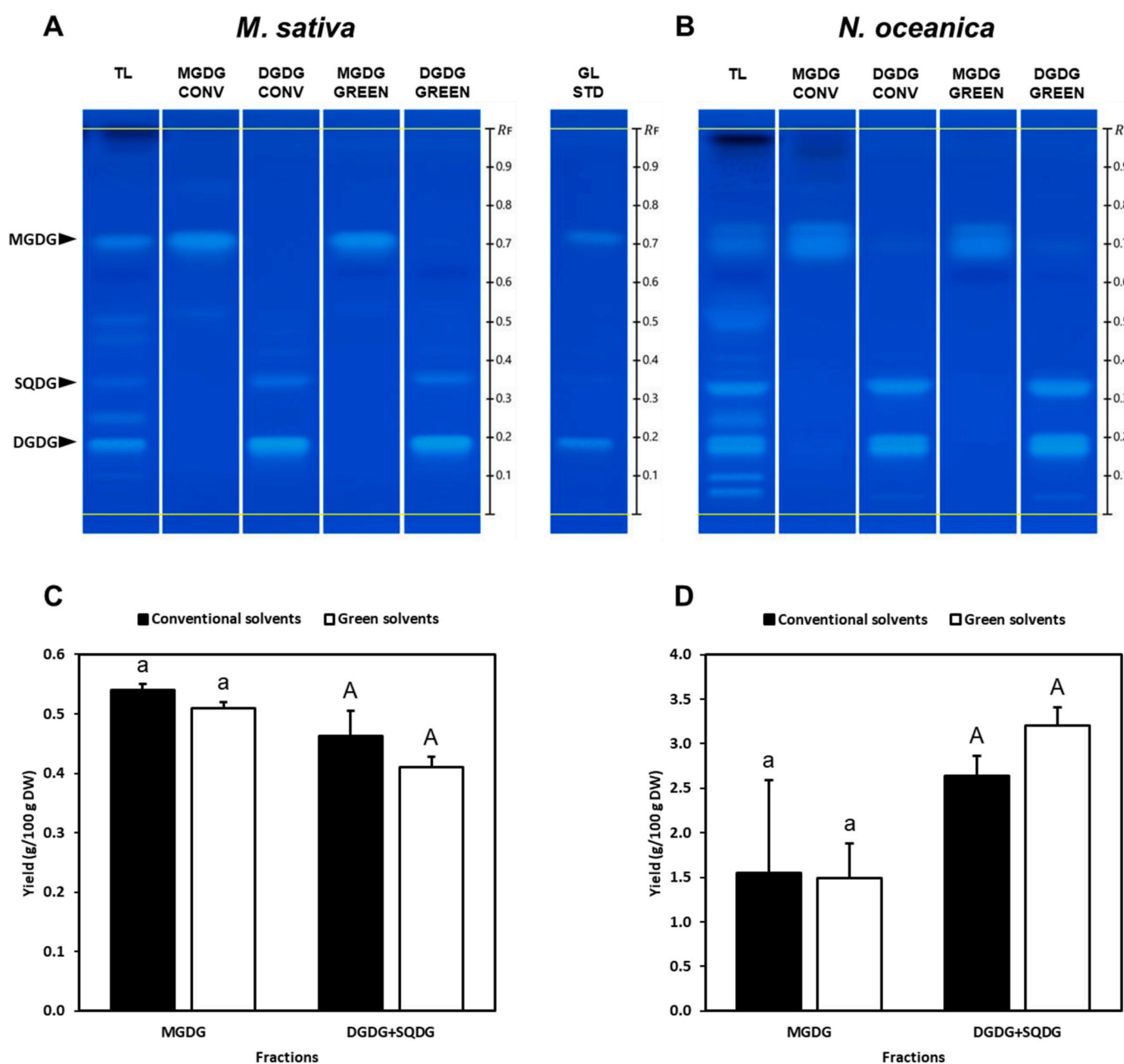


Fig. 3. Comparison of glycolipid fractions obtained by silica gel fractionation using conventional solvents and green solvents, from *M. sativa* (A, C) and *N. oceanica* (B, D). TL: total lipids, GL STD: glycolipid standard, CONV: conventional solvent fractionation, GREEN: green solvent fractionation. Different letters indicate significant differences ($p < 0.05$).

For *N. oceanica*, two narrowly separated bands were observed for both DGDG and MGDG, characteristic of their various aliphatic chains, as previously reported by Tancrède et al. (1980). Their galactolipid nature was confirmed by glycolipid-specific derivatization using 1-naphthol (Supplementary Fig. 1).

Although the conventional and green solvent fractionation approaches effectively isolated galactolipids, the obtained fractions contained minor impurities. In conventional MGDG fractions, faint bands were observed with $R_F \approx 0.55$ and 0.85 . In all DGDG fractions, sharp bands of SQDG were observed with $R_F \approx 0.34$. A few faint bands ($R_F \approx 0.42$ – 0.48 for *M. sativa* and $R_F \approx 0.04$ and 0.69 for *N. oceanica*) corresponding to unidentified polar lipids were also detected along with DGDG and SQDG. In addition, dark bands were observed in green MGDG fractions from *M. sativa* and *N. oceanica* ($R_F \approx 0.62$), in the conventional MGDG fraction from *N. oceanica* ($R_F \approx 0.95$), and all the green DGDG fractions ($R_F \approx 0.62$). Before derivatization, these dark bands appeared green under white light (data not shown) and were therefore identified as chlorophylls. Indeed, although it was obvious that most pigments were eluted in the neutral lipids fraction, which exhibited a dark green color, the fractionation process yielded light-colored galactolipid fractions (data not shown) likely containing residual pigments. Gravimetric yield did not differ significantly when the fractionation was carried out with conventional solvents or green solvents (Fig. 3C and D).

As in conventional extraction, conventional fractionation of total lipid extracts is usually performed using hazardous solvents. This study demonstrates that a combination of low-toxicity solvents can provide galactolipid fractions with a composition and yield comparable to those obtained using a conventional method. Firstly, using *n*-butyl acetate as the first eluent efficiently and selectively removed neutral lipids and most lipophilic pigments. This result is consistent with Sánchez et al. (2023) who obtained a fraction containing over 96.2 % neutral lipids using *n*-butyl acetate for the first fractionation step on silica gel, and a subsequent ethanol fraction containing 97.6 % polar lipids. Then, ethyl

acetate proved to be highly selective for MGDG recovery, which aligns with its slightly higher eluotropic strength ($\epsilon^\circ = 0.38$) compared with *n*-butyl acetate ($\epsilon^\circ = 0.33$) (Snyder, 1966). Ultimately, acetone was retained in the green method due to its widespread use for the selective recovery of glycolipids (Ryckebosch et al., 2012; Servaes et al., 2015; Jiménez Callejón et al., 2022).

3.3. Purification of DGDG by elution scheme improvement and anion exchange chromatography on DEAE-C using green solvents

The purity of DGDG fractions collected from silica gel fractionation was improved by removing undesirable compounds. Fig. 4 shows that, by dividing the initial volume of acetone used to collect the DGDG from *N. oceanica* ($V_1 + V_2 + V_3 = 58$ mL/g of silica gel) in three smaller volumes (V_1 , V_2 , and V_3), DGDG was efficiently isolated from the other lipid species. The first 7.3 mL of acetone per g of silica gel (V_1) eluted mostly undesirable compounds (SQDG, pigments, and unidentified lipid species) and few DGDG. The following 14.5 mL/g (V_2) yielded most of the DGDG and minor amounts of SQDG. Eventually, the last 36.2 mL/g (V_3) yielded most SQDG and unidentified species along with a few DGDG. Thus, for the next experiments, only V_2 was collected for further DGDG purification.

DGDG from the V_2 acetone subfraction of *N. oceanica* was then purified by removing residual SQDG using DEAE-C and ethanol as the mobile phase. As shown in Fig. 4, this purification step yielded a pure DGDG fraction. The same procedure was applied to the DGDG fraction from *M. sativa*, leading to comparable results (Fig. 4). Although this step is typically carried out using chloroform-methanol (Rouser et al., 1961; O'Brien and Benson, 1964), ethanol proved to be a highly efficient green alternative under the experimental conditions of the present study.

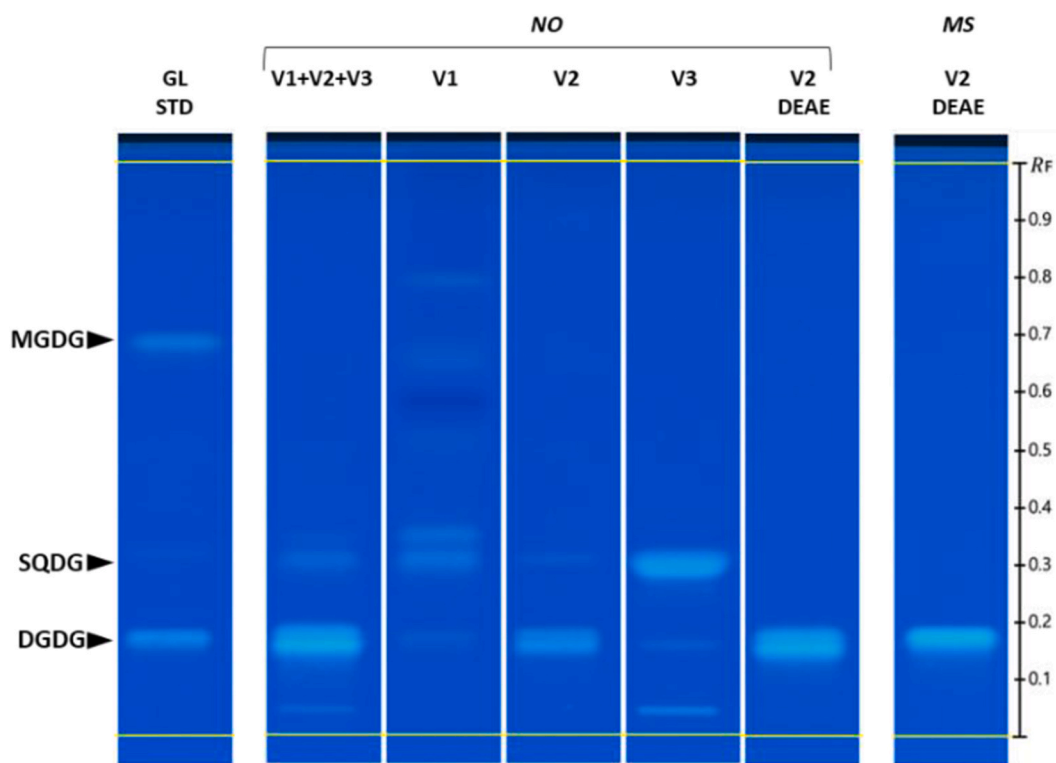


Fig. 4. Lipid composition of *N. oceanica* and *M. sativa* acetone fractions. These fractions were collected after removal of neutral lipids and monogalactosyldiacylglycerols with *n*-butyl acetate and ethyl acetate, respectively. NO: *N. oceanica*, MS: *M. sativa*, V_1 : first 7.3 mL of acetone per g of silica gel, V_2 : following 14.5 mL/g, V_3 : following 36 mL/g, V_2 DEAE: V_2 after purification on a DEAE-C column.

3.4. Pigment removal from crude lipid extracts using activated carbon for MGDG purity improvement

Most pigments (chlorophylls and carotenoids) were removed together with neutral lipids during fractionation. However, residual pigments remained in galactolipid fractions (Table 3). Non-depigmented MGDG fractions had the highest pigment contents (1.23 and 2.27 mg/100 mg of total lipids for *M. sativa* and *N. oceanica*, respectively). Non-depigmented DGDG fractions contained mostly chlorophylls, with the DGDG fraction from *M. sativa* having a much higher pigment content than the DGDG fraction from *N. oceanica* (0.98 and 0.35 mg/100 mg, respectively).

To improve the purity of the subsequent galactolipid fractions, pigments were removed from crude lipid extracts before fractionation, using various amounts of activated carbon (Fig. 5A and B). For *M. sativa*, 2.5 mg of activated carbon per mg of total lipids removed 92.3 % of total pigments. For *N. oceanica*, 1.6 mg/mg removed 85.9 % of total pigments. Higher amounts of activated carbon led to greater pigment removal (up to 99.9 %). However, activated carbon also unselectively adsorbed other compounds, to the extent that at elevated concentrations, it removed all lipids from the extracts (Fig. 5C and D).

After subsequent fractionation of depigmented extracts, the galactolipid fractions obtained from *M. sativa* contained 0.12 and 0.00 mg/100 mg of total pigments for MGDG and DGDG, respectively (Table 3). This corresponded to a decrease of 91 % and 100 % compared with the fractions obtained from non-depigmented extracts. For *N. oceanica*, the galactolipid fractions contained 0.93 and 0.22 mg/100 mg of total pigments, corresponding to a decrease of 59 % and 38 %, for MGDG and DGDG, respectively.

Pigment removal from microalgal or plant biomass is usually achieved prior to lipid extraction using chloroform (Yarnpakdee et al., 2022) or hexane (Amara et al., 2010), by saponification (Li et al., 2016) or by adsorption using bleaching clays (Tang et al., 2023). Only a few studies reported the use of activated carbon, and it was typically applied in column chromatography in the presence of chloroform rather than as part of a green isolation approach (Yamaguchi et al., 2012; Hazahari et al., 2018). To the best of our knowledge, no studies have explored the use of activated carbon for pigment removal from plant or microalgal lipid extracts in the context of green solvents. The present study shows that pigment removal from crude extracts using activated carbon and ethanol efficiently reduced the pigment content in total lipids and galactolipid fractions, although the reduction was greater for *M. sativa* than for *N. oceanica*.

Subsequently, the following preparative scale experiment was carried out with extracts depigmented using an activated carbon concentration of 2.5 mg/mg for *M. sativa* and 1.6 mg/mg for *N. oceanica*, to remove most of the pigment while minimizing the loss of lipids.

3.5. Preparative scale experiment

Preparative-scale experiments using the conditions described above were carried out on large biomass batches. These conditions included: green extraction (maceration in ethanol for *M. sativa* and PLE 125 °C for

N. oceanica), pigment removal (2.5 mg and 1.6 mg of activated carbon per mg of lipids for *M. sativa* and *N. oceanica*, respectively), green fractionation with three acetone subfractions, and DEAE-C purification of DGDG. Galactolipid yield, purity, and fatty acid composition of these fractions were then determined. Lipids were extracted and depigmented from 100 g of *M. sativa* and 72 g of *N. oceanica* in a single run. The fractionation process was scaled up to handle up to 1800 mg of lipids per column, representing a 45-fold increase compared with the initial 40 mg previously processed on silica gel cartridges. DGDG purification was performed on up to 600 mg of dried acetone fraction (DGDG + SQDG).

Galactolipids were quantified at each step of the process, using HPLC-ELSD. Yields obtained using the green extraction method at the preparative scale were compared to those obtained using the reference method (chloroform-methanol, at the analytical scale). Subsequently, the recoveries of galactolipids after depigmentation and purification processes were compared to the total amounts extracted by the green method, to assess the recovery performance of the purification process (Fig. 6).

The preparative-scale extraction using maceration in ethanol yielded 0.70 ± 0.03 and 0.55 ± 0.01 g of MGDG and DGDG, respectively, per 100 g of dried *M. sativa*. There was no significant difference compared to the reference extraction method. After depigmentation, the recovery of MGDG was 80 % (yielding 0.56 ± 0.02 g/100 g), and 96 % for DGDG (0.52 ± 0.01 g/100 g), relative to the initial amounts. Following purification, the recovery of MGDG remained unchanged (0.57 ± 0.07 g/100 g), while DGDG recovery decreased to 75 % (0.41 ± 0.06 g/100 g).

These results indicate that, despite a slightly lower overall lipid extraction efficiency (see Fig. 2), maceration in ethanol was as effective as chloroform-methanol for the specific extraction of galactolipids at the preparative scale. However, the depigmentation step resulted in a slight loss of MGDG, while DGDG was fully recovered. This may be attributed to the higher polarity of DGDG, which makes it more soluble in ethanol, thereby reducing its adsorption by activated carbon. At the final stage, the purification process led to minor losses of DGDG, but no additional loss of MGDG. This difference is likely due to the two-step purification procedure applied to DGDG (silica gel followed by DEAE-C), whereas MGDG purification involved only silica gel. Overall, the purification process allowed efficient recovery of galactolipids, with MGDG being slightly more preserved than DGDG.

For *N. oceanica*, the preparative-scale extraction using PLE at 125 °C yielded significantly more MGDG (1.02 ± 0.03 g/100 g) than the reference extraction method (0.76 ± 0.02 g/100 g). A similar trend was observed for DGDG, with yields of 1.00 ± 0.02 and 0.86 ± 0.03 g/100 g, respectively, although the difference was not significant. After depigmentation, the recovery of MGDG was 89 % (yielding 0.90 ± 0.01 g/100 g), and 98 % for DGDG (0.98 ± 0.02 g/100 g), relative to the amounts initially extracted. However, for both MGDG and DGDG, yields were not significantly different from those obtained after the green extraction step. Following purification, the recovery of MGDG remained unchanged (0.91 ± 0.08 g/100 g), while DGDG recovery decreased to 75 % (0.75 ± 0.11 g/100 g).

The higher galactolipid yield obtained using PLE at 125 °C, compared with the reference extraction method, supports the hypothesis

Table 3

Pigment content in non-depigmented and depigmented galactolipid fractions from *M. sativa* and *N. oceanica* (mg per 100 mg lipids).

	MGDG		DGDG	
	non-depigmented	depigmented	non-depigmented	depigmented
<i>M. sativa</i>				
Chlorophylls	0.82 ± 0.06	0.08 ± 0.01	0.89 ± 0.03	0.00 ± 0.00
Carotenoids	0.40 ± 0.05	0.04 ± 0.01	0.09 ± 0.00	0.00 ± 0.00
Total	1.23 ± 0.01	0.12 ± 0.02	0.98 ± 0.03	0.00 ± 0.00
<i>N. oceanica</i>				
Chlorophylls	1.47 ± 0.31	0.23 ± 0.00	0.27 ± 0.02	0.19 ± 0.01
Carotenoids	0.81 ± 0.12	0.76 ± 0.06	0.08 ± 0.01	0.03 ± 0.00
Total	2.27 ± 0.41	0.93 ± 0.06	0.35 ± 0.02	0.22 ± 0.01

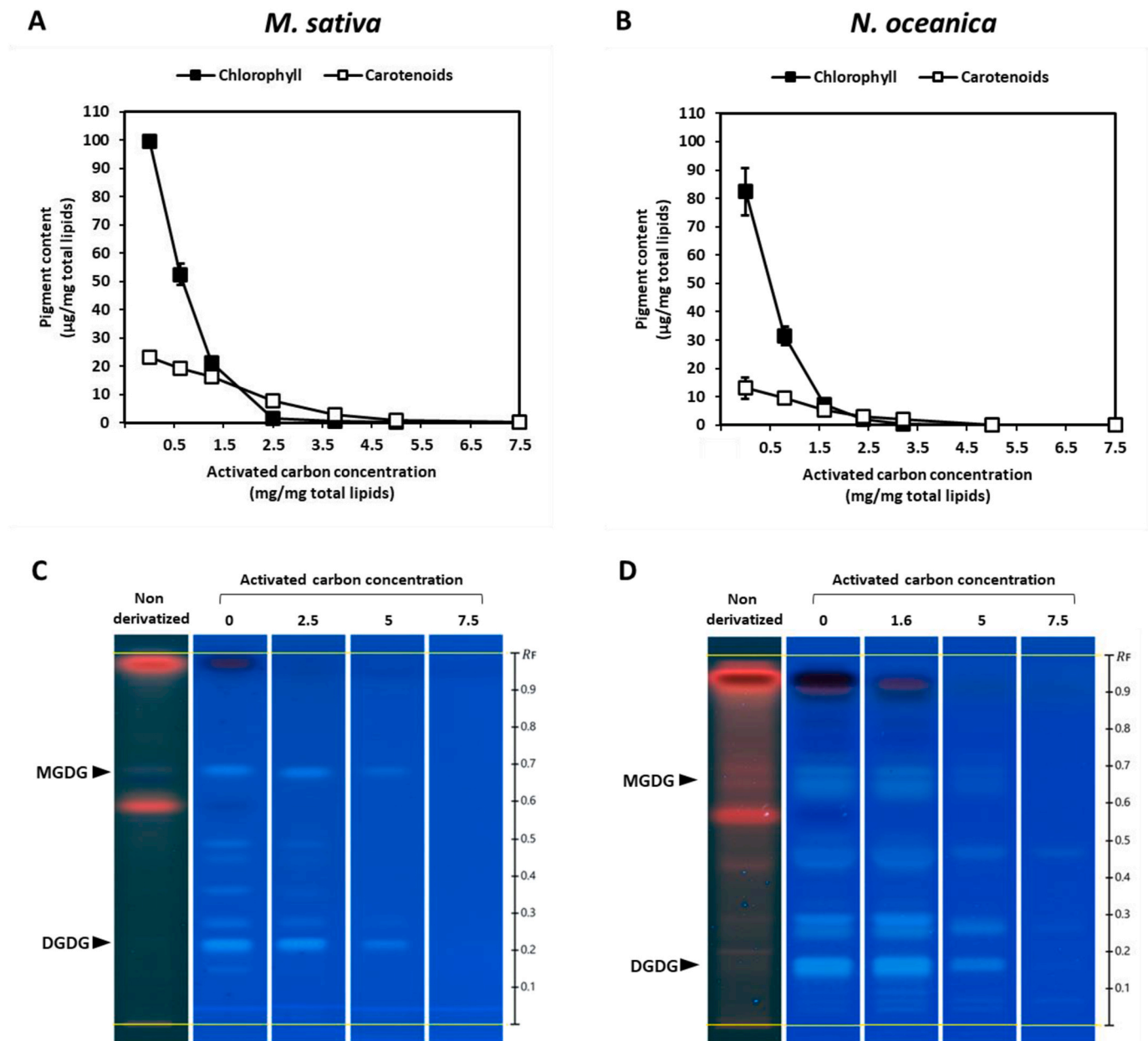


Fig. 5. Effect of activated carbon concentration on pigment content and lipid composition of crude lipid extracts from *M. sativa* (A, C) and *N. oceanica* (B, D). Activated carbon concentrations are expressed as mg of activated carbon per mg of total lipids. In panels C and D, the first tracks on the left were not derivatized.

that chloroform-methanol may not extract the total lipid content of *N. oceanica*, as discussed in section 3.1. Although both methods resulted in similar total lipid yields (Fig. 2), PLE at 125 °C appeared to be more efficient for the specific extraction of galactolipids. As observed for *M. sativa*, the purification process allowed efficient recovery of galactolipids from *N. oceanica*, with MGDG being slightly better preserved than DGDG.

Besides yield and recovery, assessing the purity and fatty acid profile of the purified galactolipids was essential to evaluate the relevance of the proposed process. The purity was assessed by HPLC-ELSD (Fig. 7) and HPTLC (Fig. 8). For all purified fractions, the galactolipid peak exceeded 98.4 % of the total area under the HPLC-ELSD chromatogram (solvent peak excluded). HPTLC analysis (1.7–3.1 µg were deposited) did not reveal any detectable impurities in the purified fractions, except for MGDG from *M. sativa*, in which a very faint band was observed with $R_F \approx 0.55$. This demonstrated the high purity of the galactolipid fractions.

All the galactolipid fractions were rich in n-3 fatty acids (Table 4). MGDG and DGDG from *M. sativa* were primarily composed of ALA (93.6 % and 79.5 % of total fatty acids, respectively). These values align with O'Brien and Benson (1964) as well as Van Der Veen and Olcott (1964), who purified *M. sativa* galactolipids using chloroform and methanol. On the other hand, MGDG and DGDG from *N. oceanica* were mostly composed of EPA (55.9 % and 29.6 %, respectively) as well as C16:0 and C16:1. This result is consistent with Conde et al. (2023), who reported that the most abundant MGDG and DGDG species from *N. oceanica* were 20:5/20:5 and 16:0/16:1, respectively.

4. Conclusion

In this study, lipids were extracted from *M. sativa* and *N. oceanica* using ethanol. The crude extracts were depigmented using activated carbon and ethanol, and non-lipid compounds were removed through ethyl acetate partitioning. The lipid extracts were then fractionated on

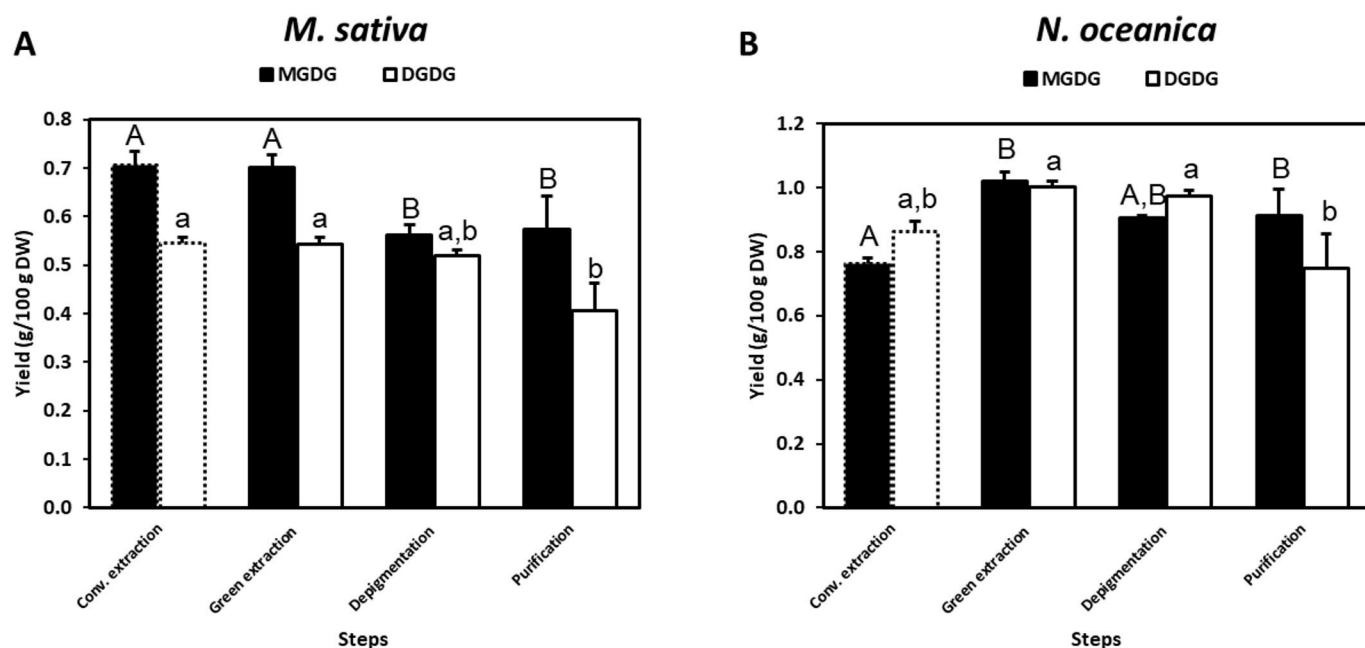


Fig. 6. Galactolipid yields from *M. sativa* (A) and *N. oceanica* (B) after each step of the green extraction and purification process at the preparative scale. Conventional extraction was included for comparison and performed at the analytical scale, whereas all the green steps were conducted at the preparative scale. Different letters indicate significant differences ($p < 0.05$); uppercase and lowercase letters refer to MGDG and DGDG, respectively.

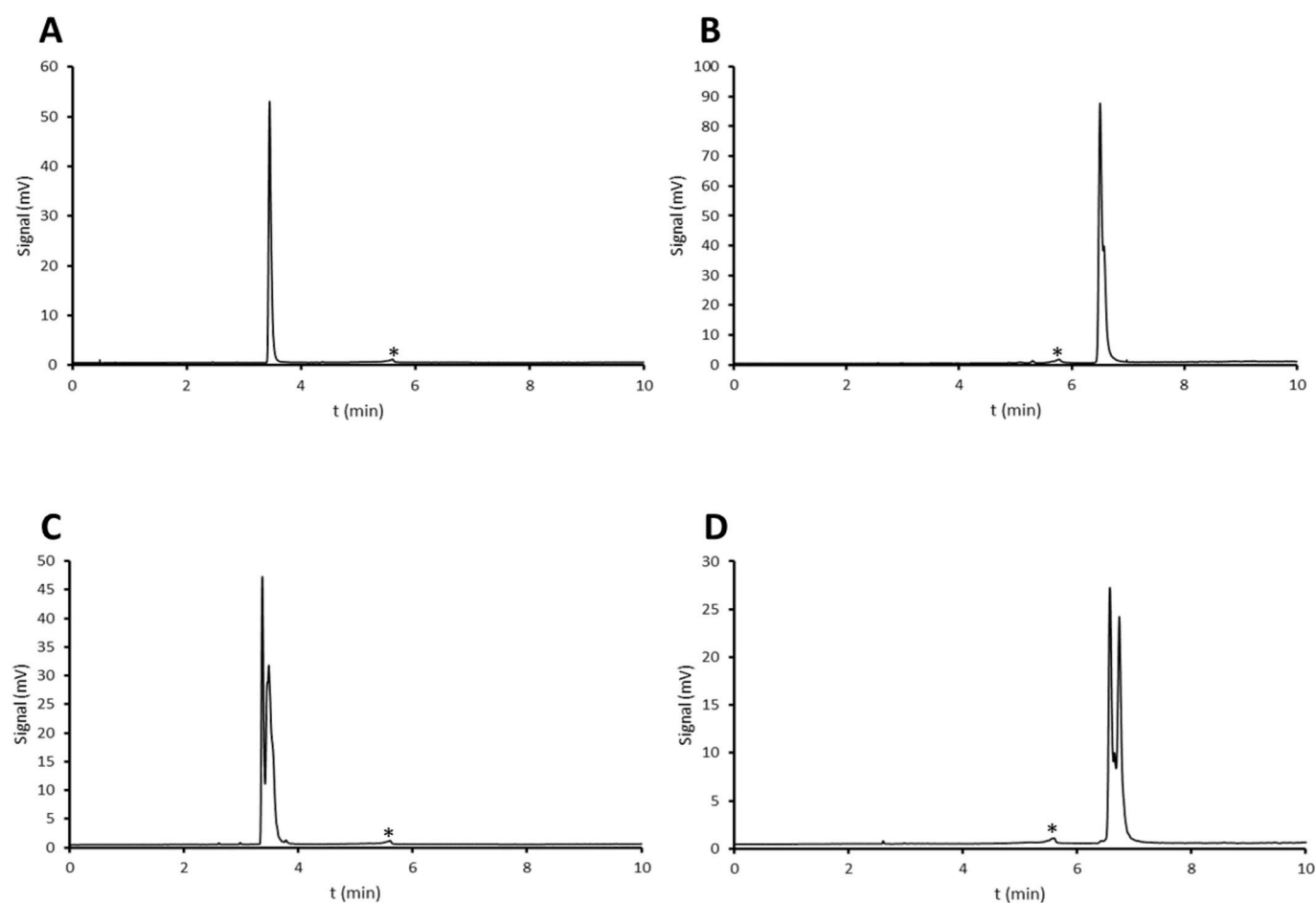


Fig. 7. HPLC-ELSD chromatograms of purified A) MGDG and B) DGDG from *M. sativa*, C) MGDG and D) DGDG from *N. oceanica*. *: solvent peak.

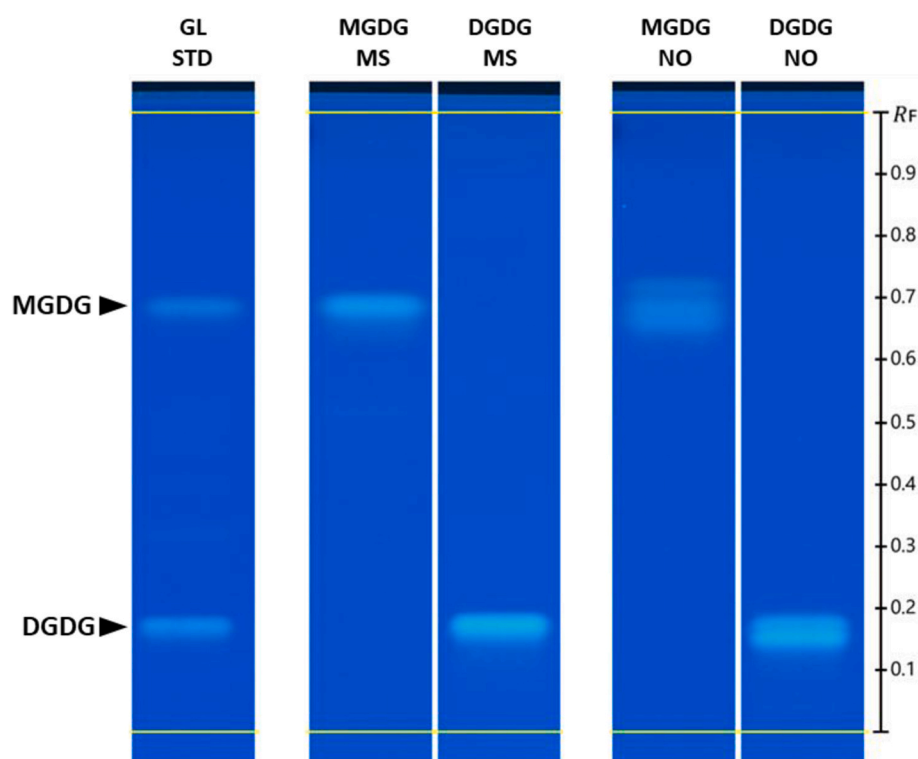


Fig. 8. HPTLC analysis of final galactolipid fractions from *M. sativa* and *N. oceanica* after preparative-scale extraction and purification.

Table 4

Fatty acid content in purified galactolipid fractions from *M. sativa* and *N. oceanica* as weight percentage of total fatty acids.

	<i>M. sativa</i>		<i>N. oceanica</i>	
Fatty acids	MGDG	DGDG	MGDG	DGDG
12:0	–	–	1.2 ± 0.1	–
14:0	–	–	9.7 ± 0.6	4.8 ± 0.0
15:0	–	–	0.3 ± 0.1	0.4 ± 0.0
16:0	1.7 ± 0.2	13.9 ± 0.9	13.3 ± 0.5	29.8 ± 1.4
18:0	0.4 ± 0.0	2.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
16:1n-7	–	–	11.1 ± 0.9	29.8 ± 1.2
18:1n-9	0.4 ± 0.1	0.6 ± 0.6	0.8 ± 0.1	1.1 ± 0.1
22:1n-9	0.4 ± 0.0	–	0.6 ± 0.4	0.5 ± 0.3
18:2n-6	3.5 ± 0.1	3.3 ± 0.2	1.6 ± 0.0	2.4 ± 0.0
20:4n-6	–	–	2.7 ± 0.1	0.6 ± 0.0
18:3n-3	93.6 ± 0.5	79.5 ± 1.2	0.4 ± 0.0	0.5 ± 0.0
20:5n-3	–	–	55.9 ± 2.2	29.6 ± 2.5
Others	–	0.5 ± 0.1	2.2 ± 0.5	0.3 ± 0.1

silica gel, sequentially eluting neutral lipids, MGDG, and DGDG using *n*-butyl acetate, ethyl acetate, and acetone, respectively. The DGDG fraction was further purified on DEAE-C using ethanol. The entire process was performed using low-toxicity solvents and it was scaled up to process larger amounts of biomass and lipid extracts, making it suitable for preparative applications. The purified MGDG and DGDG fractions exhibited high purity and high n-3 fatty acid content, making them suitable for a wide range of applications, including *in vitro* digestion and *in vivo* studies.

This method was designed for galactolipid purification, but could potentially be optimized for efficient purification of neutral lipids and phospholipids as well. Although these lipid classes can currently be recovered, *n*-butyl acetate – used to elute neutral lipids – is challenging to remove by evaporation. Replacing it with tert-butyl acetate, which has a lower boiling point, may offer a more convenient alternative despite its higher cost. In addition, phospholipids may have been only partially recovered under the experimental conditions applied in this

study. Using mixed green solvent systems instead of ethanol alone could improve their extraction, while pH adjustment may enhance their elution during fractionation. This work contributes to the development of improved green purification methods for lipids from various sources. Future research should explore the applicability of this method to other lipid-rich species and assess both its scalability and economic viability at larger production scales for potential industrial implementation.

CRediT authorship contribution statement

Andres Lopez: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sébastien Marze:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Alice Kermarrec:** Writing – review & editing, Methodology, Investigation. **Ha Phuong Ta:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used OpenAI's ChatGPT-4-turbo in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clet.2025.101049>.

Data availability

Data will be made available on request.

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