WALAILAK JOURNAL
http://wjst.wu.ac.th Applied Sciences

Monitoring of Biochemical Compounds and Fatty Acid in Marine Microalgae from the East Coast of Thailand

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Received: 16 November 2017, Revised: 19 June 2018, Accepted: 30 July 2018

Abstract

Determinations of fatty acid profiles of five microalgae; *Amphora* sp., *Chaetoceros* sp., *Melosira* sp., *Bellerochae* sp., and *Lithodesmium* sp., from the east coast of Thailand were evaluated by conventional Gas Chromatography-Flame Ionization Detector (GC-FID). The results exhibited that the fatty acids suitable for biodiesel production were the most frequent entities encountered in all microalgae profiles. The GC chromatogram of fatty acid profiles in microalgae showed that both Amphora sp. and Chaetoceros sp. comprised essential omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Additionally, this study assessed whether Fourier Transform infrared (FT-IR) microspectroscopy could be used to evaluate and monitor the biochemical compositions of microalgae, including lipid, carbohydrate, and protein profiles, by using colorimetric methods. Results showed that FT-IR spectra combined with biochemical values of lipid, carbohydrate, and protein contents were used as predictive models generated by partial least square (PLS) regression. Cross-validation of the lipid, protein, and carbohydrate models showed high degrees of statistical accuracy with RMSECV values of approximately 0.5 - 3.22 %, and a coefficient of regression between the actual and predicted values of lipids, carbohydrates, and proteins were 92.66, 95.73, and 96.43 %, respectively. The RPD values were all high (> 3), indicating good predictive accuracy. This study suggested that FT-IR could be a tool for the simultaneous measurement of microalgae composition of biochemical contents in microalgae cells.

Keywords: Marine microalgae, Biochemical compounds, East coast of Thailand, Fatty acid, FT-IR

Introduction

The coastal environment in Thailand is supported by various types of coastal ecosystems including sandy shores, mangrove areas, estuaries, and mudflats [1]. Therefore, the coastal areas have a substantial amount of various nutrient supplies in response to a wave exposure gradient. The nutritional value and chemical compositions of microalgae's biomass depend on nutrient availability in their environment, as it helps increase microalgal biomass and productivity and shifts species compositions [2]. Algae blooms in Chonburi, a province on the east coast of Thailand, have been observed because they can lower levels of dissolved oxygen, causing fish deaths and poor water clarity, as well as lowering shoreline property values [3]. Vulnerable marine ecosystems can adversely affect tourism and, most notably, human settlements in coastal regions, leading to a decrease in marine organism diversity [4]. Marine microalgae have potential benefits in the protection of the environment, as well as in pharmaceuticals, biofuel production, and cosmetics. Therefore, they are considered to be valuable sources of food supplements for

humans and animals [5]. They are also important as feed sources in aquaculture, due to their nutritional value and their ability to synthesize and accumulate great amounts of proteins, amino acids, pigments, antioxidants, vitamins, minerals, and essential fatty acids including ω3-polyunsaturated fatty acids (PUFAs) [6], such as 22:6n-3 (DHA) and 20:5n-3 (EPA) [7,8]. According to the biochemical changes previously reviewed, an investigation of changes in microalgae lipid production throughout a bloom is of particular interest, as lipids provide a primary source of energy and essential fatty acids to higher tropic levels [9]. In particular, PUFAs have important physiological functions. Therefore, the screening of microalgae nutrients is primarily evaluated, and the quantifications of the biochemical compositions of lipid (fatty acid), proteins and carbohydrates are primarily individually evaluated using traditional and conventional methods to discover the microalgae compounds. In general, most conventional methods used to measure nutritional and physiological changes in microalgae require several types of instruments and multi-step procedures, which are time-consuming, intensive samples, and hazardous reagents [10,11]. The biochemical composition of microalgal biomass is usually quantified using a diverse range of techniques, typically involving chemical extraction, followed by gravimetric determination, spectrophotometry, or mass spectroscopy. Analyses are determined using conventional gravimetric or colorimetric SPV or high performance liquid chromatography (HPLC) and gas chromatography (GC) [5, 10-12]. The conventional sulfo-phospho-vanillin (SPV), anthrone, and micro-biuret methods are alternatives for the analysis of lipids, carbohydrates, and proteins similar to the analysis of biochemical contents in microalgae [13,14]. Moreover, several techniques have been investigated for more rapid and reproducible measurement of microalgal compositions, including spectrophotometry, Raman spectroscopy [15], Nuclear Magnetic Resonance (NMR), and Fourier Transform Infrared (FT-IR) microspectroscopy [16,17]. FT-IR microspectroscopy is a powerful and useful technique in many research studies, used either to compare the metabolic or biochemical fingerprints in macrobiological and microbiological sources in different growth conditions [10]. FT-IR microspectroscopy has been widely used to study the chemical compositions of biological materials; it provides an in situ and nondestructive chemical analysis of individual algal cells [11]. An infrared spectroscopy with high spatial resolution allows for the analysis of macromolecular pools (e.g., proteins, lipids, carbohydrates) in the intracellular structures of individual cells [12-14].

The present study of microalgae in the east coast of Thailand includes: (i) an investigation of potential microalgae lipid profiles using GC-FID, which can provide a valuable indication of the physiological and metabolic status of microalgae, (ii) the establishment of a rapid method to identify biochemical compositions with lipids, proteins, and carbohydrates by using FT-IR, compared against conventional methods, and (iii) an evaluation of cross validation to determine the biochemical compositions in microalgae by FT-IR microspectroscopy.

Materials and methods

Microalgae cultivation and harvest

Marine microalgae, Amphora sp., Chaetoceros sp., Melosira sp., Bellerochae sp. and Lithodesmium sp., were collected from Chonburi Province, located on the east coast of Thailand. All microalgae strains were categorized based on their fast growth responses and taxonomical diversity by the Institute of Marine Sciences, Burapha University, Thailand. They were grown in f/2 medium. Stock cultures were grown in Erlenmeyer flask places on a shaking platform at 25 °C in 12 h light/ dark cycles. Microalgae strains were identified by Asst. Prof. Amonrat Kanokrung (The Institute of Marine Sciences, Burapha University, Chonburi 20131, Thailand). The microalgae culture was harvested by centrifugation (6000 g for 10 min) and washed using normal saline three times. The cell pellets were determined for biochemical content using FT-IR-microspectroscopy and the conventional lipid sulfo-phospho-vanillin (SPV), carbohydrate anthrone, and micro-biuret protein methods.

Polyunsaturated fatty acid profile analysis in microalgae

PUFAs of marine microalgae were analyzed by GC-FID, the method modified according to the procedures reported by [18]. Microalgae 20 mg dry weight was methylated with 5 % sulfuric acid in

methanol (3 ml) by sonicator at 60 - 80 °C for 60 min. The resulting fatty acid profiles were extracted with 3 ml of hexane and washed with 10 ml of 5 % NaOH in water. To analyze the fatty acids, hexane extract and standard PUFA No. 1 from Supelco (Bellefonte, PA, USA) were injected into an Agilent 7890 GC. The gas chromatogram was obtained by an Agilent Technology 7890A gas chromatograph GC-FID, equipped with a fused silica capillary column HP-5 (30 m × 0.32 mm i.d., 0. 5 µm film thicknesses). The oven temperature was programmed from 150 °C and, after 2 min, rose to 270 °C at 4 °C/min and was kept for 10 min at 270 °C. The oven temperature was set at 270 °C and the temperature of the FID detector was set at 250 °C. The flow rate of carrier gas (Helium) in splitless mode was maintained at 1 ml/min in a constant flow mode. The injection volume was 1 µl for all of the samples. Retention time of fatty acid was quantified with ChemStation for GC systems. The compositions were elucidated by comparison of peak areas of standard PUFA No. 1. The analyses were done in triplicates.

Biochemical composition analysis by conventional methods

Cellular lipids, proteins, and carbohydrates were analyzed with the modification method of [13,14]. Briefly, microalgae of 3 - 5 mg dry weight was resuspended in 480 µl of R1 (25 % methanol in 1 N NaOH), and 20 µl phosphate buffer (0.05 M, pH 7.4) added; the pellets were disrupted using sonication for 1 h, then 100 µl of the lysate cells was added to each well of the microplate and was reacted with anthrone reagent and high concentrations of sulfuric acid. Plates were then mixed and placed for 10 min at 4 °C. Subsequently, plates were incubated for 20 min at 100 °C. After heating, a cooling step treatment for 20 min at room temperature before reading absorbance at 600 nm triplicate sample were done in a microplate reader. Quantification was performed using a glucose standard curve measured at 600 nm [14].

The remaining sample was saponification under heating at 80 °C for 30 min and cooled down. Then, the samples were centrifuged at 10,000×g for 5 min at 20 °C, and then 100 µl of supernatants were used to estimate the protein content in the extract using microbiuret method [18]. Briefly, 50 µl of copper sulphate (0.21 % CuSO4. 5H2O in 30 % NaOH) was added to the samples and color formation measured at 340 nm. Bovine serum albumin (BSA, Sigma Aldrich) was used as the standard for calibration.

Another aliquot, 500 µl of sample was pipetted to mixture solvent (chloroform:methanol, 2:1 v/v) in an eppendorf tube and vortexed for 2 min. The mixture was centrifuged at 10,000×g for 3 min to get two separated phases. The organic phase for 100 µl was used to evaluate lipid content in the extract using SPV [14]. The sample was loaded into microplates and solvent was used for evaporation. Concentrated sulfuric acid of 100 µl was added and incubated on a water bath at 80 °C for 10 min, and then cooled down at room temperature. The microplate was used to measure background absorbance at 540 nm. After that, 100 µl of vanillin-phosphoric acid reagent was added and, after 5 min of color development, the plate was measured at 520 nm. Olive oil and standard cholesterol (sigma) were used as the standard for calibration.

FT-IR microspectroscopy

To evaluate biochemical compositional, microalgae cells were fixed on low-e slides (MirrIR, Kevley Technologies) and analyzed using FT-IR microspectroscopy [19,20]. The infrared spectra of microalgae samples were performed at BL4.1: IR Spectroscopy and Imaging, Synchrotron Light Research Institute (Public Organization), Thailand. The Bruker Hyperion 2000 microscope (Bruker Optics Inc., Ettlingen, Germany), equipped with a nitrogen cooled MCT (HgCdTe) detector with a 36×IR objective, coupled to a Bruker Vertex 70 spectrometer, was used for IR data acquisition. All the spectra were acquired at 4.0 cm^{-1} resolution with 32 scans co-added in a spectra range from 4000 to 600 cm^{-1} by using OPUS 7.2 (Bruker Optics Ltd, Ettlingen, Germany).

Peak attribution was monitored according to [19,20]. Lipid has a characteristic absorption band of approximately 1480 - 1330 cm^{-1} and 1740 cm^{-1} representing the CH_2 bending and ester C=O stretching band of approximately 3000 - 2800 cm^{-1} representing the C–H stretching vibration in acyl chains of lipids. Protein has characteristic absorption bands at 1650 cm^{-1} (amide I) and 1540 cm^{-1} (amide II) representing C=O stretching vibration and N-H bending vibrations, respectively. Polysaccharide has a

characteristic absorption band of 1200 - 950 cm^{-1} representing the C-O-C vibration, and its absorption strength can be used to quantify the total carbohydrate content.

Calibration and quantitative analysis

The recorded FT-IR spectra, together with the results from biochemical conventional colorimetric methods, were analyzed using the partial least-square (PLS) regression. PLS calibration development using cross validation was performed with the QUANT 2 module of the OPUS 7.2 software e (Bruker), according to the multivariate calibration techniques described by Conzen [21]. The PLS analysis was applied for developing the calibration models to determine the lipid, protein, and carbohydrate contents of the microalgae samples based on spectral regions, as shown in **Figure 2**. The FT-IR spectra or their first derivatives were pre-processed by vector normalization to achieve optimal results. PLS regressions use a number of PLS principal components to predict the biochemical contents of microalgae. All PLS regression models were checked by cross-validation to determine the correlation coefficient of determination (R^2) of the respective model. The R^2 represents the difference between true and recomputed biochemical contents (lipids, proteins, and carbohydrates), with an R^2 of 1 indicating a perfect fit. Therefore, an R^2 of 0.9 indicates that 90 % of the variance can be explained by the respective PLS regression. The root mean square error of cross-validation (RMSECV) serves as the standard value for the quality of the fit and gives the approximate standard error between the predicted and reference values for all data points. The residual predictive deviation (RPD) is a qualitative measure for the assessment of validation results [22,23].

Calibration models correlating the FT-IR spectra and chemical data in both reflection and transmission modes were carried out employing PLS regression. The accuracy and performance of the models were assessed by the value of R^2, and RMSECV, the bias value, and the RPD value. The most capable QUANT 2 is the one with the lowest RMSEP value and the highest RPD value. Moreover, the bias value should be as close as possible to zero.

Statistical analysis

The results were reported as mean ± standard deviation (SD) (n = 3). The biochemical composition of the different microalgae species were statistically investigated using one-way analysis of variance (ANOVA) with least significant difference (LSD). A statistical probability (p value) of less than 0.05 indicated a statistically significant difference between groups.

Results and discussion

Fatty acid profile of microalgae

Marine microalgae *Amphora subtropica, Chaetoceros muellerii, Melosira dubia, Bellerochae* sp., and *Lithodesmium* sp. of Bacillariophyceae were collected from Chonburi, a coastal province on the east coast of Thailand, and were identified by Dr. Amonrat Kanokrung (Bangsaen Institute of Marine Science, Burapha University, Thailand) [24,25] based on their fast growth responses and taxonomical diversity. As marine microalgae are a good source of fatty acids vital for marine organisms, fatty acid compositions in five marine microalgae were evaluated [14,26]. The results presented the fatty acid compositions of *Amphora* sp., *Chaetoceros* sp., *Melosira* sp., *Bellerochae* sp., and *Lithodesmium* sp. using GC-FID method, as exhibited in **Table 1** and **Figure 1**. Most importantly, the fatty acids suitable for biodiesel production, such as myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1), and Oleic acid (C18:1) [4,6,27], were the most frequent entities found in all microalgal profiles, as shown in **Figure 1**. Microalgae contain high fatty acids because the structures of cells accumulate lipids, while environmental factors can affect growth performance and fatty acid profiles of diatom [28].

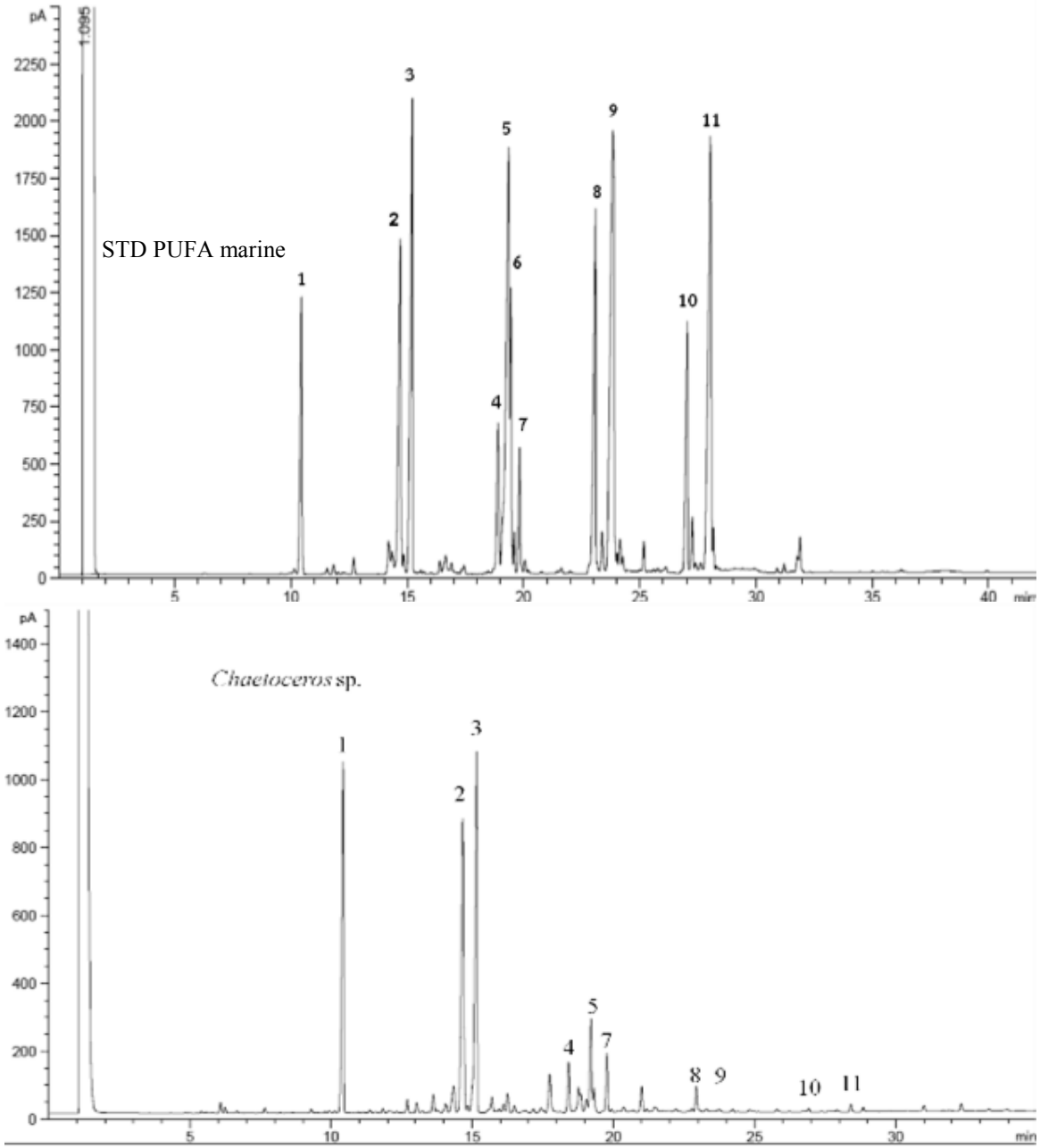
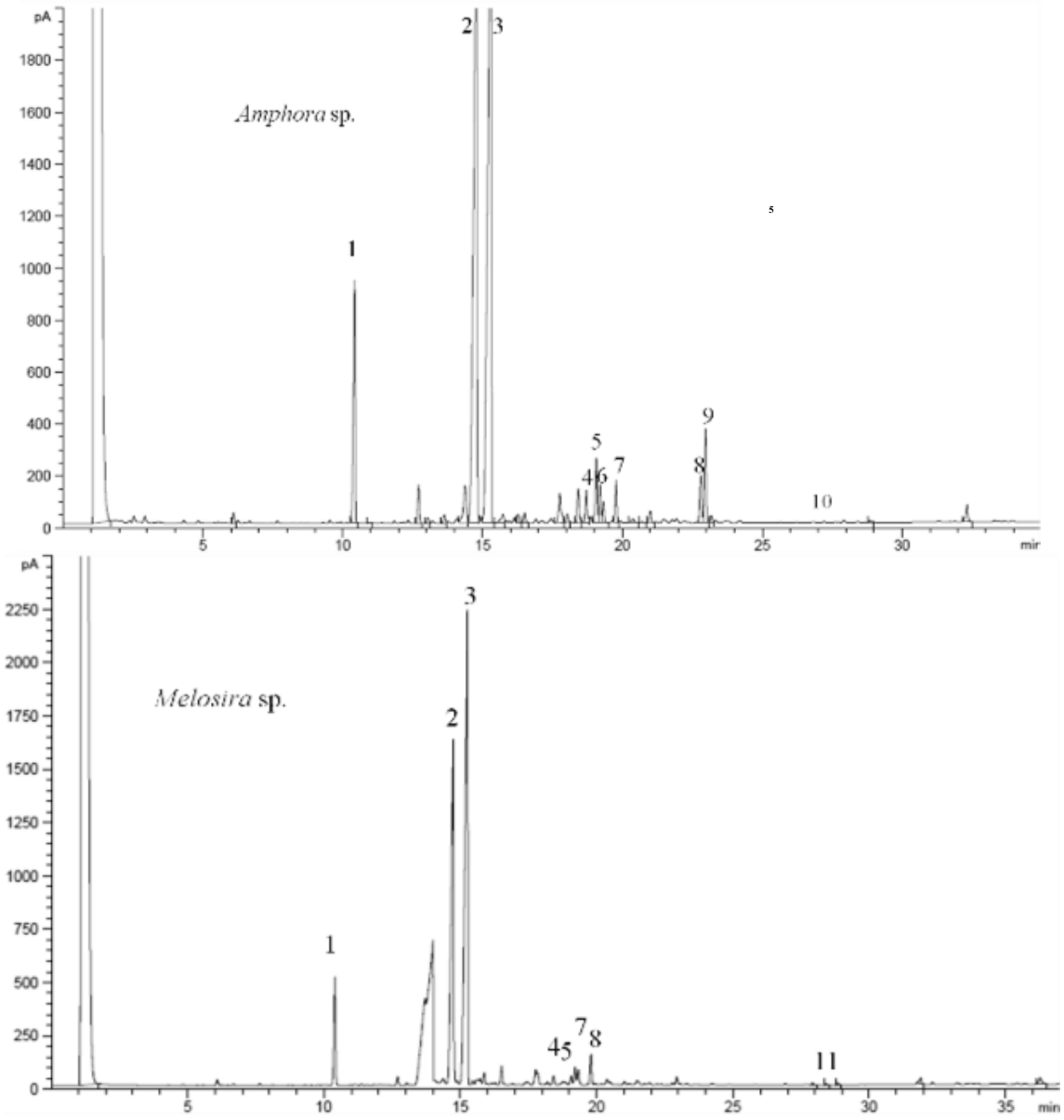


Figure 1 GC chromatogram of standard fatty acid (PUFA No.1) and five marine microalgae fatty acids by using GC-FID peak (1) C14:0 Myristic acid (2) C16:0 Palmitic acid (3) C16:1 Palmitoleic acid (4) C16:2 Hexadecadienoic acid (5) C18:1 Oleic acid (6) C18:3 linolenic acid (7) C18:0 Stearic acid (8) C20:5 Eicosapentaenoic acid (9) C20:1 Eicosenoic acid (10) C22:6 Docosahexaenoic acid and (11) C22:1 Docosenoic acid.



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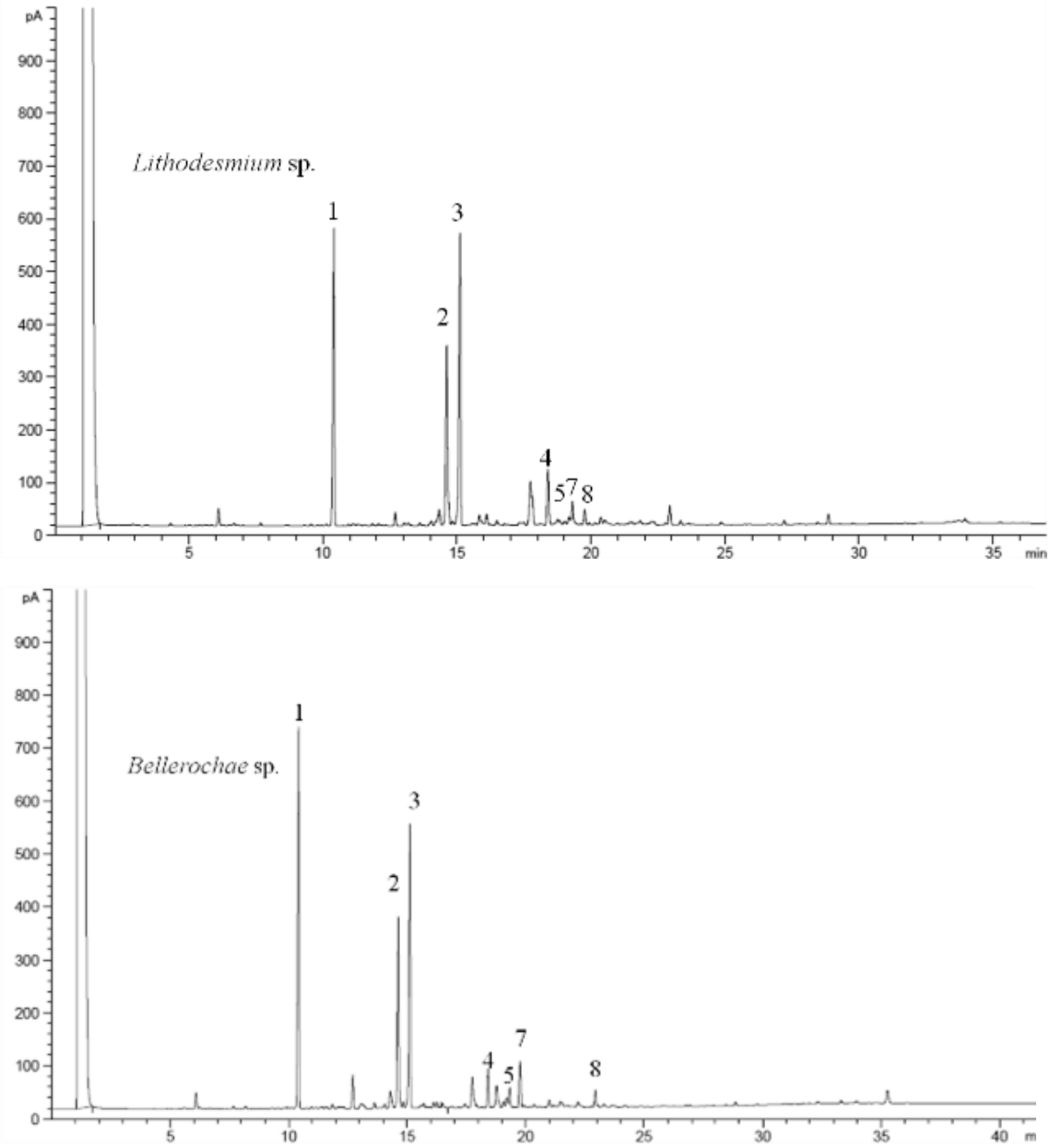

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Table 1 Fatty acid methyl esters compositions of five microalgae using GC-FID analysis

Peak	Retention time (min)	PUFA (%)	*Chaetoceros* sp.	*Amphora* sp.	*Melosira* sp.	*Lithodesmium* sp.	*Bellerochae* sp.
1	10.45	C14:0 Myristic acid	✓	✓	✓	✓	✓
2	14.74	C16:0 Palmitic acid	✓	✓	✓	✓	✓
3	15.25	C16:1 Palmitoleic acid	✓	✓	✓	✓	✓
4	18.93	C16:2 Hexadecadienoic acid	✓	✓	✓	✓	✓
5	19.30	C18:1 Oleic acid	✓	✓	✓	✓	✓
6	19.49	C18:3 linolenic acid	No	✓	No	No	No
7	19.88	C18:0 Stearic acid	✓	✓	✓	✓	✓
8	23.12	C20:5 Eicosapentaenoic acid	✓	✓	✓	✓	✓
9	23.78	C20:1 Eicosenoic acid	✓	✓	No	No	No
10	27.05	C22:6 Docosahexaenoic acid	✓	✓	No	No	No
11	28.04	C22:1 Docosenoic acid	✓	No	✓	No	No

✓ means GC chromatogram was observed at the peak of fatty acid profile of microalgae.
No means GC chromatogram was not observed at the peak of fatty acid profile of microalgae.

The GC chromatogram of fatty acid profiles in microalgae showed that both Amphora sp. and Chaetoceros sp. comprised essential fatty acids ω3, including 22:6n-3 (DHA) and 20:5n-3 (EPA) [5-7]. The PUFA content varied among species, probably due to differences in the distributions and accumulations of lipid classes in their intracellular structures [29,30]. Only Amphora sp. was dominated by linolenic acid (C18:3) - an essential fatty acid providing cardiovascular-protective, anti-cancer, neuro-protective, anti-osteoporotic, anti-inflammatory, and antioxidative effects [31] for human health. Therefore, *Amphora* sp. and *Chaetoceros* sp. might be suitable as beneficial materials for various applications in animal feed with high nutrition and, following the fatty acid profile, these two species are the most interesting species for further use. The biochemical constituents of these microalgae were different from those found in previous reports. An amount of the biochemical content, including polyunsaturated fatty acid, was found to correlate with a decrease in the environmental system [25,32,33]. Therefore, the data would be useful for the determination of environmental changes. Coastal pollution on the east coast of Thailand has caused negative changes in the ecosystem and environment, as well as problems in coastal land uses leading to concern about bad effects on the diversity of marine microorganisms in the ecosystem [5]. The study of microalgae and other microorganisms would provide useful biochemical profile data significant for the establishment of an effective strategic conservative plan.

Simultaneous monitoring of biochemical compound using conventional methods and FT-IR microspectroscopy

A small amount of each of the five marine microalgae, *Amphora subtropica*, *Chaetoceros muellerii*, *Melosira dubia*, *Bellerochae* sp., and *Lithodesmium* sp. of Bacillariophyceae, were simultaneously monitored for their biochemical compositions using conventional colorimetric methods and FT-IR, which required suitable spectra, as biomolecules are often recoverable. This study applied FT-IR

microspectroscopy in a reflection method with a few microalgae samples, whereby the samples were deposited on low-e slides [20,21]. Currently, FT-IR spectroscopy is an extremely useful technique to rapidly compare metabolic fingerprints in different algal species from different growth conditions [18,34]. Average fingerprint FT-IR spectra of five microalgae species recorded were between 4000 - 700 cm^{-1}, as shown in **Figure 2**. The calibration regions of biochemical macromolecules from each FT-IR fingerprint were measured, including lipids (3000 - 2800 cm^{-1}, 1740 cm^{-1}, and 1480 - 1330 cm^{-1}), proteins (1718 - 1479 cm^{-1}), and carbohydrates (1300 and 829 cm^{-1}), based on their chemical bonds within the functional groups of biochemical molecules with distinct vibrational properties, as shown in **Figure 2**.

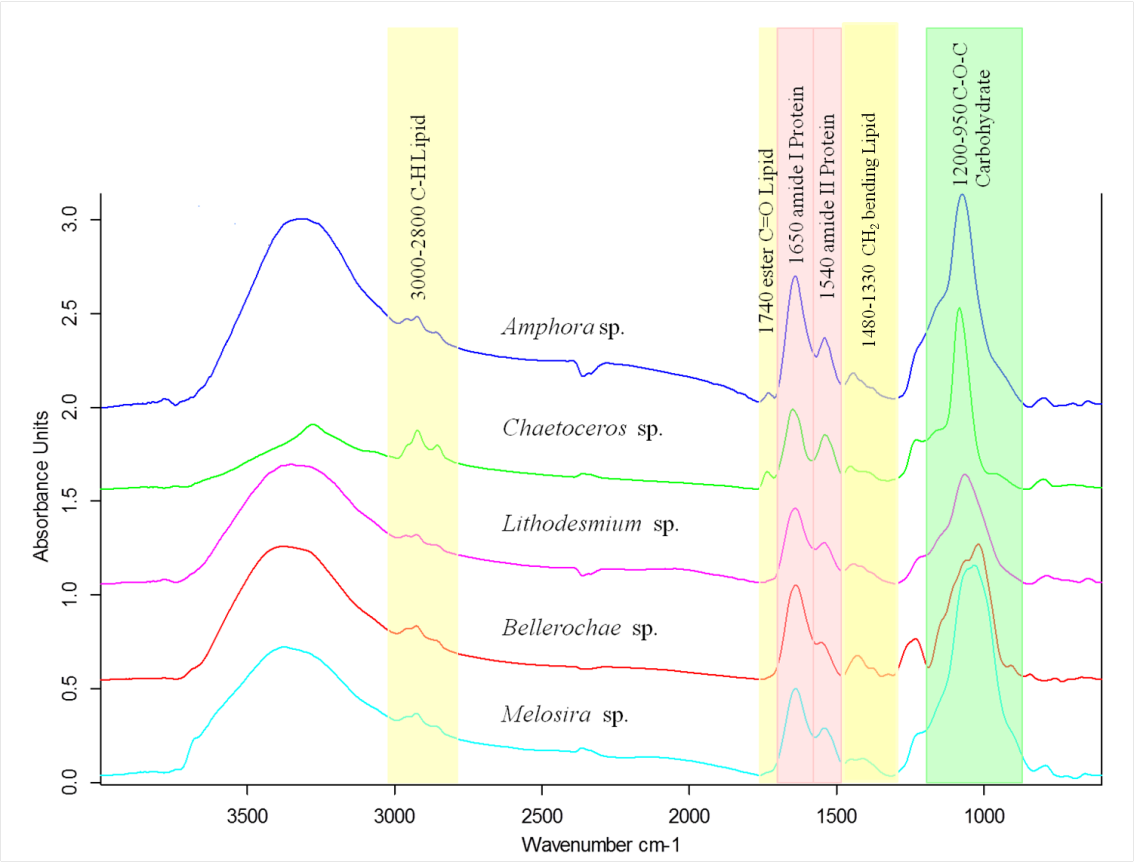

Figure 2 Analysis of five microalgae species using FT-IR microspectroscopy. Average FT-IR spectra obtained from *Amphora* sp., *Chaetoceros* sp., *Melosira* sp., *Bellerochae* sp., and *Lithodesmium* sp.

The presented data were further validated by a comparison of the biochemical contents, including lipids, carbohydrates, and proteins, obtained by the application of FT-IR microspectroscopy to colorimetric measurement. The FT-IR spectral data of different ranges of biochemical profiles were evaluated in order to reach the best prediction results. The best frequency regions, as presented in **Figure 2**, were selected for calibration between FT-IR and colorimetric measurement values for lipids, carbohydrates, and proteins contents. The overall data of microalgae spectra were used for calibration. The models for the determination of lipid, carbohydrate, and protein contents in microalgae were built by PLS regression using FT-IR spectra of the selected spectral regions in absorption modes. **Table 2** illustrates the correlation between colorimetric measurement and FT-IR values for the biochemical compounds in the microalgae samples from the calibration model using cross validation. The

conventional methods of biochemical profiles were determined; the lipids contents were determined by specific colorimetric SPV method. The results present actual data of the highest lipid contents in *Bellerochae* sp. and *Chaetoceros* sp., which were not significantly different ($p < 0.05$) of 65.7±2.0 and 62.7±1.7 % (w/w), similar to the predicted lipid contents, 65.2±3.6 and 61.0±6.4%. Meanwhile, the amount of carbohydrate contents presented were high in *Melosira* sp. significantly different from *Lithodesmium* sp., with values of 59.3±1.0 and 44.8±0.8 % (w/w), respectively ($p > 0.05$). The predicted carbohydrate contents were 58.7±2.9 and 42.7±3.8 %. The protein constituents in microalgae were measured by microbiuret method and found to be lower than those of lipid and carbohydrate contents. *Amphora* sp. and *Lithodesmium* exhibited the highest protein contents with values of 12.6±0.8 and 11.9±0.6 % (w/w), showing no significant difference ($p < 0.05$). Finally, the predicted protein contents were 12.6±0.6 and 11.3±0.7 %, measured by the infrared method (**Table 2**).

Table 2 Biochemical compositions of the selected microalgal species (in dry weight percentage) by using colorimetric measurement and FT-IR predicted value. All measurements represent independent analyses of a single batch of biomass in triplicate.

No.	Microalgae	Lipids		Carbohydrates		Proteins	
		% Lipid (SPV)	FTIR prediction	% Carb. (Anthrone)	FTIR prediction	% Protein (Micro-biuret)	FTIR prediction
1	*Amphora* sp.	38.0±2.0[a]	39.4±2.9 [a]	23.8±0.4 [a]	24.5±2.2 [a]	12.6±0.8 [a]	12.6±0.6 [a]
2	*Lithodesmium* sp.	45.5±1.2	44.2±2.9	44.8 ±0.8	42.7±3.8	11.9±0.6 [a]	11.3±0.7 [a]
3	*Melosira* sp.	39.4±1.6[a]	40.9±1.9 [a]	59. 3±1.0	58.7±2.9	6.3±0.1 [b]	5.4±0.4 [b]
4	*Chaetoceros* sp.	62.7.±1.7[b]	61.0±6.4 [b]	26.9±0.6 [a]	26.8±2.7 [a]	5.7±0.7 [b]	5.9±0.5 [b]
5	*Bellorechea* sp.	65.7.±2.0[b]	65.2±3.6 [b]	24.3±0.9 [a]	27.8±3.1 [a]	7.4±0.3	7.4±0.5

Data were shown as the mean ± SD, $n = 3$
Data with the same superscript [a, b] in the same column are not significantly different ($p < 0.05$)

As shown in **Figure 3**, the results of validation plots of PLS regression models showed good prediction based on the values of R^2, RMSECV, RPD, and bias, useful for distinguishing the accuracy of the FT-IR method compared to the colorimetric measurement in analysis of lipid, carbohydrate, and proteins contents (**Figure 2**). Cross-validation of the lipid, protein and carbohydrate models show high degrees of statistical accuracy, with RMSECV values of approximately 0.5 - 3.22 %, and the regression coefficients between the actual and predicted values (slope) of lipids, carbohydrates, and proteins were 92.66, 95.73, and 96.43 %, respectively (**Figure 3** and **Table 3**). The RPD values were all high (> 3), indicating good predictive accuracy [23,35]. The bias values of lipids, carbohydrates, and proteins were all < 0.01 %, indicating that the regression relationships were reliable [35].

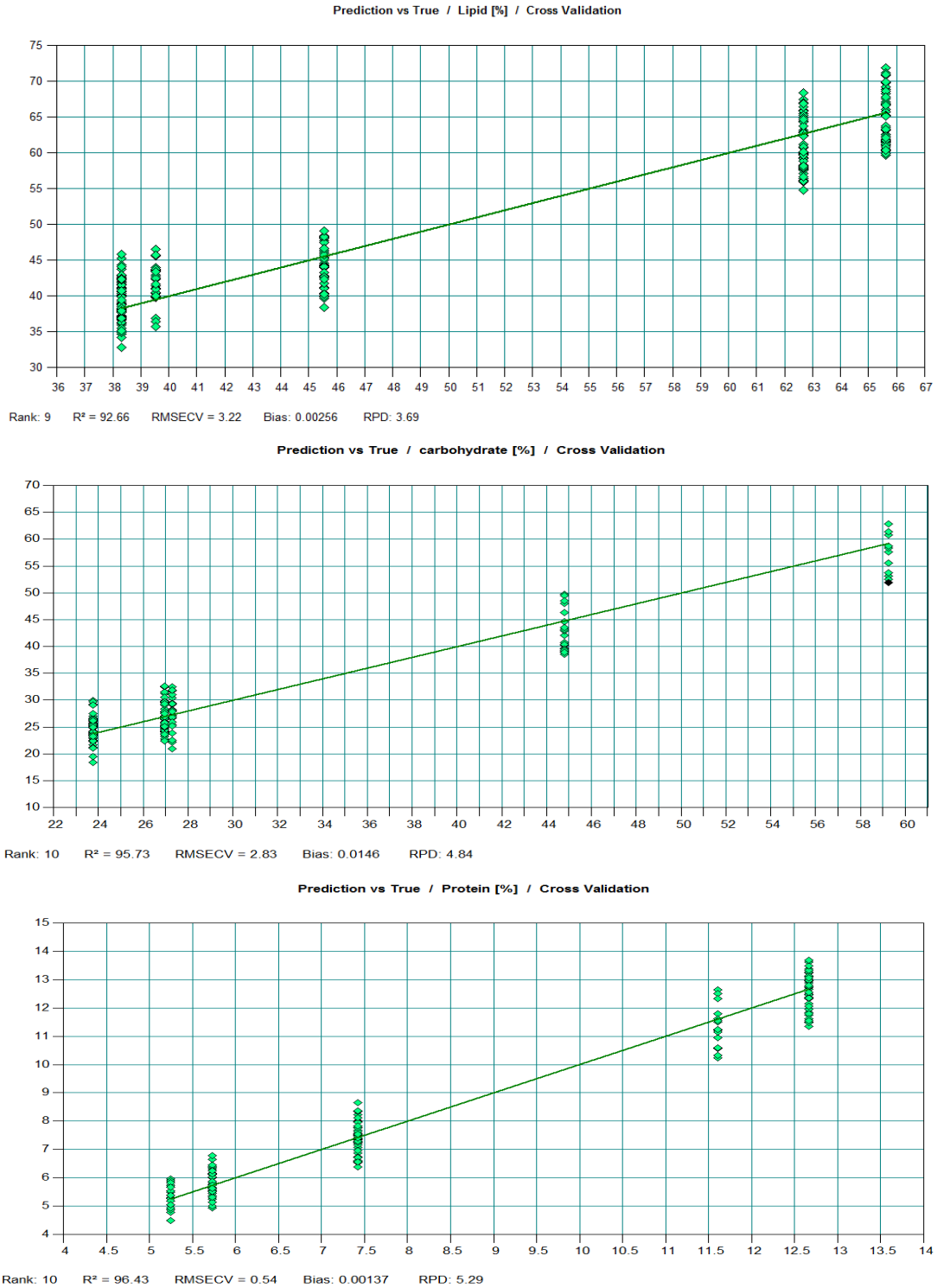

Figure 3 Cross validation plots of PLS regression models for the prediction of lipid, carbohydrate, and protein contents versus actual biochemical values of each biochemical component (% DW).

Table 3 Performance of statistical cross validation models for prediction of biochemical content in microalgae.

Components	R^2 (%)	RMSECV	bias	RDP
Lipids	92.66	3.69	0.00256	3.69
Carbohydrates	96.73	2.83	0.00146	4.84
Proteins	96.43	0.54	0.00137	5.29

R^2: coefficient of determination
RMSECV: root mean square error of cross-validation
RDP: residual predictive deviation

All results indicate that the infrared methods developed in this study could rapidly and simultaneously monitor and quantify changes in microalgal biochemical compositions in different microalgae species. Furthermore, the presented methods are not only restricted to microalgal cells but also to multicellular organisms, such as cancer cells, fungal cells, or bacterial cells, or in higher plants. One of the most well-known examples is the determination of oleic and linoleic acid contents in sunflower seeds using calibration between FT-IR and GC value based PLS regression methods [22]. Further studies may include the comparison of these methods in the evaluation of the fatty acid contents in microalgae and GC-MS. If these methods are able to detect living cells, it can be proved that the use of FT-IR spectroscopy can significantly improve the analysis of nutritional value and biochemical changes in the microalgae community.

Conclusions

In conclusion, polyunsaturated fatty acid profiles of five microalgae from the east coast of Thailand showed different fatty acid profiles and could probably be suitable as beneficial materials for various applications in animal food or as nutraceutical or pharmaceutical products based on the essential PUFA, especially for hexadecadienoic acid (C16:2), linoleic acid (18:3), eicosapentaenoic acid (EPA) (C20:5), and docosahexaenoic acid (DHA) (C22:6) in *Amphora* sp. and *Chaetoceros* sp. The application of FT-IR microspectroscopy for biochemical contents, including lipids, carbohydrates, and proteins, provided results which were statistically similar to conventional analyzing methods. The prediction models can determine the concentrations of lipids, carbohydrates, and proteins with reasonable accuracy and achieve a correlation of R^2 higher than 90 %. The results are also directly comparable with those from previous studies which applied conventional methods. However, relatively small sample volumes, and no toxic chemical reagents, were needed. The use of this approach should enable the more ready generation of the types of data required for the optimization of cultivation strategies and configuration of mathematic models describing the growth of microalgae. The next challenge is, thus, to speed up sample preparation to enable the approach to become closer to real-time capability, and this FT-IR technique might be used to classify microalgae species using PCA, multivariate data analysis. Additionally, the developed method could be easily used in algae industries for a rapid and reliable analysis of the biochemical content or fatty acid determination in microalgae.

Acknowledgements

This work was financially granted by Burapha University through the National Research Council of Thailand (Grant no. 2/2558) under the Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn. The authors would like to express their gratitude to the Institute of Marine Sciences, Burapha University, Synchrotron Light Research Institute (Public Organization); Nakhonratchasima, Faculty of Marine Technology, and Faculty of Pharmaceutical Sciences, Burapha University, Thailand, for their facility and staff support.

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