Short Communication

Investigation of major and trace element distribution in the extraction–transesterification process of fatty acid methyl esters from microalgae *Chlorella* sp.

Bruno M. Soares [a], Augusto A. Vieira [a], Juliana S. Lemões [a], Clarissa M.M. Santos [b], Márcia F. Mesko [c], Ednei G. Primel [a], Marcelo G. Montes D'Oca [a], Fábio A. Duarte [a,*]

[a] *Escola de Química e Alimentos, Universidade Federal do Rio Grande, 96203-900 Rio Grande, RS, Brazil*
[b] *Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil*
[c] *Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 96010-610 Pelotas, RS, Brazil*

ARTICLE INFO

ABSTRACT

This work reports, for the first time, the determination of major and trace elements (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, La, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Sn, Sr, Ti, Tl, U, and Zn) in the fractions of the synthesis of fatty acid methyl esters (FAMEs). These include fresh microalgae, residual biomass, lipid fraction, crude FAMEs, insoluble fraction and purified FAMEs from microalgae *Chlorella* sp. A microwave-assisted digestion procedure in closed vessels was applied for sample digestion and subsequent element determination by inductively coupled plasma-based techniques. The proposed method was suitable for the multielement determination in FAMEs and its fractions obtained from microalgae. The element concentration was compared with results found in the literature and a careful discussion about the use of residual biomass for different applications was performed.

© 2012 Elsevier Ltd. Open access under the Elsevier OA license.

1. Introduction

Microalgae have been recently reported for biodiesel production as one of the most promising biomass with potential to replace fossil fuels. The production of biodiesel from microalga oil involves the lipid extraction from microalga biomass followed by its conversion to fatty acid methyl esters (FAMEs) based on a transesterification process (D'Oca et al., 2011a). For the production of FAMEs, several species of microalgae with different amounts of lipids can be used, such as *Isochrysis* sp. (25–33% lipids), *Nannochloris* sp. (20–35% lipids), *Nannochloropsis* sp. (31–68% lipids), *Neochloris oleoabundans* (35–54% lipids), *Tetraselmis sueica* (15–23% lipids) and *Chlorella* sp. (28–32% lipids) (Chisti, 2007; Gog et al., 2012).

Several inorganic contaminants may occur in vegetable oils, mainly due to the absorption of some minerals from the soil. In addition, the origin of some elements in biodiesel is in the production process (Lepri et al., 2011). The occurrence of some elements in diesel engines can damage their performance and result in decomposition, formation of precipitates or corrosion of metallic parts. The monitoring of Na and K needs to be performed due to their ability to form undesirable substances in the engines (Edlund et al., 2002). Some elements such as Ca, Co, Cu, Fe, Mg, Mn, and

Ni can promote oxidative degradation and affect the quality and conservation of oil and fats directly (Gonzálves et al., 2010). Moreover, some elements such as Cu, Pb, and Zn can catalyze the biodiesel oxidation (Lepri et al., 2011). In Brazil, the Agência Nacional do Petróleo, Gás Natural e Biocombustíveis (ANP) has established the maximum limits for Na, K, Ca and Mg (5 mg kg^{-1}), P (10 mg kg^{-1}), and S (50 mg kg^{-1}) in biodiesel. However, the limits for other elements have not been established yet (Resolução ANP No. 7, 2008).

Trace element determination in edible oils and biodiesel has been performed mainly by spectrometric techniques (Lepri et al., 2011). Many problems have been associated to sample preparation procedures of organic matrices due to its complex nature (Antes et al., 2010; Pereira et al., 2009). Digestion procedures for elemental determination in edible oils and biodiesel, mainly by spectrometric techniques, have been applied to mitigate or eliminate organic content, reduce possible interference and make it easier to place the sample in the instruments (Lepri et al., 2011). Among the procedures reported for sample digestion of biodiesel and edible oils, the microwave-assisted digestion especially in closed vessels, have been described as successful tools (Flores et al., 2007).

The purpose of this study was to determine major and trace elements (Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, La, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Sn, Sr, Ti, Tl, U, and Zn) in all steps of the synthesis of FAMEs from microalgae *Chlorella* sp. by inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled

plasma mass spectrometry (ICP-MS) after microwave-assisted digestion. The determination was performed in *Chlorella* sp. biomass after the extraction–transesterification process and in other fractions such as residual biomass, lipid fraction, crude FAMEs, insoluble fraction and purified FAMEs. It is important to point out that no study with this focus was found in the literature. Therefore, this study enables a quite singular discussion about the occurrence of inorganic elements in the production of FAMEs from microalgae.

2. Methods

2.1. Instrumentation

A microwave sample preparation system (Multiwave 3000, Anton Paar, Graz, Austria) equipped with eight high-pressure quartz vessels (80 mL) was used for sample digestion. Temperature and pressure were controlled during the heating program (maximum pressure and temperature were 80 bar and 280 °C, respectively).

An inductively coupled plasma mass spectrometer (Perkin–Elmer, Model ELAN DRC II, Thornhill, Canada) and an inductively coupled plasma optical emission spectrometer with axial view configuration (Model Spectro Ciros CCD, Spectro Analytical Instruments, Kleve, Germany) were used for element determination. Instrumental performance and other operational conditions are described in Table 1. For ICP-MS and ICP OES determinations, argon 99.996% (White Martins–Praxair, São Paulo, Brazil) was used.

2.2. Reagents and standards

Water obtained from a Milli-Q system (Resistivity 18.2 MΩ cm, Millipore, Billerica, USA) was used to prepare all standard solutions. Concentrated nitric acid (Merck, Darmstadt, Germany) was doubly distilled in a model duoPUR 2.01E sub-boiling system (Milestone, Bergamo, Italy) and glass materials were soaked in 10% (m/v) HNO_3 for 48 h before use. A multielement stock solution containing 10 mg L^{-1} Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, La, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Sn, Sr, Ti, Tl, U, V, and Zn (SCP33MS, SCP Science, Quebec, Canada) was used to prepare reference solutions by sequential dilution in 5% HNO_3 (v/v), in the range of 0.025–10 µg L^{-1} for ICP-MS and 5–100 µg L^{-1} for ICP OES. Sulfur and phosphorus analytical solutions, both in the range from 0.1 to 2.5 mg L^{-1}, were prepared right before use by serial dilution of stock solutions containing 1000 mg L^{-1} (Spex CertiPrep, Metuchen, USA) and 326.1 mg L^{-1} (Merck) were used, respectively.

2.3. Samples

Microalga *Chlorella* sp. samples were commercially obtained from Galena Company and the other samples used in this work were obtained from synthesis; the procedure was similar to the one performed by D'Oca et al. (2011b). For the synthesis, 100 g dry biomass was mixed to 300 mL of the solvent system chloroform:methanol 2:1 in a 600 mL round bottom flask; then, the extraction was carried out for 120 min at room temperature (20 °C) under magnetic stirring (700 rpm). The mixture was filtered and the organic phase was evaporated under reduced pressure. After the lipid extraction, the reaction was carried out using H_2SO_4 as catalyst (3% in relation to the lipid mass) under constant stirring for 4 h at 60 °C. The molar ratio of alcohol:lipids was 30:1. When the reaction was completed, the excess of methanol was removed under reduced pressure and hexane was added. The mixture was vacuum filtered through a Büchner funnel. The filtrate was separated and the hexane was removed under reduced

Table 1
Operational parameters for element determination by ICP-MS and ICP OES.

Parameters	ICP-MS	ICP OES
RF power (W)	1400	1500
Plasma gas flow rate (L min^{-1})	15	14
Auxiliary gas flow rate (L min^{-1})	1.2	1.0
Nebulizer gas flow rate (L min^{-1})	1.15	0.9
Sampler and skimmer cones	Pt	–
Nebulizer	Concentric	Cross flow
Spray chamber	Cyclonic	Double pass–Scott type
Isotopes (m/z)	^{109}Ag, ^{75}As, ^{9}Be, ^{209}Bi, ^{111}Cd, ^{59}Co, ^{52}Cr, ^{65}Cu, ^{139}La, ^{7}Li, ^{55}Mn, ^{98}Mo, ^{60}Ni, ^{208}Pb, ^{82}Se, ^{120}Sn, ^{86}Sr, ^{46}Ti, ^{203}Tl, ^{238}U, ^{51}V, and ^{66}Zn	–
Wavelengths (nm)	–	Al (167.078), Ba (455.404), Ca (317.933), Cr (267.716), Cu (327.296), Fe (238.204), K (766.491), Mg (279.553), Mn (260.569), Na (589.592), P (177.495), S (180.731), Sr (421.552), Ti (334.187), and Zn (213.856)

pressure to obtain the crude product. The fatty acid methyl esters were purified in a silica gel/Al_2O_3 column to obtain pure FAMEs.

The determinations were performed in the following samples: microalga biomass; residual biomass; lipid fraction; crude FAMEs; insoluble fraction; and purified FAMEs.

2.4. Sample preparation procedure

Sample decomposition was performed by microwave-assisted digestion in high-pressure closed vessels. In this case, up to 500 mg sample were weighed in quartz vessels and 6 mL concentrated nitric acid was added and the following irradiation program was used: 1400 W (15 min of ramp), 1400 W for 10 min, and 0 W for 20 min (cooling step). After cooling, digests were diluted to 30 mL in polypropylene vessels with ultrapure water for further determination by ICP-MS and ICP OES. After each run, vessels were soaked in concentrated HNO_3 for 10 min followed by rinsing with ultrapure water.

A certified reference material (CRM) of peach leaves (NIST SRM 1547) was used for evaluation of method accuracy. All statistical calculations (95% significance level) were performed using the GraphPad InStat (GraphPad InStat Inc., Version 3.00, 1997) software.

3. Results and discussion

The total element concentration (means ± standard deviation, µg g^{-1}) was measured in the fractions obtained from the synthesis of FAMEs from microalgae *Chlorella* sp. Results are shown in Table 2. It is important to emphasize that most elements in this kind of samples were determined for the first time and that no study with this focus was found in the literature.

3.1. Microalga biomass

As can be seen in Table 2, results (for most elements) in the microalga *Chlorella* sp. samples were similar to those reported by Marín et al. (2010) and de la Rocha et al. (2009). However, for some elements, such as Al, Co, Cr, Fe, Ni, P, and Pb, the concentration was

Table 2
Results for element concentration in fractions obtained after the synthesis of FAMEs from microalgae *Chlorella* sp. using MAD for sample digestion and determination by ICP OES and ICP-MS (values represent the mean in µg g^{-1} and standard deviation, $n = 3$).

Elements	Microalgae biomass		Residual biomass		Lipid fraction		Insoluble fraction		Crude FAMEs		Purified FAMEs	
	ICP OES	ICP-MS	ICP OES	ICP-MS	ICP OES	ICP-MS	ICP OES	ICP-MS	ICP OES	ICP-MS	ICP OES	ICP-MS
Al	125 ± 4	nd	118 ± 4	nd	3.9 ± 1.0	nd	52 ± 1	nd	2.9 ± 0.8	nd	5.3 ± 0.52	nd
As	nd	0.84 ± 0.04	nd	0.43 ± 0.04	nd	2.5 ± 0.1	nd	2.9 ± 0.1	nd	2.7 ± 0.2	nd	<0.009
Ba	35 ± 1	nd	35 ± 1	nd	<0.43	nd	<1.1	nd	<0.28	nd	<0.09	nd
Ca	2237 ± 51	nd	2250 ± 80	nd	40 ± 1	nd	48 ± 1	nd	15.1 ± 1.4	nd	24 ± 4.3	nd
Cd	nd	0.039 ± 0.001	nd	0.030 ± 0.002	nd	<0.002	nd	0.005 ± 0.001	nd	0.006 ± 0.001	nd	<0.001
Co	nd	0.25 ± 0.01	nd	0.20 ± 0.01	nd	0.097 ± 0.004	nd	0.127 ± 0.019	nd	0.093 ± 0.007	nd	<0.001
Cr	1.10 ± 0.08	1.10 ± 0.03	1.10 ± 0.16	0.91 ± 0.04	0.43 ± 0.22	0.40 ± 0.06	0.65 ± 0.13	0.56 ± 0.04	0.32 ± 0.06	0.53 ± 0.08	<0.69	<0.050
Cu	2.8 ± 0.2	3.2 ± 0.1	2.6 ± 0.2	2.6 ± 0.1	0.99 ± 0.56	0.92 ± 0.05	1.40 ± 0.20	1.15 ± 0.01	0.91 ± 0.20	0.69 ± 0.03	1.04 ± 0.06	<0.020
Fe	515 ± 3	nd	478 ± 17	nd	7.4 ± 0.7	nd	<0.6	nd	5.8 ± 0.4	nd	<3.5	nd
K	8434 ± 97	nd	7546 ± 204	nd	2258 ± 68	nd	2858 ± 13	nd	1228 ± 36	nd	<26	nd
La	nd	0.096 ± 0.008	nd	0.079 ± 0.009	nd	<0.001	nd	0.010 ± 0.001	nd	<0.001	nd	<0.001
Mg	2708 ± 45	nd	2525 ± 73	nd	1815 ± 57	nd	2106 ± 40	nd	1619 ± 70	nd	<4.9	nd
Mn	51 ± 1	56 ± 1	47 ± 2	47 ± 1	1.40 ± 0.08	1.40 ± 0.01	1.40 ± 0.16	1.60 ± 0.03	1.10 ± 0.04	1.30 ± 0.16	<0.26	<0.009
Mo	nd	0.23 ± 0.01	nd	0.198 ± 0.006	nd	0.009 ± 0.001	nd	0.017 ± 0.001	nd	0.006 ± 0.001	nd	<0.001
Na	811 ± 9	nd	685 ± 19	nd	306 ± 9	nd	331 ± 3	nd	256 ± 12	nd	10.4 ± 0.08	nd
Ni	nd	0.67 ± 0.04	nd	0.43 ± 0.01	nd	0.82 ± 0.11	nd	0.90 ± 0.02	nd	0.80 ± 0.07	nd	<0.067
P	12,849 ± 159	nd	11,848 ± 447	nd	5259 ± 108	nd	5833 ± 78	nd	4901 ± 166	nd	<10.0	nd
Pb	nd	0.69 ± 0.07	nd	0.69 ± 0.09	nd	<0.004	nd	<0.006	nd	<0.003	nd	<0.005
S	6428 ± 81	nd	5968 ± 253	nd	1961 ± 167	nd	19,061 ± 339	nd	14,174 ± 824	nd	<4.4	nd
Se	nd	0.089 ± 0.017	nd	0.121 ± 0.049	nd	0.036 ± 0.006	nd	<0.03	nd	0.063 ± 0.013	nd	<0.025
Sn	nd	0.093 ± 0.001	nd	0.062 ± 0.001	nd	0.067 ± 0.003	nd	0.080 ± 0.010	nd	0.090 ± 0.010	nd	<0.116
Sr	19.6 ± 0.2	21 ± 1	19.1 ± 0.6	17.6 ± 0.9	0.46 ± 0.01	0.50 ± 0.05	0.62 ± 0.02	0.67 ± 0.04	0.22 ± 0.01	0.23 ± 0.02	<0.23	<0.006
Ti	10.1 ± 0.3	10.9 ± 1.0	9.8 ± 0.4	9.9 ± 0.2	0.41 ± 0.23	0.45 ± 0.08	0.45 ± 0.36	0.41 ± 0.17	<0.29	0.27 ± 0.06	<0.58	<0.08
Tl	nd	0.008 ± 0.001	nd	0.007 ± 0.001	nd	0.002 ± 0.001	nd	<0.001	nd	<0.001	nd	<0.001
U	nd	0.008 ± 0.001	nd	0.009 ± 0.001	nd	<0.005	nd	<0.01	nd	<0.004	nd	<0.009
V	nd	0.183 ± 0.004	nd	0.27 ± 0.01	nd	0.103 ± 0.036	nd	<0.06	nd	0.003 ± 0.001	nd	<0.05
Zn	20 ± 1	25 ± 1	20 ± 1	21 ± 1	1.90 ± 0.33	1.80 ± 0.52	3.2 ± 0.2	2.9 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	<1.42	<0.33

nd = not determined.

Table 3
Results from the determination in NIST 1547 (Peach Leaves). Values represent the mean in µg g^{-1} and standard deviation, n = 3.

Element	ICP OES	ICP-MS	Certified value NIST 1547
Al	232 ± 9	nd	249 ± 8
As	nd	0.092 ± 0.013	0.060 ± 0.018
Ba	121 ± 4	nd	124 ± 4
Ca	13,780 ± 333	nd	15,600 ± 200
Cd	nd	0.027 ± 0.002	0.026 ± 0.003
Co	nd	0.07 ± 0.01	0.07[a]
Cr	0.92 ± 0.15	0.87 ± 0.05	1[a]
Cu	3.4 ± 0.5	3.4 ± 0.5	3.7 ± 0.4
Fe	191 ± 4	nd	218 ± 14
K	21,383 ± 766	nd	24,300 ± 300
La	nd	8.1 ± 0.6	9[b]
Mg	3970 ± 110	nd	4320 ± 80
Mn	98 ± 4	87 ± 7	98 ± 3
Mo	nd	0.050 ± 0.003	0.060 ± 0.008
Na	25 ± 3	nd	24 ± 2
Ni	nd	0.89 ± 0.10	0.69 ± 0.09
P	1394 ± 55	nd	1370 ± 70
Pb	nd	0.68 ± 0.09	0.87 ± 0.03
S	1883 ± 71	nd	2000 ± 94
Se	nd	0.17 ± 0.04	0.12 ± 0.01
Sn	nd	0.080 ± 0.010	<0.2
Sr	55 ± 2	53 ± 9	53 ± 4
Ti	17.1 ± 1.4	14.0 ± 1.0	13[b]
Tl	nd	0.019 ± 0.001	0.012 ± 0.001[c]
U	nd	0.011 ± 0.001	0.015[a]
V	nd	0.31 ± 0.01	0.37 ± 0.03
Zn	18.0 ± 0.7	17.1 ± 0.7	17.9 ± 0.4

nd = not determined.
[a] Informed CRM values.
[b] Informed values from Ivanova et al. (2001).
[c] Informed values from Stephens and Calder (2004).

1.1–2.6 times higher than those observed for seaweed samples (*Porphyra* and *Laminaria*). Among these elements, P and Fe had the highest concentrations, 12,849 and 515 µg g^{-1} in the *Chlorella* sp. samples used in this study, respectively. It may be supposed that these values are higher because Fe is one of the essential elements which is usually added to a growth medium (Chisti, 2007). Moreover, $FeCl_3$ and $Fe_2(SO_4)_3$ are commonly used as flocculants or coagulants in the flocculation step in the microalgal biomass production process (Grima et al., 2003). Some toxic (As, Cd, Cu, Mo, and V) and major (Ca, K, Mg, and Na) elements showed total concentration 1.7–29 times lower than the one found in the literature (Marín et al., 2010; de la Rocha et al., 2009). This difference can be explained by the high salt content associated with the marine environment of the seaweeds which were previously reported (Marín et al., 2010; de la Rocha et al., 2009).

3.2. Lipid fraction and residual biomass

Samples were digested and total element concentration was measured in lipid fraction and residual biomass. After extraction ($CHCl_3$:CH_3OH, 2:1), elements were noticed to remain almost totally in residual biomass (78.6–99.6%, w/w), except As (54.3%, w/w). These features enable the residual biomass from FAME production processes to be potentially used as animal feed (Chisti, 2007). According to Cromwell, (1997), trace elements, such as Cu, Fe, Zn, Mn, I, and Se, are essential elements in the dietary requirements of livestock and poultry. The values found for these elements in residual biomass were 2.6, 478, 20, 47, and 0.12 µg g^{-1}, respectively, except for iodine which was not determined (Table 2). Thus, residual biomass could be used as feedstock for the production of animal supplements. Copper is one of the elements required for animal nutrition; it could be used for swine supplement (3–6 µg g^{-1}); iron for swine (40–100 µg g^{-1}), poultry (38–120 µg g^{-1}),

cattle (50 µg g^{-1}), dairy cattle (50–100 µg g^{-1}), and sheep (30–50 µg g^{-1}); zinc for sheep (20–33 µg g^{-1}); manganese for poultry (17–60 µg g^{-1}); and selenium for swine (0.10–0.30 µg g^{-1}), poultry (0.05–0.20 µg g^{-1}), and sheep (0.10–0.20 µg g^{-1}) (Cromwell, 1997). It is important to point out that these values are in the range of those found in residual biomass. By comparing the values of the macronutrient Ca, K, Mg, P and S found in residual biomass (Table 2) with those found in some feedstuffs commonly used for dairy cattle, e.g. bakery by-products, the concentrations were 1.1–4.6 times higher. For the blood meal, the concentrations of Ca, K, Mg and P were 2.3–8.4 times higher, while Na and S were 5.8 and 1.29 times lower, respectively (Clark et al., 2001). Kolesárová et al. (2011) have reported the concentrations of Ca (7900, 800, 3100, 1100, 500, 3100, 24,500, 1300, and 3000 µg g^{-1}) and P (10,600, 6700, 1100, 7400, 11,100, 8500, 11,100, 6900, and 13,000 µg g^{-1}) for residual biomass from different oils: canola, coconut, cottonseed, groundnut, mustard, palm kernel, sesame, soy bean, and sunflower, respectively. Results (Table 2) showed concentrations of 2250 ± 80 µg g^{-1} for Ca and 11,848 ± 447 µg g^{-1}, for P. In addition, all elements determined in residual biomass showed lower concentrations than the maximum tolerable level (MTL) for rodents, poultry, swine, horse, cattle, sheep, and fish. MTL is defined as the dietary level that, when fed for a defined period of time, will not impair animal health and performance (Klasing et al., 2005).

3.3. Crude FAMEs and insoluble fraction

For crude FAMEs, it was possible to notice that for most elements, except for Cd, Cr, S, Se, Sn, and Zn, concentrations were lower than for the lipid fraction. The most significant difference (7.2 times higher) was observed for sulfur concentration and can be attributed to the use of H_2SO_4 as catalyst (3% in relation to the lipid mass). For the insoluble fraction, an increase in the sulfur content during the extraction was observed. It can be explained by the small sample mass (about 2.4 g) and by the possibility of forming insoluble salts, such as sulfates from sulfuric acid added during the extraction–transesterification process.

3.4. Purified FAMEs

For all the elements, including the toxic ones, concentration was lower than the limit of detection (LOD), except for Al, Ca, Cu, and, Na (5.3 ± 0.52, 24 ± 4.3, 1.04 ± 0.06, and 10.4 ± 0.077 µg g^{-1}, respectively). For elements with maximum limits established by ANP, such as Ca, K, Mg, and Na (5 mg kg^{-1}), P (10 mg kg^{-1}), and S (50 mg kg^{-1}), only Ca and Na showed concentrations that were 4.8 and 2.1 times higher, respectively (Resolução ANP No. 7, 2008). Although Ca concentration was not significantly higher than the recommended one, it must be monitored once Ca can promote oxidative degradation, affecting the quality and conservation of oils and fats directly (Gonzálves et al., 2010). In addition, Na can form undesirable substances in the engines (Edlund et al., 2002).

3.5. Analytical performance

The method validation for FAMEs samples from microalgae (*Chlorella* sp.) and their fractions was carried out by ICP OES and ICP-MS, for major and minor elements, respectively. Analytical figures of merit were obtained using multielemental, sulfur, phosphorus reference solutions in 5% HNO$_3$ (v/v). The linear working range was from 0.025 to 10 µg L^{-1} for ICP-MS and from 5 to 100 µg L^{-1} for ICP OES, for most elements, and from 0.1 to 2.5 mg L^{-1} for sulfur and phosphorus. The LOD was defined as three times the standard deviation (3σ) after ten measurements of the blank. The limit of quantification (LOQ) was defined as three times the LOD. For the

determinations by ICP OES and ICP-MS, the linear correlation coefficients of the calibration curves were higher than 0.999, indicating suitable linearity. It is important to point out that, for most elements, the concentration was below the LOD, except for Al, Ca, Cu, and Na.

The accuracy was evaluated using a CRM (NIST SRM 1547). The CRM was digested and diluted in the same way as the biodiesel and its fractions. Results are shown in Table 3. Likewise, precision was estimated by the relative standard deviation (RSD) which was lower than 23%, indicating a reasonable precision, considering a multielemental analysis. For some elements which do not have certified or informed values, concentrations were compared data provided by Ivanova et al. (2001) and Stephens and Calder, (2004). In addition, a *t*-test was performed to compare the results obtained by ICP OES and ICP-MS and no significant difference was observed, except for Cu, Mn, Sr, and Zn in biomass and for Cr and Zn in crude FAMEs. It is important to point out that all values that presented significance difference ($P < 0.05$) were similar to the significance level ($\alpha = 0.05$).

4. Conclusions

This study demonstrated, for the first time, the determination of 27 elements in fractions from the synthesis of FAMEs from microalgae *Chlorella* sp. The method was suitable for the multielement determination in FAMEs and the results obtained in this study can contribute to suggest a way for the reuse of residual biomass. Moreover, because of its high content of major elements, residual biomass has also been used as fertilizer and to produce methane by anaerobic digestion. After lipid extraction, most elements remain in the residual biomass in sufficient amount to be used in the production of animal feed.

Acknowledgements

References

Antes, F.G., Duarte, F.A., Mesko, M.F., Nunes, M.A.G., Pereira, V.A., Müller, E.I., Dressler, V.L., Flores, E.M.M., 2010. Determination of toxic elements in coal by ICP-MS after digestion using microwave-induced combustion. Talanta 83, 364–369.
Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306.
Clark, J.H., Beede, D.K., Erdman, R.A., Goff, J.P., Grummer, R.R., Linn, J.G., Pell, A.N., Schwab, C.G., Tomkins, T., Varga, G.A., Weiss, W.P., 2001. Nutrient Requirements of Dairy Cattle, seventh ed. National Academies Press, Washington DC.
Cromwell, G.L., 1997. Trace elements and animal production. In: Fisher, P.W.F., L'Abbé, M.R., Cockeli, K.A., Gibson, R.S. (Eds.), Trace elements in man and animals-9: Proceedings of the ninth international symposium of trace elements in man and animals, NRC Research press, Ottawa, pp. 290-293.
D'Oca, M.G.M., Haertel, P.L., Moraes, D.C., Callegaro, F.J.P., Kurz, M.H.S., Primel, E.G., Clementin, R.M., Morón-Villarreyes, J.A., 2011a. Base/acid-catalyzed FAEE production from hydroxylated vegetable oils. Fuel 99, 912–916.
D'Oca, M.G.M., Viêgas, C.V., Lemões, J.S., Miyasaki, E.K., Morón-Villarreyes, J.A., Primel, E.G., Abreu, P.C., 2011b. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. Biomass Bioenergy 35, 1533–1538.
de la Rocha, S.R.F., Muniz, J.S., Juaristi, M.G., Marín, M.T.L., 2009. Trace elements determination in edible seaweeds by an optimized and validated ICP-MS method. J. Food Compos. Anal. 22, 330–336.
Edlund, M., Visser, H., Heitland, P., 2002. Analysis of biodiesel by argon-oxygen mixed-gas inductively coupled plasma optical emission spectrometry. J. Anal. At. Spectrom. 17, 232–235.
Flores, E.M.M., Barin, J.S., Mesko, M.F., Knapp, G., 2007. Sample preparation techniques based on combustion reactions in closed vessels: a brief overview and recent applications. Spectrochim. Acta, Part B. 62, 1051–1064.
Gog, A., Roman, M., Toşa, M., Paizs, C., Irimie, F.D., 2012. Biodiesel production using enzymatic transesterification - current state and perspectives. Renewable Energy 39, 10–16.
Gonzálves, A., Ghanjaoui, M.E., El Rhazi, M., de la Guardia, M., 2010. Inductively coupled plasma optical emission spectroscopy determination of trace element composition of argan oil. Food Sci. Technol. Int. 16, 61–75.
Grima, E.M., Belarbi, E.H., Fernández, F.G.A., Medina, A.R., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491–515.
Ivanova, J., Korhammer, S., Djingova, R., Heidenreich, H., Markert, B., 2001. Determination of lanthanoids and some heavy and toxic elements in plant certified reference materials by inductively coupled plasma mass spectrometry. Spectrochim. Acta, Part B 56, 3–12.
Klasing, K.C., Goff, J.P., Greger, J.L., King, J.C., Lall, S.P., Lei, X.G., James, G.L., Nielsen, F.H., Spears, J.W., 2005. Mineral Tolerance of Animals, second ed. The National Academies Press, Washington.
Kolesárová, N., Hutnan, M., Bodík, I., Spalková, V., 2011. Utilization of biodiesel by-products for biogas production. J. Biomed. Biotechnol. 2011, 1–15.
Lepri, F.G., Chaves, E.S., Vieira, M.A., Ribeiro, A.S., Curtius, A.J., Oliveira, L.C.C., Campos, R.C., 2011. Determination of trace elements in vegetable oils and biodiesel by atomic spectrometric techniques – a review. Appl. Spectrosc. Rev. 46, 175–206.
Marín, M.T.L., Alfonso, M.S.P., Juaristi, M.G., Muniz, F.J.S., de la Rocha, S.R., 2010. Validation of an ICP-OES method for macro and trace element determination in *Laminaria* and *Porphyra* seaweeds from four different countries. J. Food Compos. Anal. 23, 814–820.
Pereira, J.S.F., Mello, P.A., Moraes, D.P., Duarte, F.A., Santos, M.F.P., Guimarães, R.C.L., Knapp, G., Dressler, V.L., Flores, E.M.M., 2009. Feasibility of microwave-induced combustion for digestion of crude oil vacuum distillation residue for chlorine determination. Energy Fuels 23, 6015–6019.
Resolução ANP No 7, de 19.3.2008 - DOU 20.3.2008 - In: http://nxt.anp.gov.br/nxt/gateway.dll/leg/resolucoes_anp/2008/mar%C3%A7o/ranp%207%20-%202008.xml, accessed at 01/07/2011.
Stephens, W.E., Calder, A., 2004. Analysis of non-organic elements in plant foliage using polarised X-ray fluorescence spectrometry. Anal. Chim. Acta 527, 89–96.