Food Innovation and Advances

ARTICLE

Open Access

https://doi.org/10.48130/fia-0024-0007

Food Innovation and Advances **2024**, 3(2): 75–87

Chemical, rheological, and volatile profiling of microalgae *Arthrospira*, *Isochrysis*, *Nannochloropsis*, and *Tetraselmis* species

Johannes Magpusao[1,2,3], Indrawati Oey[1,2] and Biniam Kebede[1*]

[1] *Department of Food Science, University of Otago, PO Box 56, Dunedin 9054, New Zealand*
[2] *Riddet Institute, Private Bag 11222, Palmerston North 4442, New Zealand*
[3] *School of Technology, University of the Philippines Visayas, Miagao, Iloilo 5023, Philippines*
* Corresponding author, E-mail: biniam.kebede@otago.ac.nz

Abstract

Microalgae are increasingly regarded as a sustainable source of novel food and functional products due to their nutritional composition. This study aimed to conduct an in-depth analysis of the chemical, microstructural and rheological, and volatile-flavour related properties of *Arthrospira*, *Isochrysis*, *Nannochloropsis*, and *Tetraselmis* species. Chemometric data analysis was employed to integrate the multivariate data, investigate the classification among the four species, and identify discriminating and distinct features. *Arthrospira* is high in protein content, and *Nannochloropsis* is lipid-rich with dominantly polyunsaturated fatty acids. *Isochrysis* is rich in carotenoids and total phenolics, while *Tetraselmis* is high in carbohydrates. Key discriminant volatile markers encompass aldehydes, terpenes, and hydrocarbons for *Arthrospira*; ketones and alcohols for *Nannochloropsis*; aldehydes, ketones, and sulfur-containing compounds for *Tetraselmis*; and furans and aldehydes for *Isochrysis*. Moreover, *Arthrospira* and *Isochrysis* demonstrate elevated viscosity and notable thickening potential. In summary, the different microalgal biomass studied in this study showcase unique compositional, rheological, and volatile properties, highlighting their potential as functional ingredients for diverse applications in the food and pharmaceutical industries.

Citation: Magpusao J, Oey I, Kebede B. 2024. Chemical, rheological, and volatile profiling of microalgae *Arthrospira*, *Isochrysis*, *Nannochloropsis*, and *Tetraselmis* species. *Food Innovation and Advances* 3(2): 75–87 https://doi.org/10.48130/fia-0024-0007

Introduction

Microalgae are chlorophyll-containing, photosynthetic aquatic organisms with basic growth requirements that allow them to sustainably yield valuable bioactive compounds, such as lipids, proteins, and carbohydrates[1]. The large quantities of macronutrients present in microalgae make it a valuable potential for food and pharmaceutical applications. In addition, the chemical composition of microalgae displays the availability of a broad array of functional properties for various food applications[2]. Currently, the main microalgal species considered as potentially promising in the food industry due to the high presence of bioactive compounds includes *Chlorella*, *Arthrospira platensis*, *Isochrysis galbana*, *Dunaliella salina*, *Porphyridium* sp. and *Haemotococcus*[3].

While market opportunities for microalgae are still not comparable to traditional food commodities, the microalgae-based industry is showing consistent and remarkable expansion[4]. This is evidenced by the substantial number of studies characterising microalgae, which have established the pronounced variation in the biochemical composition due to different factors, such as species, seasonal changes, cultivation conditions, and developmental stage during harvest[5]. Given the diversity of microalgal species, they must undergo a comprehensive physicochemical characterisation[6]. Such investigations are particularly important since the direct incorporation of microalgal biomass into food formulation results in nutritional supplementation and modifications in the structural properties of products[7]. Bernaerts et al. have reviewed the functionality of microalgae and their polymers for their structuring and texturing potential, which pointed towards rheological improvement when microalgae is incorporated into food[8]. In addition, algae have different volatile flavour properties that increase their potential as ingredients in various food products[9]. Nevertheless, while the nutritional aspects are substantially studied, there is a need for a comprehensive study that combines chemical composition, rheological properties, and volatile flavour attributes of microalgae in a more harmonised way. The lack of a more integrative strategy in previous studies have made it difficult to make conclusive remarks regarding the suitability of microalgae biomass as functional ingredients for specific food applications.

The objective of this research work was to comprehensively characterise the chemical composition, microstructural and rheological properties, and volatile-flavour related attributes of four microalgal species, namely *Arthrospira*, *Isochrysis*, *Nannochloropsis*, and *Tetraselmis* species. These microalgal biomass are commercially available and could easily be obtained as functional ingredients that may be directly incorporated into food. Among these, the blue-green cyanobacteria *Arthrospira* sp. is the most largely cultivated, produced, and marketed species. It is known to be rich in proteins, vitamins, antioxidants, and fatty acids and has been popularly utilised for human and animal health[10]. *Arthrospira* sp. has been added directly to different food products, such as baked goods, pasta, and meat products, to improve their nutritional properties. *Isochrysis* sp. is a rich source of vitamin A and provitamin as well as carotenoids that have antioxidative and inflammatory

activities[11]. Unlike *Arthrospira* sp., food incorporated with *Isochrysis* sp. is still very limited to mostly baked goods, albeit with promising nutritional benefits[12]. *Nannochloropsis* sp. has the promising benefits for commercialisation as a source of eicosapentaenoic acid (EPA), which has been utilised as a dietary supplement of omega-3 fatty acid to manage cholesterol levels in the treatment of cardiovascular diseases[13]. Incorporation of *Nannochloropsis* sp. biomass to dough has had considerable impact on the colour, although not so much on texture properties[12]. *Tetraselmis* sp. is known to have high polyunsaturated fatty acids (PUFA), chlorophyll content, α-tocopherol, and vitamin A while having proteins and polysaccharides that show favourable emulsifying and foaming properties[14]. Incorporation of *Tetraselmis* sp. has been mostly explored in dough and other baked goods. Nevertheless, while *Arthrospira sp.* has long been a part of human diet, *Isochrysis* sp., *Nannochloropsis* sp., and *Tetraselmis* sp. are typically only used in aquaculture feed[7].

Due to the great taxonomic diversity of microalgae, even within species, evaluation of relevant properties is still necessary prior to utilization in the food sector. Understanding the microalgal biodiversity and the corresponding heterogeneity variation in biochemical, morphological, and structural properties is crucial in choosing the microalgal species for food applications. The strength of this research lies in the multiplatform analytical approach, which integrates untargeted fingerprinting and targeted profiling with chemometrics data analysis. Advanced chemometrics were employed to integrate proximate composition, microstructural, and rheological properties with the volatile flavour-related attributes. This allowed for the determination of unique and discriminant markers that reflect the distinct characteristics of each microalgal species, indicating their potential as functional ingredients.

Materials and methods

Microalgal species and samples preparation

Lyophilised biomass of *Arthrospira* was supplied by Bio-Balance, New Zealand (NZ). Wet pastes of *Isochrysis*, *Nannochloropsis*, and *Tetraselmis* were bought from Reed Mariculture (USA). Reed Mariculture grew the microalgal biomass in sealed photobioreactors with saltwater media and harvesting was done by flowthrough centrifugation. This was followed by resuspension in buffer salt solution before being immediately transferred in plastic containers. *Nannochloropsis* sp. and *Tetraselmis* sp. were kept frozen at −20 °C while *Isochrysis* sp. was maintained under refrigerated conditions (4 °C) during storage and transport. The samples were kept at refrigerated conditions and freeze-dried within 48 h of arrival in the laboratory of the Department of Food Science, Otago University, New Zealand. After being freeze-dried, the powders were vacuum-packed and kept at −20 °C until they were analysed.

Proximate analysis was performed on lyophilised samples. All other analyses were conducted on microalgal suspensions. To prepare the microalgal suspension, lyophilised biomass was dispersed in distilled water at 8% (w/v) concentration, stirred overnight (10 °C), homogenised using a homogeniser (ULTRA-TURRAX®, Krackeler Scientific, NY, USA) for a homogenous suspension, and quick-frozen with liquid nitrogen[15]. Samples for particle size distribution were stored at −20 °C until analysis. All experiments were conducted in triplicate using independently prepared suspensions.

Chemical composition

Proximate composition

Moisture and ash contents were determined by drying samples in a vacuum oven (65 °C) until constant weight loss and decomposition in a muffle furnace (550 °C), respectively[16]. Average moisture values were used in calculating percentages of chemical composition as % dry basis (DB). Lipid was extracted using the modified Bligh and Dyer method[17]. Nitrogen value was determined using the Kjeldahl method[18]. Protein content was calculated by converting the measured nitrogen using specific conversion factors for *Arthrospira* (5.95), *Nannochloropsis* (4.95), *Isochrysis* (4.59), and *Tetraselmis* (4.8)[19,20]. Total carbohydrate was determined by subtracting the sum of the moisture, crude fat, crude protein, and ash values from 100.

Pigment content

The pigment content of all microalgal samples was determined based on a modified methanol extraction method, wherein microalgal suspension was mixed in methanol, vortexed, incubated, and supernatant was collected after centrifugation[21]. Supernatant absorbance was measured at a spectrum of 350 to 850 nm, wherein maximum absorbance of chlorophyll *a* is 666 nm, chlorophyll *b* is at 653 nm, and total carotene at 470 nm. Chlorophyll *a*, chlorophyll *b*, and carotenoids were determined as $\mu g \cdot mL^{-1}$ using Eqns (1), (2), and (3)[22] and converted to mg/g dry weight of microalgae:

$$Chlorophylla\ (C_a) = 15.65\,A_{666} - 7.34\,A_{653} \quad (1)$$

$$Chlorophyllb\ (C_b) = 27.05\,A_{653} - 11.21\,A_{666} \quad (2)$$

$$Carotenoids = \frac{1000\,A_{470} - 2.86\,C_a - 129.2\,C_b}{221} \quad (3)$$

Total phenolic content

Total phenolic content was estimated using a modified Folin-Ciocalteau method[23] . Absorbance was measured at 765 nm against methanol as blank. Total phenolic content of samples was quantified with reference to the gallic acid standard curve (0–500 $mg \cdot L^{-1}$) and reported as gallic acid equivalent (GAE)/g dry weight of microalgae.

Microstructural and rheological properties

Morphological characteristics

An optical microscope (Ceti Magnum, Medline Scientific, UK) equipped with a video camera and interfaced with the software ToupView (ToupTek Photonics, China) was used. Images were taken by 40 × dry objective lens.

Particle size distribution

Sample particle size distribution (PSD) was evaluated by a laser diffraction particle size distribution analyser (Partica LA-950V2, Retsch Technology GmbH, Germany) with distilled water as dispersant[24]. Particle size parameters of the volume distribution were determined using the LA-950 software.

Rheological properties

Rheological analysis was perfomed using a controlled-stress rheometer (20 °C) (HAAKE Rheostress 1, Germany) with a double gap cylinder (DG 41-bob and DG 43-cup, 5.1 mm gap)[15]. Steady-state measurements were performed to determine the flow behaviour of suspensions at a shear rate of 0.1 to 100 s^{-1}).

Volatile flavour-related attributes

Fatty acid profile using GC-FID

Fatty acid methyl esters (FAME) were prepared from extracted lipid with a combination of diethyl ether as organic

solvent, boron trifluoride as derivatisation agent, and pentadecaenoic acid (C15:0) as internal standard[25]. FAME was analysed using a GC system coupled with a flame ionisation detector (GC-FID) (689A; Agilent Technologies, USA) equipped with an autosampler (7683 series injector, Agilent Technologies, USA) and fitted with a BPX70 capillary column (SGE, Australia). Sample injection was carried out in split mode with hydrogen as carrier gas. The GC oven temperature regime involved a starting temperature of 120 °C that increased to 225 °C at 3 °C/min, followed by a 10 °C/min ramp up to 245 °C, and kept at 245 ° for 2 min. The detector temperature was set at 250 °C.

Obtained chromatograms were analysed with GC ChemStation (Build 4.01, Agilent Technologies, USA). FAME peaks were manually identified by matching retention time with commercial standards (FAMQ-005, AccuStandards, USA). After manual peak alignment and removal of interfering background was done, the proportion of signal abundance of each fatty acid was calculated in % abundance of total signal abundance.

Volatile profile using HS-SPME GC-MS

Volatile analysis was performed using headspace solid-phase microextraction technique coupled with gas chromatography-mass spectroscopy (HS-SPME GC-MS) technique. HS-SPME was carried out on vialed samples (1:2:4 sample : ultrapure water : saturated NaCl solution). Volatile extraction was done with a pre-conditioned HS-SPME fibre coated with 30/50 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, USA) that was exposed to the vial headspace and desorbed into the GC injection port. Chromatographic separation was carried out in a ZB-Wax column (60 m × 0.32 mm × 0.5 μm) (Phenomenex) with helium as carrier gas. GC oven temperature was: 50 °C for 5 min, increased to 210 °C at 5 °C/min, ramped to 240 °C at 10 °C/min for 5 min, and then cooled to 50 °C. Mass spectra were obtained by electronic ionisation at 70 eV and scanned from 35 to 400 m/z. MS ion source and MS quad temperatures were 230 and 150 °C, respectively[26]. Six replicates were performed for each sample.

Resulting chromatograms were processed through a series of preprocessing steps using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software (Version 2.72, National Institute of Standards and Technology (NIST), USA) and Mass Profiler Professional (MPP) software (Version 14.9.1, Agilent Technologies, USA). Compound identification was made using the NIST mass spectral library (NIST14, Version 2.20, NIST, USA) and validated.

Univariate statistical analysis

All experiments were conducted in triplicates except for the volatile analysis. Experimental error was determined for the triplicate assays and expressed as standard deviation. Statistical analyses (ANOVA and Tukey tests) at $p < 0.05$ were carried out using Minitab 18 software (Minitab Inc., USA).

Multivariate data analysis (MVDA)

MVDA was employed on data matrix containing the chemical composition, microstructural and rheological parameters, and volatile flavour-related attributes. Initially, all data were mean centred and variables were given equal variance. MVDA was performed in two stages using Solo (Version 8.2.1, Eigenvector Research, USA): principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA).

Selection of discriminant variables was performed with variable identification (VID) coefficient calculation. Only variables with an absolute VID higher than 0.80 were deemed important by confirming significant testing.

Results and discussion

Chemical composition of microalgae

Proximate composition

The chemical composition of the selected microalgal biomass (Table 1) have been corrected for the moisture contents of each dried biomass. Each biomass composition was distinct but generally consistent with reported literature. The wide differences among microalgal species are in accordance with their different taxonomic position.

Lipid content of the selected microalgae ranged from approximately 10% and 20% of the biomass. Lipid content of *Arthrospira* (15.60%) is considerably higher than cited in the literature[6]. Lipid content of *Isochrysis* (12.01%), *Nannochloropsis* (17.62%), and *Tetraselmis* (8.80%) were generally comparable to reported ranges[27].

Arthrospira had the highest protein content (57.92%) and was comparable to the literature[19,28]. The relatively high protein content of *Arthrospira* could be attributed to the abundance of water-soluble phycobiliproteins, which are known to have a direct and significant relationship with the amount of protein in algal biomass[29]. The protein content of *Nannochloropsis* (25.44%) was similar to the literature[30]. In contrast, protein content of *Isochrysis* (10.11%) and *Tetraselmis* (15.54%) were both lower than previously reported[6,31].

Ash content of *Isochrysis* (38.55%) and *Tetraselmis* (33.50%) were relatively higher[28]. This may result from the varying salinities in cultivation mediums used by other authors from the conditions utilised for the microalgal biomass in this study. Additionally, harvested biomass was resuspended in buffer salt solution prior to packing for delivery as a wet paste, a step not included in sample preparation by others[28]. Low ash content of freshwater algae *Arthrospira* (5.81%) was comparable to that reported in the literature[30]. Available carbohydrate of *Tetraselmis* was highest (25.42%) while the other species were in the same range relatively at 14.06%–19.68% and were comparable to the literature[28].

The considerable diversity of the chemical compositions among the microalgal species is attributable to differences in cultivation practices, seasonal and geographical influences, and genetic modifications[5]. It should be noted that the use of freeze-dried microalgal biomass was reported to have minimal impact on the chemical composition, particularly protein and lipid. Hence, it is widely applicable to microalgal-based analytical and extraction procedures[32].

Pigments

Pigment content of samples (Table 1) calculated from the spectra of the methanol extracts included chlorophyll *a*, chlorophyll *b*, and carotenoids. Chlorophyll *b* was present at significantly lower amounts than chlorophyll *a* and carotenoids for all species except *Tetraselmis*. Unlike chlorophyll *a,* which is ubiquitous in all algal classes, chlorophyll *b* is exclusive to some algal classes only, specifically Chlorophyceae and Cryptophyceae[33]. As such, only chlorophyll *a* was included in Table 1

Isochrysis had highest chlorophyll *a* (0.070 ± 0.001 mg/g DM) and carotenoids (0.137 ± 0.003 mg/g DM) values. These pigments have been previously reported in this microalgae[34].

Table 1. Chemical composition and physical properties of the microalgal biomass used in this study.

Parameters	*Arthrospira* sp.	*Isochrysis* sp.	*Nannochloropsis* sp.	*Tetraselmis* sp.
Proximate composition				
Crude lipid (%, DB)	15.60 ± 1.12^{a}	12.01 ± 0.24^{b}	17.62 ± 0.84^{a}	8.80 ± 0.22^{c}
Crude protein (%, DB)	57.92 ± 0.52^{a}	10.11 ± 0.12^{d}	25.44 ± 0.79^{b}	15.54 ± 0.49^{c}
Total ash (%, DB)	5.81 ± 0.02^{d}	38.55 ± 0.08^{a}	25.82 ± 0.15^{c}	33.50 ± 0.39^{b}
Carbohydrate (%, DB)	14.06 ± 0.40^{c}	18.75 ± 0.05^{b}	19.68 ± 0.04^{b}	25.42 ± 0.65^{a}
Pigments				
Chlorophyll *a* (mg/g DM)	0.051 ± 0.003^{b}	0.070 ± 0.001^{a}	0.008 ± 0.001^{c}	0.046 ± 0.001^{b}
Carotenoids (mg/g DM)	0.023 ± 0.001^{b}	0.137 ± 0.003^{a}	0.005 ± 0.000^{d}	0.014 ± 0.000^{c}
Total phenolic content (mg GAE/100 g DM)	245.64 ± 7.92^{a}	242.80 ± 2.92^{a}	195.63 ± 8.55^{c}	221.50 ± 9.24^{b}
Particle size distribution				
d (0.1) (μm)	5.65 ± 0.10	2.69 ± 0.02	1.40 ± 0.00	5.80 ± 0.06
d (0.5) (μm)	9.08 ± 0.13	4.21 ± 0.03	2.35 ± 0.01	8.82 ± 0.03
d (0.9) (μm)	14.48 ± 0.16	6.44 ± 0.08	4.27 ± 0.04	13.13 ± 0.05
Rheological properties				
Consistency coefficient, K (Pa·s^{n})	0.013 ± 0.000^{b}	0.020 ± 0.001^{a}	0.008 ± 0.000^{c}	0.004 ± 0.000^{d}
Flow behaviour index, n (–)	0.900 ± 0.002^{a}	0.774 ± 0.002^{c}	0.802 ± 0.006^{b}	0.907 ± 0.013^{a}
Yield stress, σ_0 (Pa)	0.003 ± 0.001^{a}	0.004 ± 0.002^{a}	0.002 ± 0.000^{a}	0.001 ± 0.000^{a}

Values are mean ± standard deviation from independent replicates (n = 3). Means with different superscripts in the same row indicate a significant difference ($p < 0.05$). % DB refers to % dry basis.

Isochrysis biomass and suspensions had a distinct yellow-brownish colour that could be attributed to this species being characteristically rich in carotenoids, which mainly comprised of fucoxanthin[35].

Arthrospira was high in green-coloured chlorophyll *a* (0.051 ± 0.003 mg/g DM) and had carotenoids (0.023 ± 0.001 mg/g DM). The blue-green colour that was observable in the samples exhibited the presence of phycobiliproteins, which has been reported in aqueous extracts of this species[36]. The relatively low amount of carotenoids of *Arthrospira* was comparable to a previous report[37].

Tetraselmis suspensions had intense green colour and contained comparable amounts of chlorophyll *a* (0.046 ± 0.001 mg/g DM) and carotenoids (0.014 ± 0.000 mg/g DM) to *Arthrospira*. Chlorophyll *b* was present in likewise significant amount (0.042 ± 0.002 mg/g DM, not shown in Table 1) in *Tetraselmis*. The presence of chlorophyll *a* has been reported on *Tetraselmis* along with chlorophyll derivatives like chlorophyll *b*[38]. The same research group reported that *Tetraselmis* have high levels of a wide variety of carotenoids, with the presence of both α- and β-carotenes and their derivatives due to the carotenoid biosynthesis pathway.

While *Nannochloropsis* displayed intense green colour, this species had the lowest chlorophyll *a* (0.008 ± 0.001 mg/g DM) and carotenoid (0.005 ± 0.000 mg/g DM) content among the samples. The low amounts of measured pigment for *Nannochloropsis* may be attributed to their rigid algal cells, which inhibited release of pigments to the soluble fraction. *Nannochloropsis* is known to have chlorophyll *a* as the dominant pigment[30] while having limited carotenoid content[35]. The application of pre-treatment, such as ultrasound treatment and drying, on *Nannochloropsis* were observed to increase recovery of pigments and other valuable compounds[39].

Total phenolic content

Although all the samples are rich in phenolic compounds (Table 1), both *Arthrospira* and *Isochrysis* have the highest amounts of total phenolics. Phenolic content of *Arthrospira* (245.64 mg GAE/g DM) is lower than previously reported[40]. Similarly, phenolic content of *Isochrysis* (242.80 mg GAE/g DM) is lower than the total content reported by others (308 mg GAE/g DM)[41]. The same research group also determined that the total phenolic content of *Isochrysis* is significantly ($p < 0.05$) higher than that of *Tetraselmis*. The variability of the results compared to the reported values can be attributed to different cultivation practices of the microalgal biomass[5].

The abundant presence of phenolic compounds in *Arthrospira* conforms with the literature, which indicated these compounds have antioxidant properties and functionalities that can improve the immune system[40]. On the contrary, the phenolic contents of other cyanobacteria and freshwater green algae were deemed not major contributors to the antioxidant properties of these microalgae[23]. The release of valuable health-relevant compounds, including pigments and phenolic compounds, from microalgae may not be readily available because of the rigid microalgal cells. Therefore, it may be beneficial to enhance the accessibility of these compounds by utilising a mechanical cell disruption technique, such as high-pressure homogenisation, bead milling, pulsed electric field, and ultrasonic processing[42].

Microstructural and rheological properties

Morphological characteristics

The optical microscopic images (Fig. 1) highlight the diverse morphological features of the microalgal biomass. The cyanobacterium *Arthrospira* is observed with multicellular cylindrical trichomes, albeit considerable detachment from the spiral structure has occurred. The individual cylindrical trichomes had diameters that were within the reported range of 2.5 to 6 μm and have thin and fragile peptidoglycan cell walls, which allow partial disruption during sample preparation[43]. The small ellipsoidal shape of *Isochrysis* (Haptophyta) cells was observed with the distinct flagellar root system. *Isochrysis* cells were determined to be 3.5 to 6 μm in size and had no distinct cell walls but consisted of plasma membrane covering only[44]. The tiny cells of the *Nannochloropsis* (Eustigmatophyceae) cells were visibly subspherical in shape. Planktonic *Nannochloropsis* cells,

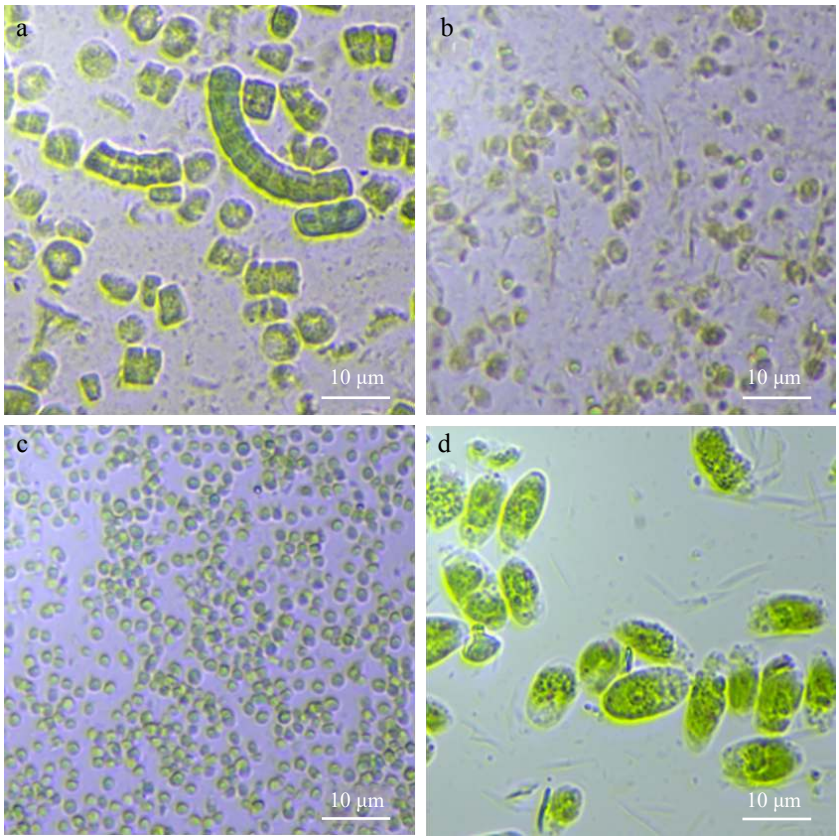

Fig. 1 Microscopic images of microalgal suspensions at 40 × magnification: (a) *Arthrospira* sp., (b) *Isochrysis* sp., (c) *Nannochloropsis* sp., and (d) *Tetraselmis* sp.

which could be either subspherical at 2 to 4 μm or cylindrical at 3–4 × 1.5 μm, have a rigid cell wall that has bilayered trilaminar sheath outer layer[45]. *Tetraselmis* (Chlorophyta) cells had elliptical shape with observable flagella appendage. *Tetraselmis* cells are typically highly motile when alive due to four flagella and can have a cell size of 9 to 15 μm[46].

Particle size distribution

Particle size distribution of all the samples (Table 1) displayed a unimodal characteristic with a singular modal peak. The particle size, which refers to sizes of a single algal cell and/or complex of cells (i.e., *Arthrospira*), ranged depending on the microalgal species. Larger cells of *Arthrospira* and *Tetraselmis* were similar in size, followed by *Isochrysis* cells, while *Nannochloropsis* cells were the smallest among the microalgae. The results correspond to the microstructure images (Fig. 1). The distribution of *Arthrospira*, *Nannochloropsis*, and *Tetraselmis* in this study was similar to that obtained by previous researchers[24]. However, a comparison for *Isochrysis* was not performed due to the unavailability of literature for this species.

Rheological properties

Flow curve of the samples (Fig. 2) generally depict shear-thinning flow behaviour, as exhibited by a decrease in viscosity with increasing shear rate. This non-Newtonian behaviour could be attributed to the composition of the microalgal suspensions, which are composed of the liquid phase with extracellular polymeric substances, algae cells, and cell debris[24].

The shear-thinning behaviour of microalgal suspensions with concentrations higher than 5 vol. % had been discussed previously by other research groups[24]. The shear stress-shear strain data were fitted into the Herschel-Bulkley mathematical model, which had a low chi-square value indicating the precision was adequate and the rheological measurements collected were reliable. The model is expressed as Eqn 4:

$$\sigma = \sigma_0 + k \cdot \dot{\gamma}^n \quad (4)$$

where σ is the shear stress (Pa), σ_0 is dynamic yield stress (Pa), k the consistency coefficient (Pa·s^n), $\dot{\gamma}$ the shear rate (s^{-1}) and n is the flow behaviour index (dimensionless). Briefly, K signifies the fluid viscosity, n reflects the shear thinning or shear thickening behaviour, and σ_0 denotes the amount of force applied to induce flow of fluid[47] (Table 1). There was a good correlation between the viscosity predicted using the Herschel-Bulkley model and experimental viscosity with a well-predicted fit (Fig. 2).

The highest K among the microalgal suspensions was observed in *Isochrysis* (0.020 Pa·s^n), which also had high viscosity. This trend exhibited by *Isochrysis* could be attributed to high ash (38.55%) and considerable carbohydrate (18.75%) contents of the biomass. Carbohydrates are an essential fraction of the microalgal biomass and are structural biopolymers that could exhibit texturising properties[8]. *Arthrospira* had the second-highest K (0.013 Pa·s^n), indicating high viscosity as well. This behaviour could be attributed to the high protein content (57%) of *Arthrospira* since higher protein-protein crosslinking interactions allow for better network formation. The gelling property could also be due to exopolysaccharides in *Arthrospira* biomass that enables the formation of a weak gel[31]. Conversely, the K of *Tetraselmis* (0.004 Pa·s^n) of was five-fold lower than that of *Isochrysis* and indicative of a weak potential for microstructural development. In terms of n, all the microalgal

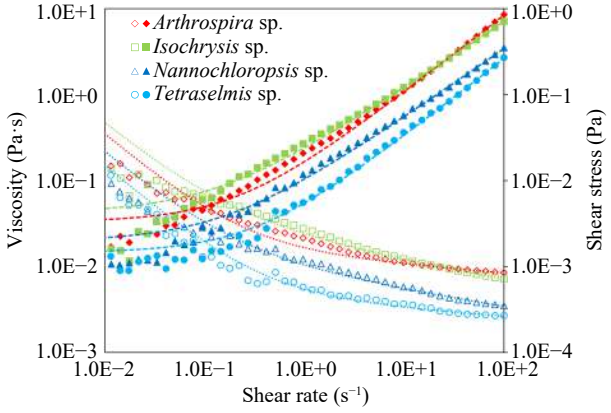

Fig. 2 Viscosity (Pa·s) and shear stress (Pa) vs shear rate (s^{-1}) of untreated microalgal suspensions of *Arthrospira* sp., *Isochrysis* sp., *Nannochloropsis* sp., and *Tetraselmis* sp. Data points are means based on three replicates. Lines represent the Herschel-Bulkley fit.

suspensions had values < 1, indicating shear-thinning behaviour. *Arthrospira* and *Tetraselmis* were at the higher range of *n* (0.900–0.907), while *Isochrysis* and *Nannochloropsis* were at the lower range (0.774–0.802). This implies that the latter two species had a greater propensity to behave as shear-thinning fluids. All samples had very low values of σ_0 that were not significantly ($p > 0.05$) different from each other and suggested that a small amount of force is needed to initiate their flow.

Volatile flavour-related attributes

Fatty acid profiles

In the relative fatty acid profiles of the samples (Table 2), 16 fatty acids were identified, with each microalga having distinct profile. Different lipid compositions may be due to species variation and differences in cultivation method and production conditions[5].

Arthrospira lipid was rich in unsaturated fatty acids, with the major fatty acids being palmitic acid (C16:0, 45.30% ± 0.95%), linoleic acid (C18:2n6c, 22.58% ± 0.6%) and γ-linolenic acid (C18:3n6, 19.61% ± 0.3%). Similar major fatty acids have been reported in *Arthrospira* at varying concentrations[37]. For *Isochrysis*, the most abundant fatty acid was arachidonic acid (C20:0, 25.01% ± 2.97%), while the other significant composition was DHA (C22:6n3, 15.99% ± 1.57%). *Isochrysis* is also identified as a rich source of PUFA, primarily EPA and DHA[27], although EPA was not identified in the current research. Other fatty acids present in moderate amounts, such as myristic acid, palmitic acid, and linoleic acid, and the abundant occurrence of DHA have previously been reported[48]. For *Nannochloropsis*, the most abundant fatty acid was EPA (C20:5n3, 32.76% ± 0.10%) followed by palmitoleic (C16:1n7, 26.87% ± 0.95%) and palmitic acid (C16:0, 21.53% ± 0.16%). These three fatty acids being the dominant in *Nannochloropsis* is consistent with other reports[49]. *Nannochloropsis* is likewise reportedly abundant in PUFA, with major relevant fatty acids such as EPA and DHA[27]. For *Tetraselmis*, the most abundant fatty acid was palmitic acid (22.37% ± 0.41%), followed by stearic (C18:0, 15.44% ± 0.85%) and α-linolenic acid (C18:3n3, 12.71% ± 0.41%). The abundance of palmitic acid in *Tetraselmis* has been reported previously[41]. In general, the abundant presence of PUFA in all samples is desirable as they can be effective in disease treatment and prevention of cardiovascular and inflammatory diseases[3,13,41].

Table 2. The selected microalgae's relative fatty acid abundance as fatty acid methyl esters (FAME) by gas chromatography coupled with a flame ionisation detector (GC-FID).

Fatty acids	*Arthrospira* sp.	*Isochrysis* sp.	*Nannochloropsis* sp.	*Tetraselmis* sp.
C12:0	ND	ND	ND	5.72 ± 0.48
C14:0	2.52 ± 0.42^{b}	12.18 ± 1.75^{a}	1.84 ± 0.33^{b}	2.22 ± 0.02^{b}
C14:1	ND	7.83 ± 1.18	ND	ND
C16:0	45.30 ± 0.95^{a}	9.86 ± 1.71^{c}	21.53 ± 0.16^{b}	22.37 ± 0.41^{b}
C16:1n7	3.34 ± 0.11^{c}	5.40 ± 0.36^{b}	26.87 ± 0.95^{a}	1.33 ± 0.15^{d}
C18:0	1.81 ± 0.80^{b}	ND	0.81 ± 0.42^{b}	15.44 ± 0.85^{a}
C18:1n9t	ND	ND	0.60 ± 0.01^{b}	1.86 ± 0.07^{a}
C18:1n9c	2.89 ± 0.18^{b}	10.16 ± 1.14^{a}	4.46 ± 0.03^{b}	10.21 ± 0.16^{a}
C18:2n6c	22.58 ± 1.08a	6.03 ± 0.42^{b}	4.01 ± 0.04^{c}	7.38 ± 0.24^{b}
C18:3n3	ND	5.20 ± 0.17^{b}	ND	12.71 ± 0.41^{a}
C18:3n6	19.61 ± 0.46^{a}	ND	0.99 ± 0.01^{c}	2.39 ± 0.10^{b}
C20:0	ND	25.01 ± 2.97^{a}	ND	8.47 ± 0.26^{b}
C20:3n3	ND	ND	ND	ND
C20:4n6	ND	ND	4.48 ± 0.12^{a}	1.39 ± 0.13^{b}
C20:5n3	ND	ND	32.76 ± 0.10^{a}	5.59 ± 0.11^{b}
C22:6n3	ND	15.99 ± 1.57	ND	ND
Total SFA	49.63 ± 1.14^{b}	47.67 ± 0.96^{b}	24.18 ± 0.58^{c}	53.47 ± 00.09^{a}
Total MUFA	6.23 ± 0.05^{d}	23.73 ± 2.76^{b}	32.14 ± 0.84^{a}	16.52 ± 0.53^{c}
Total PUFA	42.20 ± 1.25^{a}	27.61 ± 1.17^{b}	42.34 ± 0.10^{a}	29.00 ± 0.26^{b}

Values are expressed as mean ± standard deviation (n = 3). Means with a different superscript in the same row indicate a significant difference ($p < 0.05$). ND means not detected.

Volatile profiles

The volatile compounds identified through an untargeted HS-SPME GC-MS technique were approximately 136 across the four microalgal species (Supplemental Table S1) with each species having unique volatile profiles. The identified compounds belonged to varied chemical classes in the present work, including aldehydes, hydrocarbons, ketones, alcohols, furans, pyrazines, esters, and terpenes.

The identified volatiles occur based on the chemical composition of the microalgal biomass, and some of the compounds have been reported in *Arthrospira*, *Nannochloropsis*, and *Tetraselmis* by other authors[9,50]. This is the first report on the volatile profile of *Isochrysis*, leading to a very limited comparison. Aldehydes, ketones, alcohols, and hydrocarbons are volatile compounds characteristically associated with lipid oxidation. These are reaction products formed when several decomposition reactions co-occur during lipid oxidation[51]. Aldehydes have a low odour threshold and are considered significant headspace volatile compounds[9]. While hydrocarbons are present as a major group of volatiles in microalgae, hydrocarbons typically have a high odour threshold, hence are not considered relevant from an aroma point of view but have biological and ecological significance as volatile markers[52]. Alcohols are present in microalgae samples in appreciable amounts, and they contribute to a strong, pungent odour of microalgae[53]. Furans can also be present and formed in foods in small amounts, with one of the most studied furan formation pathways being lipid oxidation[54].

Investigating the inter-relationship of different attributes using MVDA

The multiplatform analytical approach followed by chemometrics could help better understand the inter-relationship between various attributes and the different microalgal species. Hence, data from the chemical, rheological, fatty acid,

and volatile assays were collated into a single data matrix and examined with MVDA. The integrated data matrix was first analysed with PCA (not shown) as an exploratory technique to detect outliers and distinguish trends or groupings. This was followed by supervised PLS-DA modelling to investigate the classification of the four microalgal species. For the model, the different attributes were considered *X*-variables and the microalgal species as categorical *Y*-variables. The first three latent variables (LVs) explained 98.30% of the cumulative variance, which denotes the presence of classification among the samples. Cross-validation was used to select the optimum LVs that can maximally explain the variance with the root mean squared error of the cross-validation (RMSECV) kept to a minimum.

A PLS-DA biplot was constructed with the first two highest LVs to visualize the classification/groupings. Level of classification can be interpreted based on the distance between the classes while the significance of compounds for classification can be explained based on their distribution on the plot. Variables projected far away from the centre of the coordinate and close to a particular species have high contribution to the classification. The *X*- and *Y*-variances explained by each LV are indicated in the respective axes. The PLS-DA biplot (Fig. 3) shows a clear separation among the species, especially with *Arthrospira* and *Nannochloropsis* being the most distant from each other and the other two species. *Isochrysis* and *Tetraselmis* showed distinct groupings but their relative closeness could indicate similarities in certain attributes.

VID technique enabled selection of discriminant compounds (Table 3), where positive VID coefficient represents higher amounts detected in the related species than the others and vice-versa. The discriminant markers associated with *Arthrospira*, *Isochrysis*, *Nannochloropsis*, and *Tetraselmis* were 45, 13, 22, and 16, respectively. To show differences among the microalgal species, selected representative markers are presented (Fig. 4).

Arthrospira

Arthrospira contains a high amount of protein, palmitic, linoleic, and γ-linolenic acid and is dominated by hydrocarbons, aldehydes, and terpenes. The prominent presence of protein (Fig. 4a) and fatty acids in this species corresponds to the literature[28,40]. Presence of these PUFA in *Arthrospira* could account for most of the dominant discriminant volatile compounds identified in the biomass through the HS-SPME GC-MS technique (Fig. 4e–h).

Between the three major chemical groups, aldehydes have lower odour threshold values and are deemed to be highly relevant headspace volatiles. Aldehydes selected in *Arthrospira* included safranal, 2-butyl-2-octenal, 2,4-dimethylbenzaldehyde, heptanal, nonanal, and octanal. Aldehydes detected in microalgal biomass have been mostly connected to the oxidation of PUFA by enzymatic reactions[55]. The high concentra-

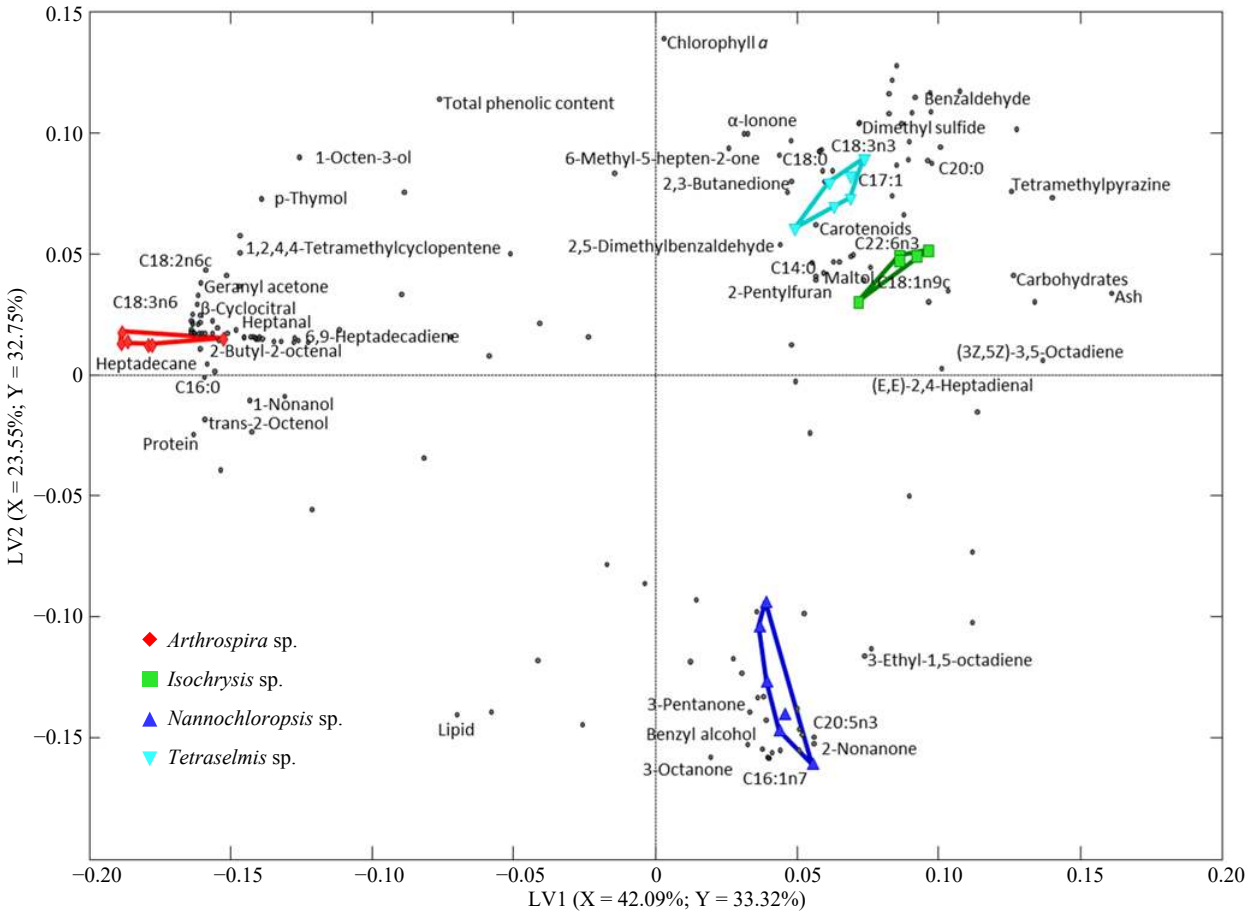

Fig. 3 PLS-DA biplots describe the variation among the selected microalgae. Differently shaped symbols represent the different microalgae: *Arthrospira* sp., *Isochrysis* sp., *Nannochloropsis* sp., and *Tetraselmis* sp. The dots represent components.

Table 3. Discriminant compounds and attributes selected per microalgal species based on the VID method confirmed with significant testing, listed in decreasing order of VID coefficient. Retention indices (RI) for the individual volatile compounds were calculated, and references were obtained from the National Institute Standards and Technology Standard Reference Database[60]. Individual fatty acids were identified by matching retention time with commercial standards.

VID	Identity	RI calculated	RI reference	Chemical class
Arthrospira sp.				
0.996	3-Ethyl-2,5-dimethylpyrazine	1438	1443	Pyrazine
0.996	Safranal	1650	1616	Aldehyde
0.995	1-Decene	1032	1050	Hydrocarbon
0.994	1R-α-Pinene	1019	1013	Terpene
0.993	2,2,6-Trimethylcyclohexanone	1317	1319	Ketone
0.993	*C18:3n6 (γ-Linolenic acid, GLA)*			
0.992	2-Butyl-2-octenal	1664	1656	Aldehyde
0.992	2,4-Dimethylbenzaldehyde	1737	1728	Aldehyde
0.982	α-Cyclocitral	1442	1425	Terpene
0.98	β-Cyclocitral	1626	1611	Terpene
0.979	β-Ionone epoxide	1997	1962	Ketone
0.975	*C18:2n6c (Linoleic acid)*			
0.97	trans-β-Ionone	1942	1940	Terpene
0.964	*Protein*			
0.957	Heptadecane	1690	1700	Hydrocarbon
0.953	Heptanal	1177	1184	Aldehyde
0.947	trans-2-Octenol	1598	1614	Alcohol
0.942	*C16:0 (Palmitic acid)*			
0.942	Nonanal	1387	1391	Aldehyde
0.937	Pentadecane	1487	1500	Hydrocarbon
0.936	Hexadecane	1587	1600	Hydrocarbon
0.929	Geranyl acetone	1846	1859	Ketone
0.925	Octanal	1281	1289	Aldehyde
0.893	Isophorone	1404	1591	Ketone
0.887	1,2,4,4-Tetramethylcyclopentene	932	–	Hydrocarbon
0.869	2,2,4,6,6-Pentamethylheptane	944	949	Hydrocarbon
0.863	β-Pinene	1091	1112	Terpene
0.858	D-Limonene	1187	–	Terpene
0.856	1-Nonanol	1643	1660	Alcohol
0.851	m-Xylene	1131	1143	Hydrocarbon
0.851	α-Ionene	1697	1565	Hydrocarbon
0.843	1-Dodecene	1227	1243	Hydrocarbon
0.818	Hexyl acetate	1261	1272	Ester
0.801	1-Octen-3-ol	1431	1450	Alcohol
−0.8	(3Z,5Z)-3,5-Octadiene	925	–	Hydrocarbon
−0.945	*Ash*			
Nannochloropsis sp.				
0.991	2-Undecanone	1591	1598	Ketone
0.986	(E)-2-Pentenal	1125	1127	Aldehyde
0.985	1,3-Pentadiene	97	624	Hydrocarbon
0.982	2-Nonanone	1381	1390	Ketone
0.98	(*Z*)-2-Pentenol	1304	1318	Alcohol
0.976	1-Penten-3-one	1016	1019	Ketone
0.974	*C16:1n7 (Palmitoleic acid)*			
0.972	*C20:5n3 (Eicosapentaenoic acid, EPA)*			
0.962	1-Heptanol	1440	1453	Alcohol
0.946	3-Octanone	1247	1253	Ketone
0.936	Benzyl alcohol	1867	1870	Alcohol
0.888	3-Pentanone	973	980	Ketone
0.885	1-Penten-3-ol	1147	1159	Alcohol
0.832	2,7-Octadienol	1666	–	Alcohol
0.815	3-Ethyl-1,5-octadiene	1019	1015	Hydrocarbon
0.812	(3E,5E)-3,5-Octadien-2-one	1566	1570	Ketone
−0.838	*Chlorophyll a*			
−0.839	*Total phenolic content*			
Tetraselmis sp.				
0.976	*C18:3n3 (α-Linolenic acid, ALA)*			
0.974	Dimethyl sulphide	743	754	Sulphur compound
0.969	*C12:0 (Lauric acid)*			
0.957	2-Ethyl-3,5,6-trimethylpyrazine	1505	1506	Pyrazine
0.955	(*Z*)-4-Heptenal	1234	1240	Aldehyde
0.947	*C18:0 (Stearic acid)*			
0.925	α-Ionone	1853	1840	Terpene
0.918	*C18:1n9c (Oleic acid)*			
0.894	*Carbohydrates*			
0.857	Benzaldehyde	1526	1520	Aldehyde
0.853	6-Methyl-5-hepten-2-one	1329	1338	Ketone
0.844	2,3-Butanedione	970	979	Ketone
−0.87	*Lipid*			
Isochrysis sp.				
0.96	*C22:6n3 (Docosahexaenoic acid, DHA)*			
0.96	*C14:1 (Myristoleic acid)*			
0.951	*C20:0 (Arachidonic acid)*			
0.948	*Carotenoids*			
0.936	3-Methyl-1,4-heptadiene	914	–	Hydrocarbon
0.933	*C14:0 (Myristic acid)*			
0.916	3-Methyl-2-(3,7,11-trimethyldodecyl) furan	2097	–	Furan
0.906	2,5-Dimethylbenzaldehyde	1746	1683	Aldehyde
0.886	Maltol	1960	1969	Ketone
0.865	(E,E)-2,4-Heptadienal	1460	1495	Aldehyde
0.835	2-pentylfuran	1221	1231	Furan

Retention indices (RI) for the individual volatile compounds were calculated and reference obtained from the National Institute Standards and Technology Standard Reference Database (National Institute of Standards and Technology n.d.). Individual fatty acids were identified by matching retention time with commercial standards.

tion of PUFA has been attributed to a greater number of linear aldehydes with the occurrence of chemical lipid oxidation and aromatic aldehydes associated with enzymatic lipid and protein oxidation[9]. This corresponds to the high amount of PUFA in *Arthrospira* as observed with linoleic acid (Fig. 4e). Among aldehydes, nonanal (Fig. 4f) was identified as a volatile biomarker to indicate changes in different growth phases[55].

Among hydrocarbons, heptadecane (Fig. 4g) was the major compound identified, followed by smaller amounts of hexadecane and pentadecane, which is in accordance with other studies[50]. Hydrocarbons are associated with neutral lipids obtained from the lipid fraction of microalgal biomass[51]. With the abundance of PUFA in *Arthrospira*, the findings corroborate the reported link of hydrocarbons to lipid peroxidation in

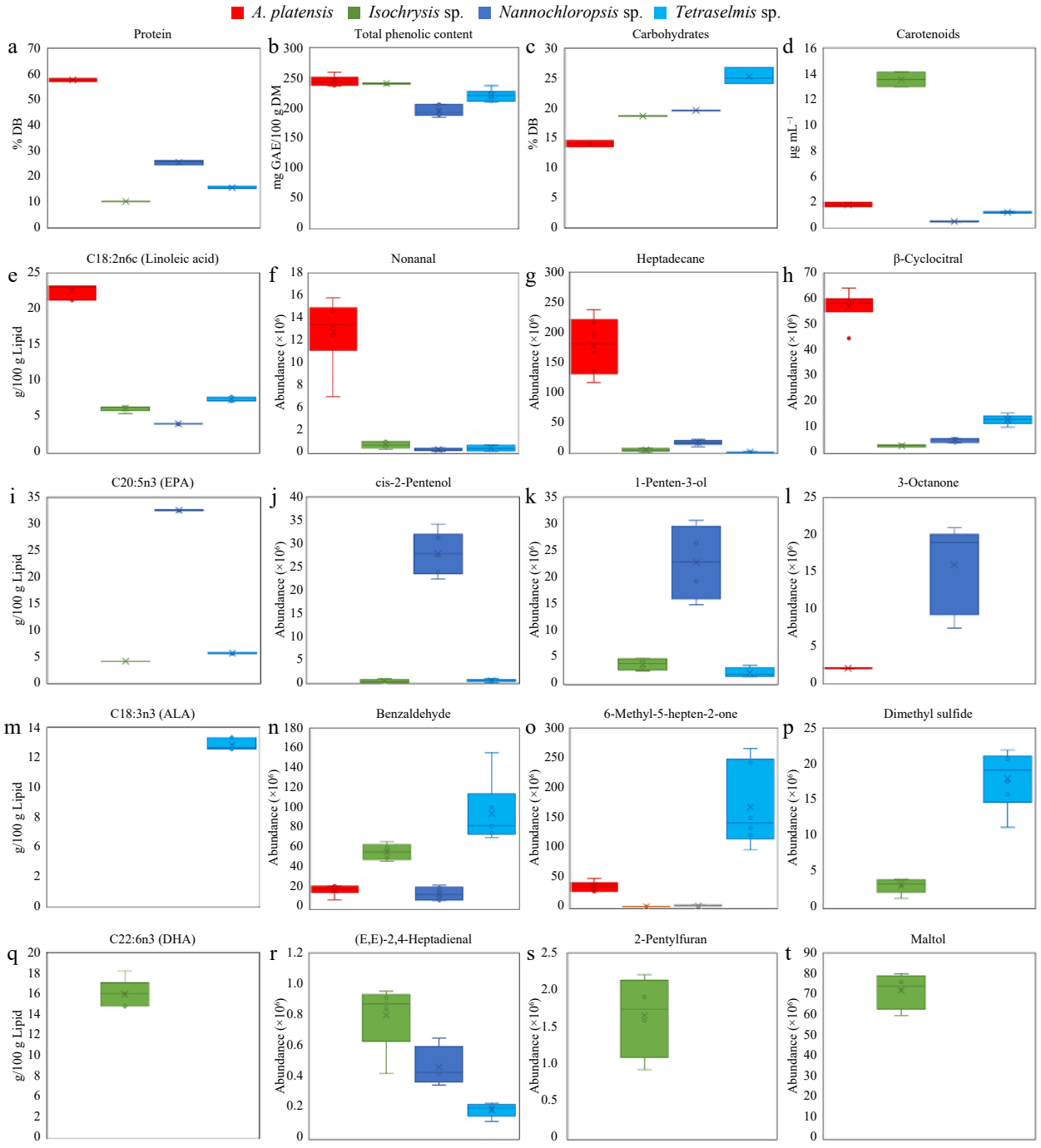

Fig. 4 Individual plots of some representative discriminant compounds show variation among *A. platensis*, *Isochrysis* sp., *Nannochloropsis* sp., and *Tetraselmis* sp. Values are mean ± standard error (n = 3).

microalgae[52]. Significant presence of terpenes is represented by *β*-cyclocitral (Fig. 4h), among others. While this is the first time that *β*-cyclocitral is reported in *Arthrospira*, this carotenoid-degradation product has been reported in other microalgae[9].

Nannochloropsis

Nannochloropsis can be characterised as an abundant source of lipid rich in PUFA and having a volatile profile that dominantly consists of ketones and alcohols (Fig. 4i–l).

Discriminant fatty acids in *Nannochloropsis* include EPA (Fig. 4i) and palmitoleic acid. Substantial amount of EPA in *Nannochloropsis* has been consistently reported in the literature and is desirable because of the numerous health benefits of this PUFA[56]. *Nannochloropsis*-derived EPA was found to control cholesterol levels and is beneficial to the cardiovascular health of the general population[13]. The abundance of palmitoleic acid has also been reported in *Nannochloropsis*[49].

Compared to other species, *Nannochloropsis* had the greatest number of alcohols identified to be discriminant. (*Z*)-2-Pentenol (Fig. 4j) and 1-penten-3-ol (Fig. 4k) were the most abundant that have also been identified by other researchers in *Nannochloropsis*[9,55]. Zhou et al. reported that the relative

contents of alcohols in *Nannochloropsis* were stable at different growth phases, whereas other microalgal species had decreasing trend[55]. While it was unclear what caused the observed trend, this could explain the predominance of alcohol volatile compounds in *Nannochloropsis*. Other studies reported short-chain alcohols, ketones, and aldehydes as the most representative volatile compounds for *Nannochloropsis* using PCA analysis[9]. Among ketones, 3-pentatone (Fig. 4k) and 1-penten-3-one has been identified previously[9]. In contrast, the generally lower values of chlorophyll *a* and total phenolic content (Fig. 4b) of *Nannochloropsis* compared to the other species are represented by these attributes' negative VID coefficient.

Tetraselmis

Tetraselmis can be described as rich in carbohydrates and α-linolenic acid with mainly ketones and aldehydes as volatile compounds (Fig. 4m–p). Superior amounts of carbohydrates (Fig. 4c) in *Tetraselmis* could be attributed to cell walls being rich in intracellular starch and complex polysaccharides[57]. The negative VID of lipid emphasizes the lower range of lipid content in *Tetraselmis* compared to other species, in accordance to other reports[57]. While α-linolenic acid (Fig. 4m) was previously found in other microalgal species[28], current work observed that this PUFA was absent in other species but richly present in *Tetraselmis*.

Aldehydes and ketones were the major discriminant volatiles in *Tetraselmis*. (*Z*)-4-heptenal and benzaldehyde could be attributed to oxidation reactions of PUFA that are relatively abundant in the biomass. High proportions of benzaldehyde (Fig. 4n) are analogous to other reports and can be accredited to phenylalanine's enzymatic and chemical degradation *via* amino acid biosynthetic pathway[9]. A significant ketone identified is 6-methyl-5-hepten-2-one (Fig. 4o) that others have consistently reported[9,53]. *Tetraselmis* had the unique presence of sulphur-containing compounds, specifically dimethyl sulphide, as discriminant. Dimethyl sulphide (Fig. 4p), the only sulphur-containing compound detected, was also found in *Isochrysis* but present at much higher concentrations in *Tetraselmis*. This compound has only been previously reported in *Tetraselmis* and *Rhodomonas* and attributed to the enzymatic and chemical degradation of dimethylsulfoniopropionate[9].

Isochrysis

Isochrysis is rich in carotenoids, DHA, arachidonic acid, myristic acid, and contained mostly aldehydes and furans. The abundance of identified fatty acids is directly related to the presence of discriminant volatiles (Fig. 4q–t). The substantially high carotenoid content (Fig. 4d) warrants this attribute as an appropriate discriminant marker for this species. Carotenoid-rich *Isochrysis* are in accordance with other studies[48,57]. Among the fatty acids, DHA, arachidonic acid, and myristic acid have sharply greater amounts in *Isochrysis* than in the other species, a feature consistently reported in the literature for this species[28,57]. Among other fatty acids, DHA (Fig. 4q) had one of the highest VID among the discriminant variables and reflects *Isochrysis* having the highest DHA level. Significantly high concentration of DHA in *Isochrysis* is desirable as they attenuate risk factors of cardiovascular and other chronic diseases[58].

Discriminant aldehydes in *Isochrysis* were 2,5-dimethylbenzaldehyde, and (E,E)-2,4-heptadienal, with the latter having been detected in *Rhodomonas* and *Botryococcus* species[9]. Additionally, an interesting compound selected to be discriminant in *Isochrysis* is 2-pentylfuran (Fig. 4s), likewise reported in *Botryococcus* and *Chlorella*[9]. Other cyanobacteria that had a detectable presence of 2-pentylfuran, an important lipid degradation product, are *Arthrospira*, *Anabaena*, and *Nostoc* genera[50]. Moreover, previous studies have identified linolenic and linoleic acids, beta-carotene, ascorbic acid, amino acids, and carbohydrates, which are considerably present in *Isochrysis*, as precursors in furan formation[54]. The ketone maltol (Fig. 4t) contributed substantially to the volatile profile of *Isochrysis*. This is the first time in this study on *Isochrysis* that maltol is identified in microalgae, although it has been reported in certain seaweeds[59]. Ketones are generally considered lipid oxidation or degradation products[51].

For all the microalgal species, it is notable that the discriminant markers were mostly health-relevant compounds that are considered to have food and pharmaceutical applications. The high protein content and richness in valuable fatty acids of *Arthrospira* verifies its distinction as a foremost microalgal biomass for commercialization since it has numerous applications as health supplement or supplementary food ingredient. Additionally, *Arthrospira* have been associated with desirable bioactive properties indicating favorable contribution to nutraceutical industry[40]. For *Nannochloropsis*, its differentiation as an EPA-rich biomass builds up to the body of evidence that this species is ideal as a functional ingredient, with reported positive effect on cardiovascular health among other health benefits[13]. *Tetraselmis* is distinguished as having PUFA-rich lipid, in the form of γ-linolenic acid, and confirms the viability of this microalgal biomass as PUFA supplement for human nutrition[13]. Meanwhile, *Isochrysis* has been differentiated for the high levels of carotenoids as well as DHA, which is a very relevant PUFA for human health. This shows its potential as a novel functional ingredient, along with the other microalgal species.

Conclusions

There is an apparent variation in the physicochemical properties of the four microalgal species. Chemometrics analysis to the multivariate data revealed the distinctness of each microalgal species based on integrated microalgal properties. The major discriminant volatile markers, indicative of each species' distinct volatile profiles were aldehydes, terpene, and hydrocarbon for *Arthrospira*, ketones and alcohols for *Nannochloropsis*, aldehydes, ketones, and sulphur-containing compounds for *Tetraselmis*, and furans and aldehydes for *Isochrysis*. The main discriminant fatty acids included γ-linolenic acid for *Arthrospira*, DHA for *Isochrysis*, EPA for *Nannochloropsis*, and α-linolenic acid for *Tetraselmis*.

As presented in this study, the rich abundance of proteins, carbohydrates, lipids, and other bioactive compounds in microalgae enables a complex association that could potentially result in microalgae-enriched biomass with promising rheological, volatile, and nutritional characteristics. The diverse profiles of microalgae allow a varied and expanded application in the food industry and the pharmaceuticals and nutraceutical sectors. Findings suggest that desirable compounds, like pigments, total phenolic contents, and other macronutrients, may be appreciated with or without the intended rheological/textural and volatile/aroma impact, depending on the type of microalgae utilised. In the case of *Arthrospira*, while it has and

can further be used for protein supplementation in health drink juices, it can also be used as a structuring/thickening agent in different food products. *Isochrysis, Nannochloropsis,* and *Tetraselmis* species have great potential as ingredients in the development of functional foods. They can be utilised in various suitable food matrices (e.g., pasta, baked products, and processed meat products) that complements their unique volatile flavour-related attributes for greater consumer acceptance.

While the present work clearly shows the effectivity of chemometrics approach in identifying the distinguishing qualities of different microalgal species, it would be worthwhile to further characterize microalgal biomass as affected by growth conditions and developmental stage. Additionally, integrating other data such as amino acid and sugar profiles could elucidate other notable unique microalgal characteristics.

The HS-SPME GC-MS fingerprinting approach employed in this research provides insights into the volatile composition of microalgae. However, it has limitations when directly correlating with the (off) flavor profile of the product. While the identified volatile compounds can be linked to reaction pathways or specific food characteristics, caution was exercised in attributing selected compounds to undesirable odor notes commonly found in microalgae-based products. (Off) flavor attributes are best assessed using descriptive sensory analysis, a facet not covered in this research. Future investigations have the potential to correlate instrumental attributes with sensory data, identifying compounds contributing to the off-flavor profile of microalgae.

Author contributions

The authors confirm contribution to the paper as follows: conceptualisation: Magpusao J, Kebede B; Investigation: Magpusao J; Methodology: Magpusao J, Oey I, Kebede B; data analysis and visualization: Magpusao J, Kebede B; supervision: Kebede B, Oey I; writing - original draft & editing: Magpusao J; writing - review & editing: Oey I, Kebede B. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The study's supporting data will be provided by the corresponding author upon reasonable request.

Acknowledgements

Johannes Magpusao acknowledges the New Zealand Development Scholarship funded through the Ministry of Foreign Affairs and Trade for her doctoral scholarship.

Conflict of interest

The authors declare that they have no conflict of interest. Indrawati Oey is the Editorial Board member of *Food Innovation and Advances* who was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and the research groups.

Supplementary Information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/fia-0024-0007)

Dates

Received 13 December 2023; Accepted 19 March 2024; Published online 22 April 2024

References

1. De Morais MG, Vaz BDS, De Morais EG, Costa JAV. 2015. Biologically Active Metabolites Synthesized by Microalgae. *Biomed Research International* 2015:835761
2. Draaisma RB, Wijffels RH, Slegers PME, Brentner LB, Roy A, et al. 2013. Food commodities from microalgae. *Current Opinion in Biotechnology* 24(2):169–77
3. Matos J, Cardoso C, Bandarra NM, Afonso C. 2017. Microalgae as healthy ingredients for functional food: A review. *Food & Function* 8(8):2672–85
4. Vigani M, Parisi C, Rodríguez-Cerezo E, Barbosa MJ, Sijtsma L, et al. 2015. Food and feed products from micro-algae: Market opportunities and challenges for the EU. *Trends in Food Science & Technology* 42(1):81–92
5. Laurens LML, Van Wychen S, McAllister JP, Arrowsmith S, Dempster TA, et al. 2014. Strain, biochemistry, and cultivation-dependent measurement variability of algal biomass composition. *Analytical Biochemistry* 452(1):86–95
6. Batista AP, Gouveia L, Bandarra NM, Franco JM, Raymundo A. 2013. Comparison of microalgal biomass profiles as novel functional ingredient for food products. *Algal Research* 2(2):164–73
7. Caporgno MP, Mathys A. 2018. Trends in microalgae incorporation into innovative food products with potential health benefits. *Frontiers in Nutrition* 5:58
8. Bernaerts TMM, Gheysen L, Foubert I, Hendrickx ME, Van Loey AM. 2019. The potential of microalgae and their biopolymers as structuring ingredients in food: a review. *Biotechnology Advances* 37:107419
9. Van Durme J, Goiris K, De Winne A, De Cooman L, Muylaert K. 2013. Evaluation of the volatile composition and sensory properties of five species of microalgae. *Journal of Agricultural and Food Chemistry* 61(46):10881–90
10. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. 2006. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* 101(2):87–96
11. Ferdous UT, Yusof ZNB. 2021. Medicinal prospects of antioxidants from algal sources in cancer therapy. *Frontiers in Pharmacology* 12:593116
12. García-Segovia P, Pagán-Moreno MJ, Lara IF, Martínez-Monzó J. 2017. Effect of microalgae incorporation on physicochemical and textural properties in wheat bread formulation. *Food Science and Technology International* 23(5):437–47
13. Rao A, Briskey D, Nalley JO, Ganuza E. 2020. Omega-3 eicosapentaenoic acid (EPA) rich extract from the microalga *Nannochloropsis* decreases cholesterol in healthy individuals: A double-blind, randomized, placebo-controlled, three-month supplementation study. *Nutrients* 12(6):1869
14. Schwenzfeier A, Helbig A, Wierenga PA, Gruppen H. 2013. Emulsion properties of algae soluble protein isolate from *Tetraselmis* sp. *Food Hydrocolloids* 30(1):258–63
15. Magpusao J, Giteru S, Oey I, Kebede B. 2021. Effect of high pressure homogenization on microstructural and rheological properties of *A. platensis*, *Isochrysis*, *Nannochloropsis* and *Tetraselmis* species. *Algal Research* 56:102327
16. AOAC. 2008. Solids (total) and loss on drying (moisture) in flour: Air oven method (AOAC 925.10-1925). In *Official Methods of Analysis of AOAC International* 665(1925). Oxford: Oxford University Press. https://doi.org/10.1093/9780197610145.003.2927
17. Burja AM, Armenta RE, Radianingtyas H, Barrow CJ. 2007. Evaluation of fatty acid extraction methods for *Thraustochytrium* sp.

ONC-T18. *Journal of Agricultural and Food Chemistry* 55(12):4795−801
18. AOAC. 2005. Protein (total) in flour (AOAC 920.87-1920). In *Official Methods of Analysis of AOAC International* 4007. Oxford: Oxford University Press. https://doi.org/10.1093/9780197610145.003.2942
19. González López CV, García MdCC, Fernández FGA, Bustus CS, Chisti Y, et al. 2010. Protein measurements of microalgal and cyanobacterial biomass. *Bioresource Technology* 101:7587−91
20. Lourenço SO, Barbarino E, Lavín PL, Lanfer Marquez UM, Aidar E. 2004. Distribution of intracellular nitrogen in marine microalgae: Calculation of new nitrogen-to-protein conversion factors. *European Journal of Phycology* 39(1):17−32
21. Kulkarni S, Nikolov Z. 2018. Process for selective extraction of pigments and functional proteins from *Chlorella vulgaris*. *Algal Research* 35:185−93
22. Wellburn AR. 1994. The Spectral Determination of Chlorophylls *a* and *b*, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *Journal of Plant Physiology* 144(3):307−13
23. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, et al. 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry* 102(3):771−76
24. Bernaerts TMM, Panozzo A, Doumen V, Foubert I, Gheysen L, et al. 2017. Microalgal biomass as a (multi)functional ingredient in food products: Rheological properties of microalgal suspensions as affected by mechanical and thermal processing. *Algal Research* 25:452−63
25. Khrisanapant P, Kebede B, Leong SY, Oey I. 2019. A comprehensive characterisation of volatile and fatty acid profiles of legume seeds. *Foods* 8(12):651
26. Kebede BT, Grauwet T, Magpusao J, Palmers S, Michiels C, Hendrickx M, et al. 2015. An integrated fingerprinting and kinetic approach to accelerated shelf-life testing of chemical changes in thermally treated carrot puree. *Food Chemistry* 179:94−102
27. Ryckebosch E, Bruneel C, Muylaert K, Foubert I. 2012. Microalgae as an alternative source of omega-3 long chain polyunsaturated fatty acids. *Lipid Technology* 24(6):128−30
28. Tokuşoglu Ö, Ünal MK. 2003. Biomass nutrient profiles of three microalgae: *Spirulina platensis, Chlorella vulgaris*, and *Isochrisis galbana*. *Journal of Food Science* 68(4):1144−48
29. Graziani G, Schiavo S, Nicolai MA, Buono S, Fogliano V, et al. 2013. Microalgae as human food: Chemical and nutritional characteristics of the thermo-acidophilic microalga *Galdieria sulphuraria*. *Food & Function* 4(1):144−52
30. Rebolloso-Fuentes MM, Navarro-Pérez A, García-Camacho F, Ramos-Miras JJ, Guil-Guerrero JL. 2001. Biomass Nutrient Profiles of the Microalga *Nannochloropsis*. *Journal of Agricultural and Food Chemistry* 49(6):2966−72
31. Bernaerts TMM, Gheysen L, Kyomugasho C, Jamsazzadeh Kermani Z, Vandionant S, et al. 2018. Comparison of microalgal biomasses as functional food ingredients: Focus on the composition of cell wall related polysaccharides. *Algal Research* 32:150−61
32. Grossmann L, Ebert S, Hinrichs J, Weiss J. 2018. Production of protein-rich extracts from disrupted microalgae cells: Impact of solvent treatment and lyophilization. *Algal Research* 36:67−76
33. Das D. 2015. *Algal Biorefinery: An Integrated Approach*. New Delhi, India: Springer International Publishing, Jointly published with Capital Publishing Company. 479 pp. https://doi.org/10.1007/978-3-319-22813-6
34. Saoudi-Helis L, Dubacq JP, Marty Y, Samain JF, Gudin C. 1994. Influence of growth rate on pigment and lipid composition of the microalga *Isochrysis* aff. *galbana clone T. iso. Journal of Applied Phycology* 6(3):315−22
35. Gheysen L, Bernaerts T, Bruneel C, Goiris K, Durme J Van. 2018. Impact of processing on n-3 LC-PUFA in model systems enriched with microalgae. *Food Chemistry* 268(June):441−50
36. Guo W, Zhu S, Feng G, Wu L, Feng Y, et al. 2020. Microalgae aqueous extracts exert intestinal protective effects in Caco-2 cells and dextran sodium sulphate-induced mouse colitis. *Food & Function* 11(1):1098−109
37. Abd El Baky HH, El Baroty GS, Ibrahem EA. 2015. Functional characters evaluation of biscuits sublimated with pure phycocyanin isolated from *Spirulina* and *Spirulina* biomass. *Nutricion Hospitalaria* 32(1):231−41
38. Di Lena G, Casini I, Lucarini M, Lombardi-Boccia G. 2019. Carotenoid profiling of five microalgae species from large-scale production. *Food Research International* 120:810−18
39. Parniakov O, Apicella E, Koubaa M, Barba FJ, Grimi N, et al. 2015. Ultrasound-assisted green solvent extraction of high-added value compounds from microalgae *Nannochloropsis* spp. *Bioresource Technology* 198:262−67
40. Matos J, Cardoso CL, Falé P, Afonso CM, Bandarra NM. 2020. Investigation of nutraceutical potential of the microalgae *Chlorella vulgaris* and *Arthrospira platensis*. *International Journal of Food Science & Technology* 55(1):303−12
41. Custódio L, Soares F, Pereira H, Barreira L, Vizetto-Duarte C, et al. 2014. Fatty acid composition and biological activities of *Isochrysis galbana* T-ISO, *Tetraselmis* sp. and *Scenedesmus* sp.: Possible application in the pharmaceutical and functional food industries. *Journal of Applied Phycology* 26(1):151−61
42. Günerken E, D'Hondt E, Eppink MHM, Garcia-Gonzalez L, Elst K, et al. 2015. Cell disruption for microalgae biorefineries. *Biotechnology Advances* 33(2):243−60
43. Sili C, Torzillo G, Vonshak A. 2012. *Arthrospira* (Spirulina). In *Ecology of Cyanobacteria II: Their Diversity in Space and Time*, ed. Whitton BA. Dordrecht: Springer. pp. 677−705. https://doi.org/10.1007/978-94-007-3855-3_25
44. Liu C, Lin L. 2001. Ultrastructural study and lipid formation of *Isochrysis* sp. *Botanical Bulletin of Academia Sinica* 42:207−14
45. Scholz MJ, Weiss TL, Jinkerson RE, Jing J, Roth R, et al. 2014. Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. *Eukaryotic Cell* 13(11):1450−64
46. Halim R, Rupasinghe TWT, Tull DL, Webley PA. 2013. Mechanical cell disruption for lipid extraction from microalgal biomass. *Bioresource Technology* 140:53−63
47. Björn A, La Monja PSde, Karlsson A, Ejlertsson J, Svensson BH. 2012. *Rheological Characterization*. In *Biogas*, ed. Kumar S. Sweden: InTech. pp. 63−76. https://doi.org/10.5772/32596
48. Shekarabi SPH, Mehrgan MS, Razi N, Sabzi S. 2019. Biochemical composition and fatty acid profile of the marine microalga *Isochrysis galbana* dried with different methods. *Journal of Microbiology, Biotechnology and Food Sciences* 9(3):521−24
49. Gu N, Lin Q, Li G, Tan Y, Huang L, et al. 2012. Effect of salinity on growth, biochemical composition, and lipid productivity of *Nannochloropsis oculata* CS 179. *Engineering in Life Sciences* 12(6):631−37
50. Milovanović I, Mišan A, Simeunović J, Kovač D, Jambrec D, et al. 2015. Determination of volatile organic compounds in selected strains of cyanobacteria. *Journal of Chemistry* 2015:969542
51. Nawar WW. 1996. Lipids. In *Food Chemistry*, ed. Fennema OR. 3rd Edition. New York, USA: Marcel Dekker Inc. pp. 225–314.
52. Achyuthan KE, Harper JC, Manginell RP, Moorman MW. 2017. Volatile metabolites emission by in vivo microalgae—an overlooked opportunity? *Metabolites* 7(3):39
53. Isleten Hosoglu M. 2018. Aroma characterization of five microalgae species using solid-phase microextraction and gas chromatography – mass spectrometry/olfactometry. *Food Chemistry* 240:1210−18
54. Kettlitz B, Scholz G, Theurillat V, Cselovszky J, Buck NR, et al. 2019. Furan and methylfurans in foods: an update on occurrence, mitigation, and risk assessment. *Comprehensive Reviews in Food Science and Food Safety* 18(3):738−52

55. Zhou L, Chen J, Xu J, Li Y, Zhou C, et al. 2017. Change of volatile components in six microalgae with different growth phases. *Journal of the Science of Food and Agriculture* 97(3):761–69
56. Bernaerts TMM, Verstreken H, Dejonghe C, Gheysen L, Foubert I, et a. 2020. Cell disruption of *Nannochloropsis* sp. improves *in vitro* bioaccessibility of carotenoids and ω3-LC-PUFA. *Journal of Functional Foods* 65:103770
57. Di Lena G, Casini I, Lucarini M, Sanchez del Pulgar J, Aguzzi A, et al. 2020. Chemical characterization and nutritional evaluation of microalgal biomass from large-scale production: a comparative study of five species. *European Food Research and Technology* 246(2):323–32
58. Buono S, Langellotti AL, Martello A, Rinna F, Fogliano V. 2014. Functional ingredients from microalgae. *Food & Function* 5:1669–85
59. Yamamoto M, Baldermann S, Yoshikawa K, Fujita A, Mase N, et al. 2014. Determination of volatile compounds in four commercial samples of Japanese Green Algae using solid phase microextraction gas chromatography mass spectrometry. *The Scientific World Journal* 2014:289780
60. National Institute of Standards and Technology (NIST). 2019. *NIST Chemistry WebBook: NIST Standard Reference Database Number 69*. https://doi.org/10.18434/T4D303