Heliyon

Received:
5 February 2019
Revised:
20 March 2019
Accepted:
14 April 2019

Cite as: Aline Terra Soares, Dayane Cristina da Costa, Armando Augusto Henriques Vieira, Nelson Roberto Antoniosi Filho . Analysis of major carotenoids and fatty acid composition of freshwater microalgae.
Heliyon 5 (2019) e01529.
doi: 10.1016/j.heliyon.2019.e01529

Analysis of major carotenoids and fatty acid composition of freshwater microalgae

Aline Terra Soares [a,*], **Dayane Cristina da Costa** [a], **Armando Augusto Henriques Vieira** [b], **Nelson Roberto Antoniosi Filho** [a]

[a] *Laboratório de Métodos de Extração e Separação (LAMES), Instituto de Química, Universidade Federal de Goiás, Campus II (Samambaia), 74690-900, Goiânia-GO, Brazil*

[b] *Laboratório de Ficologia, Departamento de Botânica, Universidade Federal de São Carlos, São Carlos, SP, Brazil*

[*] Corresponding author.

E-mail address: alinets30@gmail.com (A.T. Soares).

Abstract

Considering the nutraceutical properties, the high commercial value from pigments and essential lipids and the environmental sustainability, the purposes of this study were to assess the major carotenoids and fatty acids composition of nine microalgae species as a source of nutraceutical compounds and as fatty raw material for biodiesel production. The carotenoid and fatty acid content were analyzed by high performance liquid chromatography tandem mass spectrometry detection method with atmospheric pressure chemical ionization mode (HPLC/APCI-MS/MS) and by high resolution gas chromatography with flame ionization detector (GC-FID). For the carotenoid analysis, the developed method presented a rapid response, a good chromatographic separation, higher sensitivity and can provides more compounds information due the mass spectrum. Among the microalgae evaluated, *Desmodesmus protuberans* (10.3 mg g^{-1}), *Desmodesmus denticulatus* var. *linearis* (8.43 mg g^{-1}) and *Chlamydomonas planctogloea* (7.4 mg g^{-1}) are good lutein sources. *Coelastrum sphaericum* (15.29 mg g^{-1}) and *Parachlorella kessleri* (22.96 mg g^{-1}) showed high astaxanthin content; the others microalgae species presents low carotenoid content. In addition, *Chlorella zofingiensis* provides high quantities of γ-linolenic acid (4.3%). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were lower than 1.1 %. Regarding for

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

biodiesel production, the promising strains are *Coelastrum sphaericum* and *Parachlorella kessleri*.

Keywords: Natural product chemistry, Analytical chemistry

1. Introduction

Over the last decades, the use of microalgae biomass has gained significant importance: they are source of a wide range of highly valuable products, including lipids, pigments, proteins, polysaccharides and phycotoxin, with potential interest for human consumption as food and as medicines. Pigments and essential fatty acids have received great attention from the researchers, especially those extracted from natural sources, as the microalgae. These compounds exhibit pharmaceutical and nutraceutical properties and are considered a safe alternative to some existing synthetic medicines, due to their effectiveness in treating different diseases. In addition, they have high commercial potential for the production of pharmaceuticals, medicinal, foods, and cosmetics (Talero et al., 2015; Suleria et al., 2016; Sathasivam et al., 2017; Tzanova et al., 2017; Wang et al., 2018).

Many microalgae species produce polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are important compounds for the treatment of different human pathologies (Talero et al., 2015; Sathasivam et al., 2017). Furthermore, these microorganisms are rich in triacylglycerides, which can be converted into fatty acids methyl esters (FAME) that can be used as feedstock for biodiesel production. This renewable fuel is less polluting than petroleum diesel, being a eco-friendly fuel (Menezes et al., 2013; Soares et al., 2013).

The most important pigments from a commercial and medicinal standpoint are the carotenoids. They are divided into two groups: (*i*) carotenes, which are molecules that have only hydrogen and carbon in their chemical structure, and (*ii*) xanthophylls, which are carotenes oxidized by the presence of oxygen. Both carotenes and xanthophylls can be found in their free form or esterified to fatty acids (Van Breemen et al., 2012; Amorim-Carrilho et al., 2014). The qualitative and quantitative analysis of carotenoids is difficult and challenging due to their instability in the presence of light, heat, acids, and oxygen, as well as their similar structures. Such compounds are susceptible to oxidation reactions, isomerization, cyclization, hydrogenation, among others, which leads to their structure modification (Amorim-Carrilho et al., 2014).

Chromatographic methods are powerful analytical tools to identify and quantify carotenoids and FAME. Liquid chromatography (LC) is the most used technique to separate and analyze pigments, in a fast, specific, sensitive, and precise way

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(Rivera et al., 2011). Coupled with ultraviolet/visible (UV/Vis) and mass spectrometry (MS) detector, it presents sensitivity and selectivity in carotenoid analysis for several matrices. The gas chromatography coupled to flame ionization detector (GC-FID) is a powerful tool to detect and characterize FAME. These substances are thermally stable (Soares et al., 2013, 2014) and it is the most applied method for the FAME analysis in vegetables, fruits, animal and microorganisms (Cossignani et al., 2011; Menezes et al., 2016; Blasi et al., 2017; Gouveia et al., 2017).

The present work aimed to identify and quantify the major carotenoids in nine microalgae species by an analytical method using mass spectrometry with atmospheric pressure chemical ionization mode (APCI-MS/MS). Also, determine the FAME composition by gas chromatography and to predict the potential species as a source of essential fatty acids and as a feedstock for the production biodiesel.

2. Materials and methods

2.1. Materials

Methanol, acetonitrile and dichloromethane were purchased from J.T. Baker (Tokyo, Japan). Ethanol, hexane and sodium sulfate were acquired from Neon Analytical Reagents (Suzano, Brazil). Deionized water was made using an Elix water purification system (Millipore, Billerica, MA, USA). The reference standards *trans*-canthaxanthin (≥95%), *trans*-astaxanthin (≥97%), β-cryptoxanthin (≥97%), *trans*-lutein (≥97%), *trans*-fucoxanthin (≥95%), *trans*-β-apo-8′-carotene (apocarotenal - ≥ 96%; internal standard), *trans*-β-carotene (≥95%) and *trans*-α-carotene (≥95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polytetrafluoroethylene sterile membrane (PTFE − 0.22 μm × 25 mm) from Nova Analítica (São Paulo, Brazil).

2.2. Microalgae sample

The microalgae species evaluated were: *Chlamydomonas planctogloea*, *Eutetramorus fottii*, *Chlorella zofingiensis*, *Selenastrum bibraianum*, *Desmodesmus protuberans*, *Desmodesmus denticulatus* var. *linearis*, *Coelastrum sphaericum*, *Parachlorella kessleri* and *Mougeotia* sp. The freshwater microalgae were isolated, identified, cultivated, lyophilized and courtesy from Federal University of São Carlos. The culture were grown in borosilicate glass cylindrical bottles containing 1.8 L of the WC culture medium (Guillard and Lorenzen, 1972) at pH = 7 and 23 ± 1 °C with constant stirring by atmospheric air bubbling enriched with 4% CO_2 at 0,1 L min^{-1} and lighting of 200 μmol photons m^{-2} s^{-1} with photoperiod (12:12). The biomasses were centrifuged, frozen and lyophilized.

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2.3. HPLC conditions and APCI-MS/MS detection

HPLC/APCI-MS/MS (Agilent Technologies, Santa Clara, CA, USA, 1290 Infinity series) including a quaternary bomb, degasser, autosampler (maintained at 10 °C), column temperature controller and needle wash function operated before and after the injection for 15 seconds. The detector was 3200 Qtrap linear ion trap quadrupole mass spectrometry, from Sciex (Ontario, Canada), equipped with a Turbo V^{TM} ion source operated in the positive APCI ionization mode. Analyst software (Version 1.5.2) was used for data acquisition and MultiQuant software (version 2.1) for data analysis.

For detection and quantification, the following APCI conditions were applied: (i) ion source temperature - 450 °C; (ii) curtain gas (CUR) - 20; (iii) nitrogen - 20 psi; (iv) collision gas (CAD) - high; (v) nebulizer current (NC) - 4 μA; (vi) entrance potential (EP) - 10; (vii) ion source gas (GS1) - 45 L mim^{-1}; (viii) channel electron multiplier (CEM) - 2100. The selected ions were monitored by MRM (multiple reaction monitoring) with a dwell time of 150 ms for each Q1-Q3 transitions.

After several trial studies, a mobile phase system of methanol/acetonitrile/water (84:14:2, v/v/v) (A) and dichloromethane (B) was found appropriate with the gradient conditions being 100% A and 0% B for 11 min, decreased to 95% A in 18 min, 90% A in 21 min, 85% A in 24 min, 80% A in 27 min, 75% A in 35 min, 50% A in 40 min, 75% A in 41 min, returned to 100% A in 43 min and maintained for 7 min. The total analysis was 50 min. The injection volume was 20 μL, the column temperature controller was set at 40 °C and the flow rate of 1 mL min^{-1}. The chromatographic separation was done using YMC C30 column (250 mm × 4.6 mm I.D. x 5μm) manufactured by Waters (Milford, MA, USA).

2.4. APCI-MS/MS optimization

Each methanolic analyte solution (10 ppb) in a mixture with eluent A-methanol/acetonitrile/water (84:14:2, v/v/v) was injected under direct infusion with a syringe pump coupled a T part to HPLC pump and the APCI source, in order to: (i) confirm the presence of the molecular ion; (ii) determine the predominant fragments formed in Q1 and (iii) optimized the compound-dependent parameters. The APCI source was operated in positive and negative mode and the optimal values (fragmentation and signal intensity) was selected to optimize the compound fragmentation and mass spectrometry conditions (CAD, CUR, NC, EP and temperature - item 2.2). The syringe pump flow rate was 10 μL min^{-1} and for quaternary HPLC pump was 200 μL min^{-1}. The mass spectrum for each carotenoid was obtained in Q1 MS scan type (1000 Da s^{-1}) with cycle of 10. Next, the declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) for each transition Q1-Q3 of each standard carotenoid solution was optimized. For the multiple reaction

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

monitoring (MRM) method was used a dwell time of 150. The most abundant transition was selected for quantification and the others for the compounds identification.

2.5. Validation parameters for HPLC/APCI-MS/MS method

The calibration curves were prepared based on internal standardization method using apocarotenal as internal standard (IS) at 10 μg mL^{-1} in methanol. For lutein, astaxanthin, α-carotene and cryptoxanthin the curves were prepared at concentrations of 5, 10, 20, 30, 40, 50 and 60 μg mL^{-1}; canthaxanthin with the concentrations of 1, 2.5, 5, 10, 20, 25 and 30 μg mL^{-1}; fucoxanthin at concentrations of 1; 2.5; 5; 10; 20; 25 and 35 μg mL^{-1}; and for β-carotene with the of concentrations of 5; 10; 20; 40; 60; 80 and 100 μg mL^{-1}. Each standard solution was injected in triplicate, and the mean peak area for each concentration was used. The standard curves were prepared by plotting concentration ratio (standard versus internal standard) against its area ratio. The regression equation and correlation coefficient (*r*) for APCI-MS/MS was used the MutiQuant software, version 2.1 (Sciex, Framingham, USA). (Anvisa, 2002; Thompson et al., 2002; INMETRO, 2010; AOAC, 2012; Magnusson and Örnemark, 2014; Ravisankar et al., 2015).

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined using three low concentrations of the carotenoid standards. Each concentration was injected into HPLC system three times. The concentrations for each analyte are demonstrated at Table 1. Each standard curve was prepared by plotting concentration against area. LOQ and LOD were calculated using Eqs. (1) and (2). S is the standard deviation of the intercepts and σ corresponds to the average value of the slope:

$$LOQ = 10 \text{ x } \frac{\sigma}{S} \quad (1)$$

$$LOD = 3 \text{ x } \frac{\sigma}{S} \quad (2)$$

The intra-day variability was carried out by five injections at the same day with the standard solutions at concentration of 4.0 μg mL^{-1} to β-carotene and lutein, 9.0 μg

Table 1. Carotenoids concentrations for LOD and LOQ determination.

Carotenoids	Concentration (μg mL^{-1})
Fucoxanthin	0.65−1.25−2.5
Astaxanthin	4.0−5.0−6.0
Lutein	2.0−3.0−4.0
Canthaxanthin	0.27−0.5−1.0
Cryptoxanthin	5.0−7.0−9.0
α-carotene	5.0−7.0−9.0
β-carotene	2.0−3.0−4.0

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

mL^{-1} to α-carotene and cryptoxanthin and 1.0; 2.5; 6.0 μg mL^{-1} to canthaxanthin, fucoxanthin and astaxanthin, respectively. Similarly, the inter-day variability was performed by measuring carotenoid concentrations on the 1st, 2nd and 3rd day with three determinations each day for a total nine determinations, using the standard concentration of 20 μg mL^{-1}. The precision assays were calculated according to Eq. (3) (Taverniers et al., 2004). RSD is the relative standard deviation, SD is the standard deviation and $\overline{X}$ is the determined average concentration:

$$RSD = \frac{SD}{\overline{X}} * 100 \quad (3)$$

2.6. Pigments extraction

The carotenoids were extracted from microalgae according to Soares et al. (2016). Approximately 500 mg of microalgae biomass was weighted in an erlenmeyer flask and 10.0 mL of the hexane:ethanol mixture (1:1) were added. This mixture was left in ultrasonic bath for 40 minutes at room temperature (23°C ± 2 °C). After that, 7.5 mL of Na_2SO_4 10% aqueous solution was added, shaken by hand for 30 seconds and left to rest until phases were separated. The upper phase, rich in carotenoids, was collected and saved in a round-bottom flask. This procedure was repeated for four times with the residue within erlenmeyer, totaling five extractions. The combined upper phases were evaporated in a rotatory evaporator (Quimis - Q344b2, Diadema, Brasil). The extracted was dissolved in 2.0 mL of methanol and filtered in a PTFE sterile membrane for HPLC analysis.

2.7. Quantification of FAME content produced by direct transesterification of the microalgal biomass

Direct transesterification of the biomass of microalgae samples was performed according Soares et al. (2014). The organic phase (upper) containing the FAME was collected and analyzed using gas chromatography. The chromatographic analysis of the FAME composition used an Agilent 7890 Gas Chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector, using a DB-WAX capillary column (30 m × 0.25 mm × 0.25 μm). The oven was operated with an initial temperature of 70 °C, heated at 10 °C min^{-1} until 240 °C, remaining at this temperature for 13 min, and then heated 5 °C min^{-1} to 250 °C. The injector temperature was maintained at 310 °C with an injection volume of 2 μL, in the split mode, with a split ratio of 10:1. The detector temperature was kept at 310 °C. Hydrogen was used as the carrier gas at a linear velocity of 42 cm s^{-1}, and nitrogen was used as the auxiliary make-up gas at 20 mL min^{-1}. The FAME were identified by direct comparison with oil samples of known composition (soybean, canola, and crambe) through the injection of FAME reference standards (Nu-ChekPrep®, code 17A and 19A) and analyses via high-resolution gas chromatography coupled

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

with mass spectrometry (HRGC-MS), as described in previous paper by Soares et al. (2014).

3. Results and discussion

3.1. APCI-MS/MS optimization and HPLC development

To investigate the MS/MS performance with APCI ionization source for detection and quantification of carotenoids, preliminary studies were done using the direct infusion method of the system by injecting each standard solution in a full scan acquisition, in both positive and negative modes. The data showed that the signal output in positive mode analyses was better than negative mode. Additionally, low in-source fragmentation was observed in negative mode, compared with their corresponding spectra in positive mode. Thus, the subsequent MS/MS experiment and compound parameters were optimized in positive mode. The mass spectra in the positive mode from the carotenoids are demonstrated on Supplementary Material.

During the analysis, the MS/MS parameters for CUR, CAD, NC, and EP barely alter the protonated molecular ion signal intensity, thus the values were 20, high, 4 μA, and 10, respectively. The source temperature ranged from 400 to 450 °C; however, most carotenoids presented better signal intensity at 450 °C, and this value was used for all analyzes. The resulting transitions (Q1-Q3) per analyte and respective settings optimized to the parameters of DP, CE, and CXP are given in Table 2. The quantitative and qualitative analyzes were carried out in MRM mode, by monitoring the m/z transitions to the precursor ion. The most intense signal was used for the quantification and the less intense transitions for the compound confirmation.

After the MRM mode optimization via infusion, the chromatographic separation condition was adjusted. The chromatographic separation was developed using a standard carotenoid mixture and comparing the separation for the substances studied. Initially a gradient solvent system of methanol/acetonitrile/water (84:14:2, v/v/v) and methylene chloride developed by Soares et al. (2016) was used to separate the carotenoid mixture using a C30 column. For this, four different flow rates were evaluated (0.4, 0.6, 0.8, and 1 ml min^{-1}). The peak chromatographic separation and the analysis time were influenced by the flow rate. Using a low flow rate, the peak separation was impaired and the time analysis was longer; the peaks resolutions were also affected. For further separation, a flow rate of 1.0 ml min^{-1} was used.

Next, the effect of column temperature was studied at 10, 20, and 40 °C. The maximum column temperature recommended by the manufacturer is 40 °C, and this showed better separation efficiency for the investigated carotenoids. On the other

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 2. Positive ion APCI tandem mass with MRM parameters of the Q1/Q3 transitions. Da = daltons.

Carotenoids (Molecular Mass - Da)	Retention Time (min)	Q1 Mass (Da)	Q3 Mass (Da)	DP	CE	CXP
Fucoxanthin (658.906)	7.31	659.500	567.200	46.0	26.0	9.0
		659.500	581.600	52.0	27.0	8.0
		659.500	641.600	40.0	20.0	8.0
Astaxanthin (596.838)	15.29	597.600	379.200	66.8	17.0	5.0
		597.600	473.200	30.0	32.0	5.0
		597.600	505.000	47.0	28.0	5.0
		597.600	561.500	62.0	16.0	5.0
		597.600	579.100	43.0	21.0	5.0
Lutein (568.871)	17,37	569.200	551.300	87.0	20.0	19.0
		551.300	533.400	63.4	20.0	7.0
		551.300	495.500	54.6	20.0	7.0
		551.300	459.300	47.4	26.2	4.8
		551.300	429.000	53.3	23.1	4.8
Apocarotenal (416.638)	21.36	417.300	333.200	50.0	20.0	8.2
		417.300	347.300	59.0	23.0	3.2
		417.300	399.200	60.0	14.0	4.4
Canthaxanthin (564.840)	23.11	565.600	427.400	64.2	18.0	18.8
		565.600	413.000	67.3	23.9	4.7
		565.600	363.400	44.8	21.7	6.8
		565.600	361.100	59.9	23.8	3.5
		565.600	459.200	55.6	25.0	17.0
Cryptoxanthin (552.872)	29.42	553.500	535.400	77.0	16.9	5.9
		553.500	385.000	88.9	18.0	9.0
		553.500	331.000	58.8	16.2	14.0
		553.500	347.200	66.8	23.9	5.8
α-carotene (536.873)	34.64	537.600	177.300	55.0	30.0	8.0
		537.600	321.100	45.5	26.3	20.9
		537.600	347.200	67.5	29.2	35.0
		537.600	413.400	56.4	25.7	22.0
β-carotene (536.873)	37.45	537.600	177.300	55.0	30.0	8.0
		537.600	321.100	57.8	28.0	8.0
		537.600	413.500	66.5	27.0	3.0

hand, the best carotenoid separation obtained by Huck et al. (2000) with a Phenomenex C18 column (250 × 2 mm, 5 μm) in isocratic gradient was at a temperature of 21 °C. In these column and gradient conditions, at temperatures higher than 34 °C, the zeaxanthin and lutein were not easily separated.

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

In addition, the gradient of the mobile phase was evaluated for the separation of the standard carotenoid mixture. The optimal proportion decreased an elution time to 50 minutes; comparing with a previous study (Soares et al., 2016), the chromatography conditions analysis reduced 10 minutes, which saves solvent. Fig. 1 shows the chromatogram obtained, the carotenoids peaks were adequately resolved.

3.2. HPLC/APCI-MS/MS validation parameters

The chromatography separation by HPLC/APCI-MS/MS demonstrated that the techniques can be used to identify and quantify carotenoids, due to excellent response for each component, with well separated chromatographic peaks, which indicated a strong selectivity of the carotenoids. In addition, the chromatographic conditions ensure good specificity, as each pigment identification was not affected by the presence of the other compounds, because a small amount of cis compounds can be reliably detected.

For MS/MS detector, the *trans* astaxanthin and *cis* canthaxanthin peaks are overlapping at 15.62 min; however, they could be differentiated using the mass spectrum extracted from this peak. It is possible to identify two peaks with different spectrums, one in 15.62 min characteristic of astaxanthin and another in 15.9 min of canthaxanthin. When the *trans* canthaxanthin peak was extracted at retention time of 23 min,

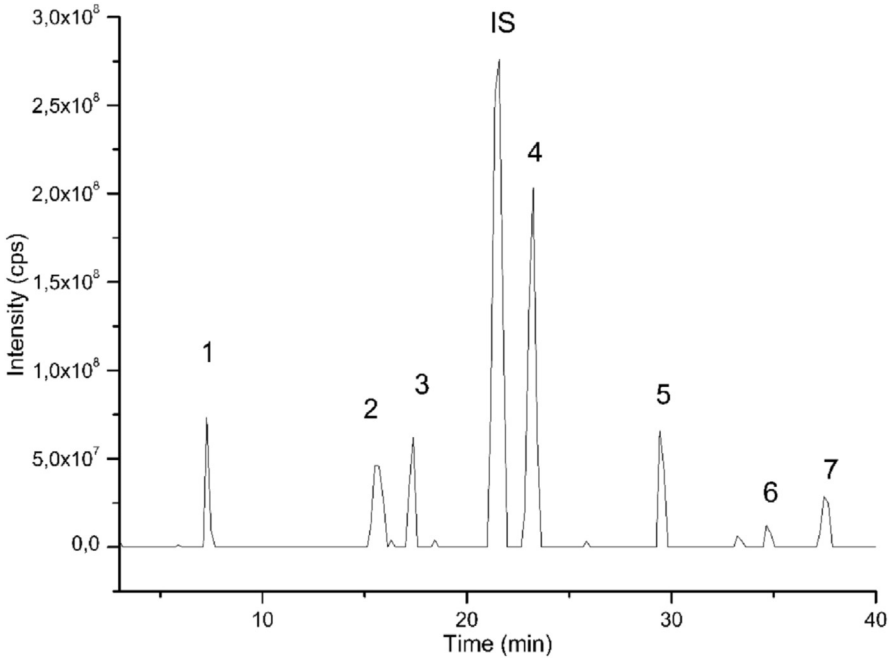

Fig. 1. Chromatogram obtained by HPLC/APCI-MS/MS in positive mode from the carotenoid standards mixture. 1 = *trans*-fucoxanthin (50 ppm/); 2 = *trans*-astaxanthin (100 ppm); 3 = *trans*-lutein (100 ppm); IS = internal standard (86 ppm); 4 = *trans*-canthaxanthin (40 ppm); 5 = *trans*-cryptoxanthin (100 ppm); 6 = *trans*-α-carotene (100 ppm); 7 = *trans*-β-carotene (120 ppm).

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

three more peaks were shown in small amount, with retention time of 15.9, 17.85, 25.98 min. These three little peaks, presenting the characteristic ions fragments identified of *trans* canthaxanthin as well as the same Q1/Q3 transitions, indicate the presence of *cis* isomer forms of canthaxanthin and one *cis* compound overlapping with *trans* astaxanthin peak. The mass spectrometry selectivity is higher for multi-compound analysis, especially when using the MRM mode, even for analytes that co-elute (Elsinghorst et al., 2011) because each analyte is confirmed by more than one Q1/Q3 transition.

The values for LOD and LOQ for each carotenoid are showed in Table 3, along with the Q1/Q3 MRM transitions. Canthaxanthin showed the lowest LOD and LOQ, followed by fucoxanthin and lutein. Using the MS/MS, the LOD and the LOQ for most analytes were <0.29 μg mL^{-1} and <0.97 μg mL^{-1}, respectively, with the exception of cryptoxanthin.

The intra- and inter-day precision for each analyte was determined according to Eqs. (3) and (4), and are shown in Table 3. The inter-day precision was evaluated on three different days, and the repeatability by five same day injections. For the tandem MS detector, the inter-day precision was higher than intra-day for α-carotene, fucoxanthin, and lutein. According to Anvisa (2002), the RSD parameters cannot be higher than 5%, while AOAC (2012) determines the RSD acceptability as a function of the analyte concentration level. Hence, for an analyte in a concentration ranging from 1

Table 3. Limit of detection (LOD), quantification (LOQ), intra-day and inter-day precision, calibration curves and the correlation coefficients (*r*) for the working range of the seven carotenoids obtained by HPLC/APCI-MS/MS in the positive mode (Q1-Q3 highest intensity transition).

Carotenoid	LOD	LOD	Intra-day precision		Inter-day precision		Linearity	
	μg mL^{-1}		CC*	RSD**	CC	RSD	curve	*r*
Fucoxanthin (659.5−641.6)	0.016	0.055	2.48	1.32	20.59	1.42	y = 0.02059x + 0.03555	0.9995
Astaxanthin (597.6−379.2)	0.202	0.672	6.21	4.77	21.17	3.97	y = 0.00426x + 0.39878	0.9900
Lutein (569.2−533.4)	0.082	0.272	4.06	2.13	21.12	3.31	y = 0.01042x − 0.01398	0.9989
Canthaxanthin (565.6−363.4)	0.004	0.014	1.03	2.77	19.52	1.64	y = 0.02103x + 0.52746	0.9904
Cryptoxanthin (553.5−347.2)	0.473	1.432	9.04	1.16	20.92	0.91	y = 0.00712x + 0.00945	0.9998
α-carotene (537.6−177.3)	0.290	0.968	8.96	1.32	19.81	3.06	y = 0.00173x − 0.00236	0.9952
β-carotene (537.6−413.1)	0.034	0.113	3.93	3.91	20.75	2.73	y = 0.00059x + 0.00027	0.9956

* Concentration calculated (CC, μg mL^{-1}).

** Relative standard deviation (RSD, %).

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ppm to 10 ppm, a RSD between 11% and 7.3% is acceptable. For concentrations between 10 ppm and 100 ppm, the maximum accepted value is between 7.3% and 5.3%, respectively (Taverniers et al., 2004). The RSD values found for the carotenoids are lower than the limits specified by the two parameters (ANVISA and AOAC). Therefore, the RSD value obtained for intra- and inter-day guarantees the precision of the quantification method for α-carotene, astaxanthin, β-carotene, canthaxanthin, cryptoxanthin, fucoxanthin, and lutein.

MS-MS/APCI calibration curve and the correlation coefficient (*r*) are reported in Table 3. The concentration working range chosen for the carotenoids shows a very strong linear relation with the analytical signal, with values of *r* than 0.99 for MS-MS/APCI. The AOAC (2012) recommend a correlation coefficient >0.99, according to Anvisa (2002), the linear correlation coefficient must be ≥0.98 and INMETRO (2010) determines r ≥ 0.90. Similar values were found for astaxanthin ($r = 0.9981$) and canthaxanthin (r = 0.9988) (Tzanova et al., 2017).

3.3. Quantitation of carotenoids in selected microalgae

Microalgae are a good source of bioactive compounds with anti-oxidant potential, such as carotenoids, that work as pharmacological molecules within therapeutic treatment/prevention of diseases (Talero et al., 2015; Suleria et al., 2016). In this context, new species of microalgae have been investigated as a natural source of medicinal substances. This study evaluated the carotenoid composition of nine microalgae. The calibration curves from MS-MS/APCI were used to quantify the carotenoids in microalgae (Table 4). The main sources of lutein are *Chlamydomonas planctogloea*, *Desmodesmus protuberans* and *Desmodesmus denticulatus* var. *linearis,* with lutein content in microalgae biomass of 7.4, 10.53, and 8.46 mg g^{-1}, respectively. These species can be used as a source of this pigment. Currently, Marigold flowers are the commercial source most used for the lutein production. The lutein production from dried flowers of Marigold *Calendula officinalis* varies from 0.04 to 0.301 mg g^{-1} (Lin et al., 2015). Therefore, these microalgae can produce around 35 to 263 times more lutein. Nevertheless, no carotenoid profile was found for these microalgae species in the literature. Taking as reference the same genus *Desmodesmus*, in Soares et al. (2016) when the carotenoids were extracted with hexane:ethanol, the lutein yield was 17% of the total carotenoids in the microalga biomass. When the biomass saponification was carried out with potassium hydroxide followed by solvent extraction, the lutein amount was 47.4%. Xie et al. (2016) also using the basic saponification, and the lutein content accounted for about 50% of total carotenoids.

Parachlorella kessleri, *Coelastrum sphaericum* and *Chlorella zofingiensis* are a natural source of astaxanthin. Although the astaxanthin is predominantly in the ester form, *C. sphaericum* presents astaxanthin in free form, which, based on the weight

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 4. Carotenoids composition in selected microalgae species obtained by HPLC/APCI-MS/MS in the positive mode.

Microalgae	Content (mg g^{-1})						
	Lutein	**Astaxanthin**	**Cryptoxanthin**	**Fucoxanthin**	**Canthaxanthin**	**β-carotene**	**α-carotene**
Chlamydomonas planctogloea	7.4	0.14	0.99	-	1.49	-	-
Eutetramorus fottii	1.72	-	-	-	0.38	-	-
Chlorella zofingiensis	0.49	5.65	0.11	-	0.18	0.29	0.09
Selenastrum bibraianum	1.73	0.41	1.34	0.41	0.03	0.16	0.08
Desmodesmus protuberans	10.53	-	-	-	0.14	-	-
Desmodesmus denticulatus var. *linearis*	8.46	-	-	0.07	-	0.40	0.21
Coelastrum sphaericum	2.75	15.29	0.42	-	0.21	0.11	-
Parachlorella kessleri	1.40	22.96	0.26	-	-	-	-
Mougeotia sp.	1.56	3.48	0.73	0.92	-	0.14	-

https://doi.org/10.1016/j.heliyon.2019.e01529

2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

of lyophilized biomass, corresponds to 6.47 mg g^{-1}. The commercial microalgae *Haematococcus pluvialis*, which is the main source used to produce astaxanthin, exhibits 5% of free astaxanthin (Ranga et al., 2009). The ester form was confirmed by the presence of the protonated molecular ion for the fatty acid palmitic (*m/z* 257.2) and oleic (*m/z* 283.1) in the mass spectrum. The highest yield of astaxanthin was from the microalga *P. kessleri*, almost 23 mg g^{-1}. Considering only the astaxanthin and lutein production based on the weight of biomass, the astaxanthin yield is around 16 times higher than lutein; however, Minhas et al. (2016) mentioned that astaxanthin productivity per day of *P. kessleri* is about two times higher than lutein productivity. The astaxanthin content in *C. zofingiensis* showed 5.65 mg g^{-1} per biomass, similar to that reported by Pelah et al. (2004), who mentioned that the cultivation of *C. zofingiensis* under stress conditions may accumulate 70% of astaxanthin and 30% of canthaxanthin and that the estimated ratio between canthaxanthin and astaxanthin content was 0.558. Here, this ratio was 0.032, because the microalga was cultivated under low light irradiance and low salinity content. Compared with shrimp samples, raw and cooked shrimp present astaxanthin content of 0.42 and 0.664 mg g^{-1}, respectively (Sanches-Silva et al., 2013). In eggs of rainbow trout species, the astaxanthin content was 0.17×10^{-3} mg g^{-1} (Tzanova et al., 2017). Thus, the microalgae studied in this work are promising astaxanthin sources.

The other microalgae species had low yield of carotenoids, but among the species evaluated, *S. bibraianum* (Fig. 2) had the highest content of cryptoxanthin (1.34 mg g^{-1}); *Chlamydomonas planctogloea* for canthaxanthin (1.49 mg g^{-1}) and *Mougeotia* sp. for fucoxanthin (0.92 mg g^{-1}). Lutein has a zeaxanthin isomer. The lutein spectrum was differentiated from its isomer by the intensity between the molecular protonated ion m/z 569 and the fragment m/z 551. For lutein, the m/z 551 fragment is more abundant than the protonated molecular ion m/z 569, whereas zeaxanthin exhibits the opposite profile (Rivera et al., 2011).

Regarding to carotenes, the levels of α-carotene and β-carotene were below of 0.40 mg g^{-1}. Considering the marine microalga *Dunaliella salina*, which is used for the β-carotene commercial production, it produces on average 5−10 mg per gram of dry weight (Deli et al., 2014), in this sense the microalgae evaluated in this study presents low yield in these carotenoids.

3.4. FAME analysis from the microalgae

The chromatogram obtained by GC-FID of the microalga *C. planctogloea* is demonstrated in Fig. 3, while the fatty acid composition of the nine species of microalgae is shown in Table 5. The chromatographic conditions that were established made it possible to highlight and separate fatty acid methyl esters with 14−24 carbon atoms such as those found in vegetable oils and animal fats in analyses lasting up to 30 minutes. However, the fatty acid content of conventional vegetable oils and animal fats,

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

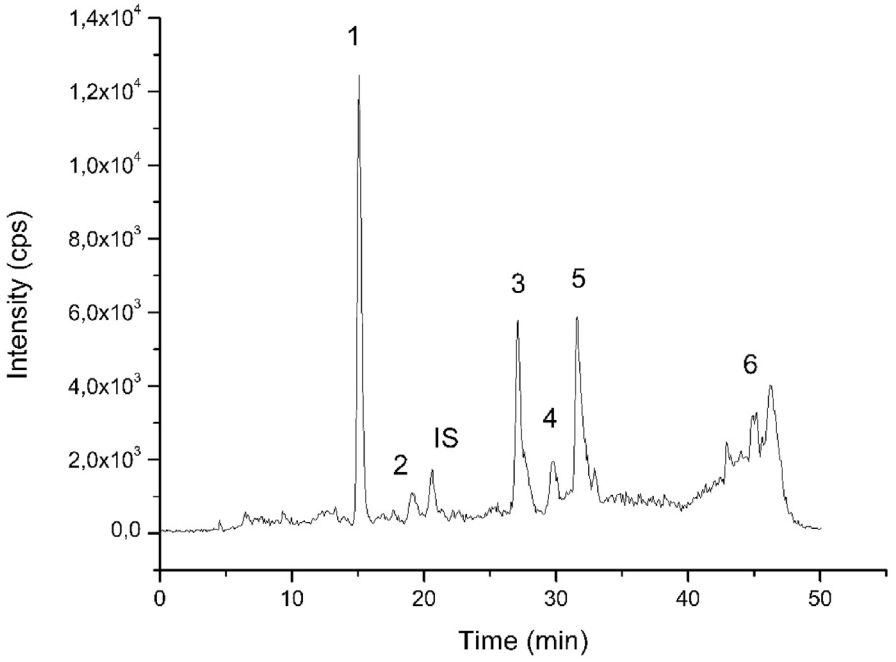

Fig. 2. Chromatogram obtained by HPLC/APCI-MS/MS in positive mode from the microalgae *Selenastrum bibraianum*. 1 = *trans*-lutein; 2 = astaxanthin; IS = internal standard; 3 = *cis*-lutein; 4 = cryptoxanthin; 5 = α-carotene; β-carotene and canthaxanthin ester; 6 = fucoxanthin ester and canthaxanthin ester.

and even of microalgae of the same class varies qualitatively and quantitatively (Cossignani et al., 2017; D'Alessandro et al., 2018; Bertoldi et al., 2019).

The essential fatty acids are precursors of prostaglandin E1, a major active biological compound required to reduce inflammation and blood pressure (Ronda and Lele,

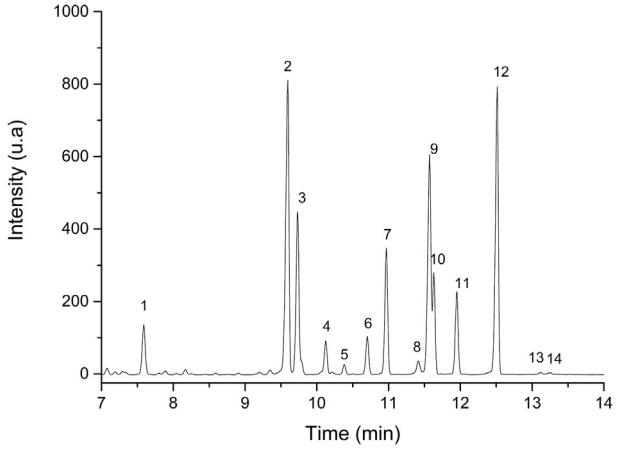

Fig. 3. Chromatogram obtained by GC/FID from *Chlamydomonas planctogloea* by direct biomass transesterification. Legend: peak 1: C14:0/ peak 2: C16:0/ peak 3: C16:1 *c*9/ peak 4: C16:2 *c*7,10/ peak 5: C17:0/ peak 6: C16:3 *c*6,9,12/ peak 7: C16:4 *c*6,9,12,15/ peak 8: C18:0/ peak 9: C18:1 *c*9/ peak 10: C18:1 *c*11/ peak 11: C18:2 *c*9,12/ peak 12: C18:3 *c*9,12,15/ peak 13: C20:0/ peak 14: C20:1 *c*9.

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

https://doi.org/10.1016/j.heliyon.2019.e01529

2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 5. FAME composition obtained by direct transesterification of microalgae biomass grown in WC medium.

FAME	Fame composition (%)								
	Chlamydomonas planctogloea	*Eutetramorus fottii*	*Chlorella zofingiensis*	*Selenastrum bibraianum*	*Desmodesmus protuberans*	*Desmodesmus denticulatus*	*Coelastrum sphaericum*	*Parachlorella kessleri*	*Mougeotia sp.*
C14:0	3.5	1.2	0.6	2.4	1.4	1.9	0.5	6.6	2.3
C16:0	22.4	27.5	31.9	23.4	28.0	21.9	31.6	14.7	32.8
C16:1 *c9*	11.5	2.8	1.6	0.9	2.4	1.8	0.4	0.3	2.6
C16:2 *c7,10*	2.4	7.8	1.6	1.1	1.6	1.5	0.3	1.0	1.6
C17:0	0.7	-	1.7	0.3	0.6	-	-	0.3	-
C16:3 *c6,9,12*	2.5	9.9	0.8	0.6	2.0	4.3	2.1	0.6	4.3
C16:4 *c6,9,12,15*	8.3	0.3	6.5	7.1	8.3	4.5	0.9	0.3	0.4
C18:0	1.1	1.7	0.9	1.6	3.6	2.7	2.7	3.8	1.6
C18:1 *c9*	16.7	8.2	16.0	27.4	21.6	33.6	38.0	44.0	16.2
C18:1 *c11*	5.6	-	9.9	-	-	-	-	-	-
C18:2 *c9,12*	5.4	22.3	12.3	11.3	7.9	6.7	11.5	13.3	11.2
C18:3 *c6,9,12*	-	-	4.3	0.2	0.9	0.7	0.4	0.2	1.1
C18:3 *c9,12,15*	19.7	18.1	9.7	20.4	19.0	16.8	7.6	11.2	25.1
C18:4 *c6,9,12,15*	-	-	1.5	2.2	2.7	2.4	1.9	1.2	0.8
C20:0	0.1	0.2	-	-	-	0.1	1.0	0.3	-
C20:1 *c9*	0.1	-	-	-	-	0.6	-	1.8	-
C20:5 *c5,8,11,14,17*	-	-	-	-	-	0.4	1.0	0.4	-
C22:6 *c4,7,10,13,16,19*	-	-	-	1.1	-	0.1	-	-	-
C24:0	-	-	0.7	-	-	-	0.1	-	-
SFA	27.8	30.6	35.8	27.7	33.6	26.6	35.9	25.7	36.7
MUFA	33.9	11	27.5	28.3	24.0	36	38.4	46.1	18.8
DUFA	7.8	30.1	13.9	12.4	9.5	8.2	11.8	14.3	12.8
TUFA	22.2	28	14.8	21.2	21.9	21.8	10.1	12	30.5
PUFA	8.3	0.3	8.0	10.4	11.0	7.4	3.8	1.9	1.2

Legend: SFA is the sum of the contents of saturated fatty acids; MUFA is the sum of the contents of mono-unsaturated fatty acids; TUFA is the sum of the contents of tri-unsaturated fatty acids; PUFA is the sum of the contents of polyunsaturated fatty acids.

2008). An exemple of these acids are: gamma linolenic acid (GLA - C18:3 *cis* 6,9,12), eicosapentaenoic acid (EPA - C20:5 ω3) and docosahexaenoic acid (DHA - C22:6 ω3). The highest amount of GLA was found in *C. zofingiensis* (4.3%), for EPA in *C. sphaericum* (1%) and DHA in *S. bibraianum* (1.1%). However, among the evaluated microalgae, only *C. zofingiensis* can be a promise from the standpoint of metabolic supplementation.

Considering the environmental sustainability, like biodiesel production, taking account that European legislation (European Committee, 2008) establishes a maximum of 12% for linoleic acid and 1% for polyunsaturated acids (PUFA) (EN 14214). In addition to linolenic acid, γ-linolenic acid (C16:3 *cis* 6,9,12), C16:3 and C17:3 may also be present in microalgae species (Soares et al., 2013; Menezes et al., 2016). Since these are similar to linolenic acid, they need to be taken into consideration. The 12% limit should include the sum of the levels of triunsaturated fatty acids rather than linolenic acid levels alone. *C. sphaericum* and *P. kessleri* have PUFA levels of 3.8% and 1.9%, respectively, also, TUFA levels of 10.1% and 12.0%, respectively, which gives these microalgae a fatty acid composition similar to that accepted by the European community. Also, these species presented a considerable amount of MUFA, 38.4% and 46.1%, respectively. To improve fatty acid composition, studies modifying culture conditions to reduce polyunsaturated acid content should be conducted.

Of the nine microalgae studied, *E. fottii* has demonstrated biodiesel production potential, with 0.3% of PUFA content. However, there is still a need to induce a reduction in the TUFA yield. This microalga showed the highest C16:3 content. Unlike, *C. planctogloea*, *C. zofingiensis*, *S. bibraianum*, *D. protuberans* and *D. denticulatus* are composed with PUFA yield higher than 7.4% of its total fatty acids. Analysis shows a high concentration of C16:4 *cis* 6,9,12,15 and C18:4 *cis* 6,9,12,15 was also found. These microalgae strains are not a suitable for biodiesel production.

4. Conclusion

The MS/MS-APCI detector is rapid and sensitive detector to determine and differentiate carotenoids, as well as has high specificity, due the mass spectrum obtained from the chromatographic peaks, which allows the elucidation of compound structure, even for peaks that co-elute. Among the evaluated microalgae species, for pigment production, *Desmodesmus protuberans*, *Desmodesmus denticulatus* var. *linearis* and *Chlamydomonas planctogloea* are good sources for lutein and *Coelastrum sphaericum* and *Parachlorella kessleri* for astaxanthin. Considering the benefits that the essential fatty acids offer to human health, *Chlorella zofingiensis* provides high levels of γ-linolenic. According to their fatty acids profiles and aiming at biodiesel production, the microalgae *Coelastrum sphaericum* and *Parachlorella*

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

kessleri are the most promising among the species of microalgae analyzed, by higher due to the composition of fatty acids that are forthcoming with the specifications laid down by the standard EN 14214.

Declarations

Author contribution statement

Aline Soares: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dayane C. da Costa: Performed the experiments.

Armando Vieira: Contributed reagents, materials, analysis tools or data.

Nelson R. A. Filho: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by the Ministry of Science, Technology and Innovation (MCTI) (FINEP (Agreement No. 01.10.0457.00) and CNPq (Case No. 407556/2013-3). Nelson R. A. Filho was supported by CAPES and CNPq (Case No. 312019/2013-0).

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2019.e01529.

References

Amorim-Carrilho, K.T., Cepeda, A., Fente, C., Regal, P., 2014. Review of methods for analysis of carotenoids. TrAC Trends Anal Chem 56, 49−73.

Anvisa, 2002. Resolução RE n°899 − Guia para validação de métodos analíticos e bioanalíticos. http://redsang.ial.sp.gov.br/site/docs_leis/vm/vm1.pdf. (Accessed 12 January 2018).

AOAC, 2012. Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals. https://www.aoac.org/aoac_prod_imis/

2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

AOAC_Docs/StandardsDevelopment/SLV_Guidelines_Dietary_Supplements.pdf. (Accessed 12 January 2018).

Bertoldi, D., Cossignani, L., Blasi, F., et al., 2019. Characterisation and geographical traceability of Italian goji berries. Food Chem. 275, 585–593.

Blasi, F., Montesano, D., Simonetti, M.S., Cossignani, L., 2017. A Simple and Rapid Extraction Method to Evaluate the Fatty Acid Composition and Nutritional Value of Goji Berry Lipid, pp. 970–979.

Cossignani, L., Blasi, F., Bosi, A., et al., 2011. Detection of cow milk in donkey milk by chemometric procedures on triacylglycerol stereospecific analysis results. J. Dairy Res.

Cossignani, L., Chiodelli, G., Ghisoni, S., et al., 2017. Changes in extra-virgin olive oil added with Lycium barbarum L. carotenoids during frying: chemical analyses and metabolomic approach. Food Res. Int. 105, 507–516.

D'Alessandro, E.B., Antoniosi Filho, N.R., Soares, A.T., et al., 2018. Viability of Biodiesel Production from a Thermophilic Microalga in Conventional and Alternative Culture media, vol. 7.

Deli, J., Gonda, S., Nagy, L.Z., et al., 2014. Carotenoid composition of three bloom-forming algae species. Food Res. Int. 65, 215–223.

Elsinghorst, P.W., Kinzig, M., Rodamer, M., et al., 2011. An LC–MS/MS procedure for the quantification of naproxen in human plasma: development, validation, comparison with other methods, and application to a pharmacokinetic study. J. Chromatogr. B 879, 1686–1696.

European Committee, 2008. Automotive Fuels. Fatty Acid Methyl Esters (FAME) for Diesel Engines. Requirements and Test Methods. EN 14214. 2003.

Gouveia, L., Oliveira, A.C., Congestri, R., et al., 2017. Biodiesel from Microalgae.

Guillard, R.R.L., Lorenzen, C.J., 1972. Yellow-green algae with chlorophyllid C. J. Phycol. 8, 10–14.

Huck, C.W., Popp, M., Scherz, H., Bonn, G.K., 2000. Development and evaluation of a new method for the determination of the carotenoid content in selected vegetables by HPLC and HPLC-MS-MS. J. Chromatogr. Sci. 38, 441–449.

INMETRO, 2010. Orientações sobre validação de métodos de ensaios químicos DOQ-CGCRE-008. http://www.inmetro.gov.br/Sidoq/Arquivos/CGCRE/DOQ/DOQ-CGCRE-8_01.pdf. (Accessed 12 January 2018).

Lin, J.-H., Lee, D.-J., Chang, J.-S., 2015. Lutein production from biomass: Marigold flowers versus microalgae. Bioresour. Technol. 184, 421–428.

https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Magnusson, B., Örnemark, U., 2014. The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics, second ed.

Menezes, R.S., Leles, M.I.G., Soares, A.T., et al., 2013. Evaluation of the potentiality of freshwater microalgae as a source of raw material for biodiesel production. Quim. Nova 36.

Menezes, R.S., Soares, A.T., Marques Júnior, J.G., et al., 2016. Culture medium influence on growth, fatty acid, and pigment composition of *Choricystis minor* var. *minor*: a suitable microalga for biodiesel production. J. Appl. Phycol. 28.

Minhas, A.K., Hodgson, P., Barrow, C.J., et al., 2016. The isolation and identification of new microalgal strains producing oil and carotenoid simultaneously with biofuel potential. Bioresour. Technol. 211, 556−565.

Pelah, D., Sintov, A., Cohen, E., 2004. The effect of salt stress on the production of canthaxanthin and astaxanthin by *Chlorella zofingiensis* grown under limited light intensity. World J. Microbiol. Biotechnol. 20, 483−486.

Ranga, R., Sarada, A.R., Baskaran, V., Ravishankar, G.A., 2009. Identification of carotenoids from green alga Haematococcus pluvialis by HPLC and LC-MS (APCI) and their antioxidant properties. J. Microbiol. Biotechnol. 19, 1333−1341.

Ravisankar, P., Naga Navya, C., Pravallika, D., Sri, D.N., 2015. A review on step-by-step analytical method validation. IOSR J. Pharm. 5, 2250−3013.

Rivera, S., Vilaró, F., Canela, R., 2011. Determination of carotenoids by liquid chromatography/mass spectrometry: effect of several dopants. Anal. Bioanal. Chem. 400, 1339−1346.

Ronda, S.R., Lele, S.S., 2008. Culture Conditions stimulating high γ-Linolenic Acid accumulation by Spirulina platensis. Braz. J. Microbiol. 39, 693−697.

Sanches-Silva, A., Ribeiro, T., Albuquerque, T.G., et al., 2013. Ultra-high pressure LC for astaxanthin determination in shrimp by-products and active food packaging. Biomed. Chromatogr. 27, 757−764.

Sathasivam, R., Radhakrishnan, R., Hashem, A., Abd_Allah, E.F., 2017. Microalgae metabolites: a rich source for food and medicine. Saudi J. Biol. Sci.

Soares, A.T., da Costa, D.C., Silva, B.F., et al., 2014. Comparative analysis of the fatty acid composition of microalgae obtained by different oil extraction methods and direct biomass transesterification. Bioenergy Res. 7.

Soares, A.T., Júnior, J.G.M., Lopes, R.G., et al., 2016. Improvement of the extraction process for high commercial value pigments from desmodesmus sp. microalgae. J. Braz. Chem. Soc. 27.

 https://doi.org/10.1016/j.heliyon.2019.e01529
2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Soares, A.T., Silva, B.F., Fialho, L.L., et al., 2013. Chromatographic characterization of triacylglycerides and fatty acid methyl esters in microalgae oils for biodiesel production. J. Renew. Sustain. Energy 5.

Suleria, H.A.R., Gobe, G., Masci, P., Osborne, S.A., 2016. Marine bioactive compounds and health promoting perspectives; innovation pathways for drug discovery. Trends Food Sci. Technol. 50, 44–55.

Talero, E., García-Mauriño, S., Ávila-Román, J., et al., 2015. Bioactive compounds isolated from microalgae in chronic inflammation and cancer. Mar. Drugs 13, 6152–6209.

Taverniers, I., De Loose, M., Van Bockstaele, E., 2004. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. TrAC Trends Anal. Chem. 23, 535–552.

Thompson, M., Ellison, S.L.R., Wood, R., 2002. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Appl. Chem. 74.

Tzanova, M., Argirova, M., Atanasov, V., 2017. HPLC Quantification of astaxanthin and canthaxanthin in Salmonidae eggs. Biomed. Chromatogr. 31, 1–8.

Van Breemen, R.B., Dong, L., Pajkovic, N.D., 2012. Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids. Int. J. Mass Spectrom. 312, 163–172.

Wang, N., Manabe, Y., Sugawara, T., et al., 2018. Identification and biological activities of carotenoids from the freshwater alga Oedogonium intermedium. Food Chem. 242, 247–255.

Xie, Y., Ho, S.-H., Chen, C.-N.N., et al., 2016. Disruption of thermo-tolerant Desmodesmus sp. F51 in high pressure homogenization as a prelude to carotenoids extraction. Biochem. Eng. J. 109, 243–251.

 https://doi.org/10.1016/j.heliyon.2019.e01529

2405-8440/© 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).