Asian Journal of Microbiology and Biotechnology
1(1): 14-24, 2016

International Knowledge Press
www.ikpress.org

MEDIA COMPOSITION AFFECTS THE FATTY ACIDS PROFILES OF THREE OLEAGINOUS MICROALGAE FROM THE PERUVIAN AMAZON

MARIANELA COBOS[1*], LIZ C. LOPEZ[1], JAE D. PAREDES[1], J. DYLAN MADDOX[2,3], GABRIEL E. VARGAS-ARANA[1], LEENIN FLORES[4], CARLA P. AGUILAR[4] AND JUAN C. CASTRO[5*]

[1]Laboratorio de Biotecnología y Bioenergética, Universidad Científica del Perú (UCP), Av. Abelardo Quiñones Km 2,5, Iquitos, Postal Code: 6024, Perú.

[2]The Field Museum of Natural History, Pritzker Laboratory for Molecular Systematics and Evolution, 1400 S. Lake Shore Drive, Chicago, IL, Postal Code: 60605, USA.

[3]Department of Environmental Sciences, American Public University System, Charles Town, WV, Postal Code: 25414, USA.

[4]Laboratorio de Biotecnología Acuática, Instituto del Mar del Perú (IMARPE), Calle Esquina General Valle s/n Chucuito-Callao, Postal Code: 07021, Perú.

[5]Unidad Especializada de Biotecnología, Centro de Investigaciones de Recursos Naturales (CIRNA), Universidad Nacional de la Amazonía Peruana (UNAP), Psje. Los Paujiles S/N, Iquitos, Postal Code: 16024, Perú.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors MC and JCC participated in the study design, obtained funds for the research, coordinated activities and preparation of the manuscript. Authors LCL and JDP realized the microalgae culture and experiments. Author JDM performed the data analysis and participated in the preparation of the manuscript. Authors GEVA, LF and CPA realized the biochemical analysis and participated in the preparation of the manuscript. All authors read and approved the final manuscript.

Received: 15th December 2016
Accepted: 9th January 2017
Published: 21st January 2017

Original Research Article

ABSTRACT

The objective of this research was to determine the effect of four culture media on the fatty acids profile of the microalgae *Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp. isolated from the Peruvian Amazon. The microalgae species were growth by triplicate in Beijerinck's medium (BM), Blue Green medium (BG-11), Bold Basal medium (BBM), and CHU-10 medium. Growth was monitored by spectrophotometric analysis at 680 nm. Biomass productivity, biochemical analyzes, and fatty acids profiling were performed using standard methods. The highest growth was obtained with BM, BBM, and CHU-10 medium for the three microalgae species and the biomass productivity was highest (> 0.23 $mg.L^{-1}.d^{-1}$) in CHU-10 medium. The highest total lipid content was reported with *Ankistrodesmus* sp. (13.77 - 21.58%). Carbohydrate content varied between 8.46 and 50.17%, with the highest percentage when the microalgae were cultured in BG-11 (27.26 – 37.80%) and CHU-10 media (30.72 – 50.17%). Also, the highest protein content was found in the three microalgae species cultured in BM medium (24.89 - 30.44%). The fatty acids profiles showed differences at the intra-species and inter-species levels. In conclusion, the three microalgae showed differences in their growth profile, biomass productivity, biochemical composition (carbohydrates, total lipids, and proteins contents), and fatty acids profiles. Likewise,

**Corresponding author: Email: mcobos@ucp.edu.pe, juanccgomez@yahoo.es;*

these growth and biochemical parameters are markedly affected by the media composition. Additionally, the fatty acids profiles of the three microalgae reveals that are a good feedstocks for biodiesel production.

Keywords: *Ankistrodesmus*; biochemical composition; biodiesel; *Chlorella*; fatty acids profiling; *Scenedesmus.*

1. INTRODUCTION

Oleaginous microalgae have a great potential for biofuel production [1,2], due to a higher biomass productivity and triacylglycerol accumulation in comparison to crop plants [3,4]. For example, some microalgae species can accumulate high amounts of triacylglycerides between 20 and 80% of their dry weight [5,6]. Extra advantages of microalgae are: can be cultured on non-arable soils and thus do not compete with human food production [7], also, these microorganisms have multiple uses such as in bioremediation [8,9], provides chemical resources for the pharmaceutical, cosmetic, and food industries [10–12].

Other additional key aspects of microalgae cells are their metabolic plasticity. This feature is expressed by changes in their biochemical compositions depending on the culture conditions. For example, modifications in the physical-chemical parameters of the culture, causes changes in nutrients content such as triacylglycerols, proteins, and carbohydrates [13–16]. Additionally, the fatty acids profile depends on the microalgae species and is significantly modified by multiple stressors and culture conditions [17–22]. The possibility to manipulate the fatty acids profile is a great advantage offered by these microorganisms. Because, the biodiesel quality and nutritive value (i.e., the polyunsaturated fatty acids content) are dependent on the fatty acids profile [2,2,23], which can provide each oleaginous microalgae species.

To date, exists a gap on the fatty acids profile of native oleaginous microalgae from the Peruvian Amazon. Therefore, to know their uses as a potential source of biodiesel feedstocks, the objective of this research was to determine the effect of four culture media on the fatty acids profile of the microalgae *Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp. isolated from the Peruvian Amazon.

2. MATERIALS AND METHODS

2.1 Microalgae Cultivation and Harvest

The oleaginous microalgae *Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp. evaluated in this investigation were obtained from the Microalgal Culture Collection of the Universidad Científica del Perú. These microalgae species were selected for their ability to *in vitro* growth under laboratory conditions. For the initial cultivation, microalgal cells cultures with an optical density at 680 nm (OD_{680}) = 0.25 were inoculated into 150 mL CHU-10 medium [24] in a 250 mL Erlenmeyer flask, incubated at 26 ± 2°C with continuous aeration under a 12:12 h light-dark cycles for two weeks using 100 μE m^{-2} s^{-1} intensity of cool-white fluorescent light. These cultures were gradually transferred to larger capacity flasks, supplemented with culture medium to obtain 5 liter cultures with an OD_{680} = 0.8 (~ 4 weeks). Subsequently, the cultures were centrifuged in a Universal 320 R (Hettich, Tuttlingen, Germany) at 1920 ×g for 10 min to harvest the microalgae cells. Pelleted cells were rinsed with 50 mL deionized water and centrifuged again.

2.2 Experimental Design

The harvested biomass of each microalgal species was inoculated (to obtain an initial OD_{680} = 0.1) and growth by triplicate in Beijerinck's medium (BM), Blue Green medium (BG-11), Bold Basal medium (BBM), and CHU-10 medium, containing different chemical composition (Table 1). The experiments were carried out in 500 mL Erlenmeyer flasks containing 400 mL of culture medium. The cultures were kept at 26 ± 2°C using 100 μE. $m^2.s^{-1}$ intensity of cool-white fluorescent light with 12 h dark:12 h ligth cycle, and continuous aeration for 7 days.

2.3 Growth Profile and Biomass Productivity

For growth profile analysis, 2 μL aliquots of the microalgal cultures were evaluated each 24 hours (between 9 and 10 hours) by spectrophotometric analysis at 680 nm using a Nanodrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, DE, USA).

After the evaluation period, microalgae cells were harvested by centrifugation under the conditions previously described. The cell pellets were rinsed with 100 mL deionized water, centrifuged again, transfer to Petri dishes, dried in an oven Ecocell 55 (MMM Group, München, Germany) at 70°C to a constant weight, and the dry weight was determined gravimetrically in an analytical balance Kern ABJ 220-4NM (Kern & Sohn GmbH, Balingen, Germany). Biomass was calculated taking into account the difference in weight of dry biomass contained in the Petri dish Petri dish using the following equation [25]:

$$P_B = B_f – B_i$$

Where: P_B = microalgae biomass production; B_f = weight of empty plate; B_i = weight of plate with dried microalgal biomass.

Table 1. Chemical composition in one liter of the four microalgal culture media

Chemical component	BM	BG-11	BBM	CHU-10
$NaHCO_3$	-	-	-	12.6 g
$NaCO_3$	-	20.0 mg	-	-
$NaNO_3$	-	1.5 g	1.50 g	40.0 g
NH_4NO_3	0.15 g	-	-	-
KH_2PO_4	0.97 g	-	1.05 mg	-
K_2HPO_4	1.18 g	40.0 mg	0.45 mg	8.7 g
$MgSO_4.7H_2O$	20.0 mg	75.0 mg	0.45 mg	36.9 g
$CaCl_2.2H_2O$	10.0 mg	36.0 mg	1.20 g	36.7 g
NaCl	-	-	0.15 mg	-
$Na_2O_3Si.9H_2O$	-	-	-	28.4 g
HCl (1 mol/L)	-	-	-	50.0 µL
EDTA.2Na	5.00 mg	1.04 g	0.05 g	0.05 g
KOH	-	-	0.31 g	-
Citric acid	-	6.0 mg	-	-
$(NH_4)_5[Fe(C_6H_4O_7)_2]$	-	6.0 mg	-	3.35g
H_3BO_3	1.00 mg	2.86 g	11.42 mg	618 mg
$MnCl_2.4H_2O$	0.15 mg	1.81 g	1.44 mg	12.6 mg
$ZnSO_4.7H_2O$	2.20 mg	0.22 g	8.80 mg	44.0 mg
$Na_2MoO_4.2H_2O$	-	0.39 g	-	12.6 mg
$CuSO_4.5H_2O$	0.15 mg	79.0 mg	1.57 mg	19.6 mg
$Co(NO_3)_2.6H_2O$	-	49.4 mg	0.49 mg	-
H_2SO_4	-	-	1.00 mg	-
$FeSO_4.7H_2O$	-	-	4.98 mg	-
MoO_3	-	-	0.71 mg	-
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.10 mg	-	-	-
$CoCl_2.6H_2O$	-	-	-	20 mg
pH	6.8	7.4	6.6	7.5
Total volume	1L	1L	1L	1L

These analyses were carried out in triplicate and the data were expressed as mean ± standard deviation (±SD).

2.4 Carbohydrates, Total Lipids, and Proteins

Carbohydrate content of the microalgal biomass was determined with the phenol-sulfuric acid method [26]. For this purpose, 5 mg of dry microalgae biomass was added to 5 mL of water. After this, a 1 mL aliquot of sample was added to 3 mL of sulfuric acid and 1 mL of 5% aqueous solution of phenol, and the mixture was stirred and incubated for 5 min at 90°C. The yellow-marron complex was analyzed by spectrophotometric analysis at 488 nm against a calibration curve based on a known concentration of glucose.

Total lipid was extracted using a modified protocol of Bligh and Dyer [27]. For each sample, ~ 50 mg of microalgal powder was mixed with 3 mL of chloroform/methanol (2:1, v/v) and homogenized in an ultrasonic Branson 2510 (Branson, CT, USA) for 5 min. The mixture was then centrifuged at 20000 ×g for 5 min, and the supernatant was transferred into a pre-weighed vial. Cellular debris was mixed with another 2 mL of chloroform/methanol (2:1, v/v) by vortex, and then centrifuged as described above. After that, the pooled organic phases were filtered through syringe filters of 0.45 µm, evaporated with nitrogen gas, and dried at 80°C in a vacuum oven Vacucell 55 – Comfort (MMM, München, Germany) to a constant weight. The lipid content and productivity of each sample was measured gravimetrically using a semi-micro analytical balance (Sartorius, MSU225S-000-DU, USA) and calculated as follows [28]:

$$\text{Total lipid (\% dry weight)} = W_L/W_A \times 100.$$

where W_L (g) is the weight of the total lipids extracted and W_A (g) is the weight of the dry microalgae biomass.

$$P_{Lipid}\ (\text{mg L}^{-1}\ \text{d}^{-1}) = W_A\ (\text{g}) \times C_{Lipid}\ (\%)/V(\text{L}) \times T(\text{d})$$

where P_{Lipid} is the lipid productivity, C_{Lipid} is the lipid content, V is the working volume, and T is the cultivation time.

Protein content was determined according the Hartree-Lowry method [29]. To 5 mg of dry microalgal biomass was added 5 mL of 0.5 M NaOH

and incubated for 10 min at 80°C, and then centrifuged for 5 min at 14000 ×g. Protein content in 1 mL of the supernatant was determined by adding 0.5 mL of 0.5 M sodium carbonate, 0.5 mL of 0.5 M sodium potassium tartrate, 0.5 M of cupper sulfate, and 2 mL of Folin-Ciocalteau reagent. The blue complex was analyzed in a Varian Cary 50 Bio UV-Visible Spectrophotometer (Agilent Technologies, CA, USA) set at 650 nm against a calibration curve based on a known concentration of BSA as a standard. All analyses were carried out in triplicate, and the data are expressed as mean ± SD.

2.5 Esterification and Fatty Acids Profiling

Fatty acid methyl esters (FAME) were prepared by acid transesterification according to Ichihara and Fukubayashi [30]. The crude lipid extract (1 mg) was dissolved in 0.2 mL of toluene, 1.5 mL of methanol, and 0.3 mL of 0.8% HCl (prepared in methanol: water 85:15 v/v), transferred into capped test tube, mixed for 5 min and then incubated at 45°C for 12 h for derivatization reaction. Then 2 mL of n-hexane and 2 mL of distilled water was added to the tube. After vortexing, the hexane phases containing FAMEs were transferred into a 1.5 mL tube and dried under a stream of nitrogen. Finally, the FAMEs were redissolved in 10 µL acetonitrile, and analyzed by using a gas chromatograph Varian CP-3800 GC (Agilent Technologies, CA, USA) equipped with automated sampler and injector, flame ionization detector and using a 30 m x 0.32 mm x 0.25 µm Stabilwax® capillary column (Restek, PA, USA). The GC conditions were as follows: injector temperature: 250°C; column temperature gradient was: 120°C for 1 min, followed by an increase to 160°C at the rate of 30°C/min, 160°C for 1 min, followed by an increase to 240°C at the rate of 4°C/min, and 240°C for 7 min. Detector temperature: 260°C. Gas pressures for He, H_2, and synthetic air were maintained at 40, 80, and 60 psi, respectively. The carrier gas (He) flow was maintained at 1 mL/min. The fatty acids were analyzed by comparing their retention time of the corresponding peaks with a known standard mixture of FAMEs (Nu-Check Prep, MN, USA), and methyl tricosanoate (Sigma-Aldrich, MO, USA) was added to each sample as internal standard. All chromatograms of the microalgal samples were analyzed by using the Galaxie™ Chromatography Data System version 1.9.3.2 (Agilent Technologies, CA, USA). All peaks spanning a peak area of more than 50 units were integrated. All analyses were carried out in triplicate, and the data are expressed as mean ± SD.

2.6 Statistical Analysis

Statistical analyses were performed using the IBM SPSS Statistics 21.0 software. The growth rate, biomass, lipid, protein, carbohydrate, and ash contents data each microalgal species growth in the four culture medium were analyzed using a one-way analysis of variance (ANOVA) and HSD Tukey test. Differences were considered significant at $P < 0.05$. All data presented here are given as mean ± SD.

3. RESULTS AND DISCUSSION

3.1 Effects of Different Culture Media on Growth and Biomass Productivity

Obtaining biodiesel from vegetable oils is currently problematic due to high land and water requirements and the labor involved with planting, growing, and harvesting [31]. However, microalgae have short cultivation periods, thus representing an alternative source of renewable oils and can be developed in wastewater with the double benefit of bioremediation, higher photosynthetic efficiency, greater effectiveness in the assimilation of nutrients, and sustained throughout the year, due to the short doubling times of microalgae [32]. Microalgae can grow optimally under suitable and sufficient nutrient conditions. The enriched culture media was used to growth various types of microalgal species isolated from freshwater [33]. To study the effects of different media compositions, the oleaginous microalgae *Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp. were cultivated in BM, BG-11, BBM, and CHU-10 media in batch culture. The highest growth was obtained with BM, BBM, and CHU-10 medium for the three species (Fig. 1), whereas the growth was lowest in *Ankistrodesmus* sp., cultivated in BG-11 media. Also, in this culture medium the others microalgae species showed a low growth. In contrast to our results, Bayona and Garcés [34] report that *Botryococcus braunii* a better growth in BG-11 medium. Similarly, George et al. [35] report that the freshwater microalgae *Ankistrodesmus falcatus* growth better in BG-11 medium than in BBM medium.

In our study, the biomass productivity of the three microalgae species was highest (> 0.23 $mg.L^{-1}.d^{-1}$) in CHU-10 medium (Table 2). This can be attributed to the fact that this medium has a high concentration of bicarbonate (12.6 g/L, see Table 1), which is a source that supplies the carbon atoms for the biosynthesis of the carbon skeletons of all the microalgae organic compounds, such as carbohydrates, lipids, and proteins. These anabolic processes begin with the fixation of CO_2 from bicarbonate by the Calvin cycle, and these organic compounds are synthesized from glyceraldehyde-3-phosphate produced in this metabolic pathway [36–38]. In addition, the Table 2, shown that *Chorella* sp. showed the highest biomass

productivity (>2.0 $mg.L^{-1}.d^{-1}$) in the four culture media, but, the highest values were obtained with BBM and CHU-10 media. Contrary to Chlorella *sp.*, the microalgae *Ankistrodesmus* sp. shown the lowest values in biomass productivity with a range between 0.07 ± 0.02 and 0.23 ± 0.07 $mg.L^{-1}.d^{-1}$. The genetic peculiarities and therefore the metabolic capabilities in the differential use of the media chemical components, could partially explain the differences in the growth profiles and biomass productivities.

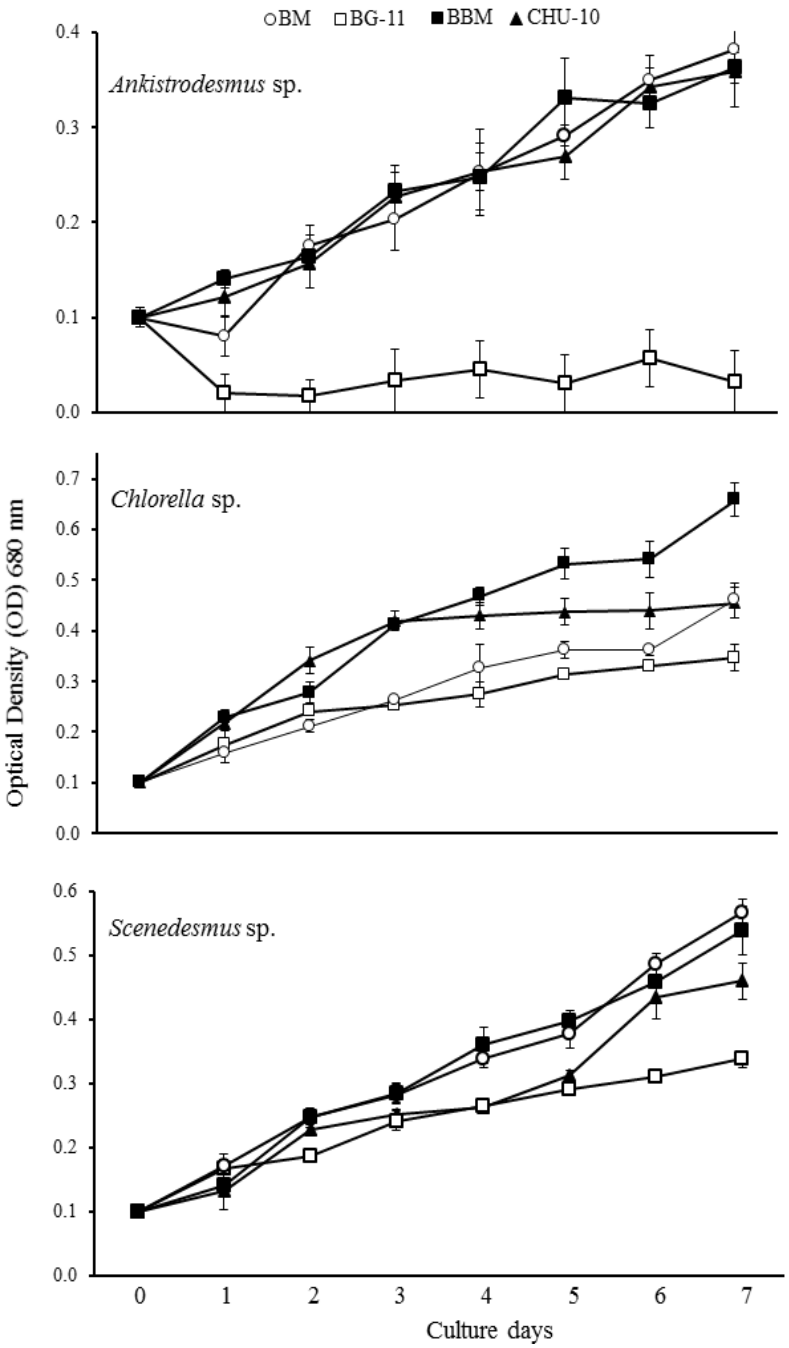

Fig. 1. Growth profiles of the three oleaginous microalgae (*Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp.) from the Peruvian Amazon in four culture media (BM, BG-11, BBM, and CHU-10)

Table 2. Biomass productivities ($mg.L^{-1}.d^{-1}$) of three native oleaginous microalgae grown in the four culture media

Oleaginous microalgae	Culture medium			
	BM	**BG-11**	**BBM**	**CHU-10**
Ankistrodesmus sp.	0.08 ± 0.02^{a}	0.23 ± 0.03^{b}	0.07 ± 0.02^{a}	0.23 ± 0.07^{b}
Chlorella sp.	2.04 ± 0.57^{a}	2.14 ± 0.48^{a}	2.21 ± 0.70^{a}	2.57 ± 0.78^{a}
Scenedesmus sp.	0.23 ± 0.023^{a}	0.44 ± 0.036^{b}	0.49 ± 0.044^{b}	1.24 ± 0.17^{c}

Means ± SD, n = 3, Different letters in the superindice indicate statistically significant differences (P < 0.05)

3.2 Effects of Different Media Composition on Carbohydrates, Total Lipids, and Proteins

The biochemical constituents of microalgae can be altered by changing the composition and concentration of nutrients in the culture medium [39]. Also, the culture conditions such as light intensity, temperature, and photoperiod can modified the biochemical constituents of several microalgae species [40]. Therefore, it is important to optimize the cultivation conditions and medium composition to achieve the best productivity of carbohydrates, lipids, and proteins [35]. In the present study, the highest total lipid content, which is a raw material for the production of biodiesel, was reported with *Ankistrodesmus* sp. (between 13.77 and 21.58%). In contrast, *Chlorella* sp. and *Scenedesmus* sp. showed values between 5.70 and 15.24% (Fig. 2). A similar study by George et al. [35] reported in *Ankistrodesmus falcatus*, grown in BBM medium, a total lipid content of 29.20%. Whereas, Mandotra et al. [41] have reported that *Scenedesmus abundance* exhibits the highest lipid content when is grown in a modified CHU-13 medium, whereas this value is low with BG-11 and BBM media (~5.70%).

In addition to lipid production, microalgae cells are an interesting alternative for the industry, due to their high content in carbohydrates and proteins [42]. Fig. 2 also shows the carbohydrate and protein content of the three microalgae species culture under different media composition. The carbohydrate content varied between 8.46 and 50.17%, with the highest percentage when the microalgae were cultured in BG-11 (27.26 – 37.80%) and CHU-10 media (30.72 – 50.17%). This increased capability for the biosynthesis and accumulation of carbohydrates, is due to the fact that both culture media have an additional source of CO_2, which are carbonate ($NaCO_3$) and bicarbonate ($NaHCO_3$), respectively (Table 1). At species level, the carbohydrates content was highest in *Chlorella* sp. cultured in CHU-10 medium, which showed significant statistical differences with respect to BM, BBM, and BG-11, media ($F = 2146.0$, $df = 3$, $P < 0.001$). *Ankistrodesmus* sp. and *Scenedesmus* sp. showed a similar pattern. A study conducted by Ho et al. [43], found that *C. vulgaris* FSP-E accumulates carbohydrates between 37.8 and 51.0% of its dry weight. Likewise, Piligaev et al. [44] reported that *S. abundans* A1175 has a carbohydrate content of 43.3%. Others investigations reported values of 8.08% in *C. vulgaris* [45] and 7.77% in *Ankistrodesmus falcatus* [35] cultured under different conditions. Consequently, the three microalgae species of our study can be used as a rich source of carbohydrates, which can be converted into valuable products as bioethanol [46].

Highest protein content was found in the three microalgae species cultured in BM medium (between 24.89 and 30.44%). These values showed differences statistically significant in comparison with the other tested media (BG-11, BBM, and CHU-10) with *Ankistrodesmus* sp. ($F = 896.1$, $df = 3$, $P < 0.001$), *Chlorella* sp. ($F = 1408.9$, $df = 3$, $P < 0.001$), and *Scenedesmus* sp. ($F = 5441.7$, $df = 3$, $P < 0.001$). Other investigations, also, report similar protein content in various microalgae species. For example, 26.0% and 47.8% in *C. Vulgaris* and between 15.7 and 33.1% in species of the genus *Scenedesmus* [42]. These results can be attributed to differences in the composition of the nitrogen sources of each culture medium. For instance, in BM medium ammonium nitrate (NH_4NO_3) is a sole nitrogen source for the biosynthesis of aminoacids and proteins. Paradoxically, the nitrogen concentration in this medium ($NH_4NO_3 = 0.15$ g/L) is lower with respect to the other tested culture media ($NaNO_3$ between 1.5 a 12.6 g/L). Consequently, ammonium nitrate is a better source of nitrogen for the biosynthesis of nitrogenous compounds in microalgae cells, such as proteins and nucleic acids. These results are corroborated by many reports, which indicated that microalgae cells can use a wide variety of nitrogen sources, such as ammonia, nitrate, nitrite, and urea [40,47].

3.3 Fatty Acids Profiling

The characteristics of biodiesel are determined by the quality of the lipids produced by the microalgae cells. Therefore, it is necessary to ensure that lipids have long chain fatty acids with a low degree of unsaturation (preferably palmitoleic and myristic acids) that can reduce toxic emissions and improve the properties of biofuels (i.e., cetane number,

oxidative stability) without compromising its flow characteristics, viscosity, and lubricity [48]. The fatty acids profiles obtained of the three microalgae species growth in the four culture media are given in Table 3. This table shows that, the fatty acid profile differs both at the intraspecific level, due to the effect of the culture medium, and at the microalgae species level. These variations may be due to a differential expression of a particular repertoire of the desaturases and elongases, which are the enzymes that determine the type and abundance of fatty acids in eukaryotes [49]. In our data, palmitic (C16:0), oleic (C18:1n-9), linoleic (C18:2n-6), and α-linolenic acids (C18:3n-3) were the most abundant fatty acids and showed greater variations within and between the species studied. Similar results were obtained by other researchers with marine and freshwater microalgae species growth in different culture conditions [50–53].

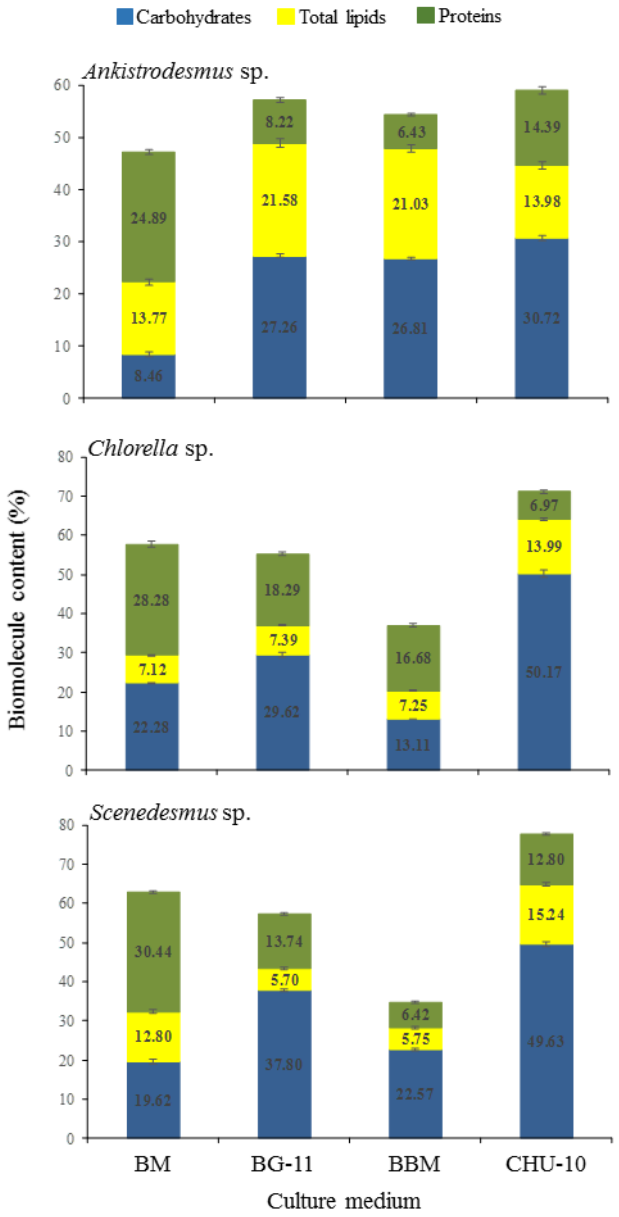

Fig. 2. Biomolecule content (carbohydrates, total lipids, and proteins) of the three oleaginous microalgae (*Ankistrodesmus* sp., *Chlorella* sp., and *Scenedesmus* sp.) obtained in four culture media (BM, BG-11, BBM, and CHU-10)

Table 3. Effect of different culture media on fatty acid profiling in three native oleaginous microalgae from the Peruvian Amazon

Fatty acids	*Ankistrodesmus* sp.				*Chlorella* sp.				*Scenedesmus* sp.			
	BM	**BG-11**	**BBM**	**CHU-10**	**BM**	**BG-11**	**BBM**	**CHU-10**	**BM**	**BG-11**	**BBM**	**CHU-10**
14:0	0.0±0.0	1.0±0.1	0.8±0.1	1.0±0.1	0.0±0.0	0.7±0.1	0.0±0.0	0.7±0.1	0.0±0.0	0.0±0.0	0.5±0.0	0.0±0.0
16:0	21.9±0.9	58.8±1.4	57.2±1.7	35.5±1.2	15.9±3.9	49.7±0.6	20.6±1.5	39.8±1.5	18.1±1.5	49.7±4.2	79.8±2.5	56.5±1.1
18:0	1.2±0.1	4.4±0.1	4.2±0.1	1.9±0.1	0.0±0.0	1.7±0.2	0.0±0.0	1.8±0.2	1.1±0.2	5.1±0.4	6.8±0.8	5.0±0.5
16:1n7	0.7±0.1	0.6±0.1	0.0±0.0	1.4±0.1	1.6±0.4	0.8±0.0	1.1±0.2	0.7±0.1	0.7±0.1	1.2±0.1	0.6±0.1	2.0±0.1
18:1n7	1.9±0.2	0.6±0.1	0.0±0.0	2.7±0.2	1.8±0.4	1.5±0.1	2.1±0.2	1.9±0.1	1.8±0.1	1.8±0.1	0.8±0.1	2.2±0.1
18:1n9	22.3±1.5	57.3±8.7	17.6±2.6	70.3±3.3	12.0±3.2	29.2±0.2	14.4±0.3	47.7±0.1	12.9±1.3	77.6±5.3	20.6±1.6	75.0±1.8
18:2n6	7.3±0.2	4.3±0.7	1.4±0.3	14.2±1.2	19.4±4.8	8.0±0.1	30.9±0.9	37.3±0.5	17.8±0.4	8.9±0.5	5.4±0.3	35.0±1.2
18:3n6	0.9±0.1	0.0±0.0	0.0±0.0	1.0±0.1	1.5±0.4	0.0±0.0	1.6±0.1	1.3±0.1	1.8±0.1	1.0±0.1	0.0±0.0	2.2±0.2
18:3n3	41.9±0.6	4.9±1.1	1.4±0.3	28.3±1.5	34.3±7.5	1.5±0.1	19.4±0.7	16.6±0.5	36.0±1.9	5.0±0.2	4.9±0.2	21.0±0.5
18:4n3	8.5±0.1	1.1±0.2	0.0±0.0	12.0±0.6	5.2±1.1	0.0±0.0	3.2±0.1	3.2±0.1	4.5±0.1	0.0±0.0	0.7±0.1	4.9±0.1
Unidentified	29.4±0.2	2.9±0.1	0.8±0.2	21.8±0.3	30.7±1.8	7.2±0.2	30.5±0.2	21.7±0.1	30.7±1.8	2.5±0.1	3.4±0.3	10.9±0.1
SFA	23.0	64.2	62.3	38.4	15.9	52.1	20.6	42.2	19.2	54.7	87.2	61.5
UFA	47.5	32.8	36.9	39.7	53.4	40.6	48.8	36.1	50.2	42.7	9.4	27.7
MUFA	25.0	58.5	17.6	74.3	15.4	31.5	17.7	50.3	15.5	80.6	22.0	79.2
PUFA	58.5	10.3	2.9	55.5	60.4	9.5	55.0	58.3	60.0	14.9	11.1	63.0

SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

Anand and Arumugam [54], also showed that the fatty acids profile of some microalgae species is characterized for their high content of saturated and monounsaturated fatty acids (54%). In addition, Talebi et al. [55] observed that oleic acid, omega-3 fatty acids, and palmitic acid were the more abundant fatty acids in several strains of *C. vulgaris*, *Amphora* sp., *Dunaliella* sp., and *D. salina*. Studies by Knothe [56] shown that oleic, palmitoleic, and palmitic acids are common fatty acids of microalgae lipids. A similar study by Mahmoud et al. [57] found that *C. vulgaris* stores about 45% of saturated fatty acids and 54% of unsaturated fatty acids. The freshwater green microalgae *Scenedesmus* sp., also have high amounts of unsaturated fatty acids (52.8%) [58]. Finally, a study by Cobos et al. [25] observed high oleic acid content (32.65%) in *S. quadricauda* in nitrogen rich conditions but was reduced to 9.88% under nitrogen depleted conditions. Based on the proposal of Talebi et al. [55] the fatty acids profile of the three microalgae species from the Peruvian Amazon are a good feedstock for the production of biodiesel.

4. CONCLUSION

The microalgae species *Ankitrodesmus* sp, *Chlorella* sp., and *Scenedesmus* sp. natives from the Peruvian Amazon, shown differences in their growth profile, biomass productivity, biochemical composition (carbohydrates, total lipids, and proteins contents), and fatty acid profile. Likewise, these growth and biochemical parameters are markedly affected by the media composition. Additionally, the fatty acids profiles of the three microalgae species shown that are a good feedstocks for biodiesel production.

ACKNOWLEDGEMENT

This work was supported by the Universidad Científica del Perú (UCP) and the Programa Nacional de Innovación para la Competitividad y Productividad (PNICP) with grant contract No. 383-PNICP-PIBA-2014.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brennan L, Owende P. Biofuels from microalgae - A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev. 2010;14:557–77.
2. Blatti JL, Michaud J, Burkart MD. Engineering fatty acid biosynthesis in microalgae for sustainable biodiesel. Curr. Opin. Chem. Biol. 2013;17:496–505.
3. Amaro H, Guedes A, Malcata F. Advances and perspectives in using microalgae to produce biodiesel. Appl Energy. 2011;3402–10.
4. Ho SH, Ye X, Hasunuma T, Chang JS, Kondo A. Perspectives on engineering strategies for improving biofuel production from microalgae - A critical review. Biotechnol. Adv. 2014;32:1448–59.
5. Ahmad AL, Yasin NHM, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: A review. Renew. Sustain. Energy Rev. 2011;15:584–93.
6. Garibay A, Vásquez R, Sánchez M, Serrano L, Martíne A. Biodiesel a Partir de microalgas. Biotecnología. 2009;13(3):38–61.
7. Li Q, Du W, Liu D. Perspectives of microbial oils for biodiesel production. Appl. Microbiol. Biotechnol. 2008;80:749–56.
8. Pereira SFL, Gonçalves AL, Moreira FC, Silva TFCV, Vilar VJP, Pires JCM. Nitrogen removal from landfill leachate by microalgae. Int. J. Mol. Sci. 2016;17.
9. Arora N, Patel A, Sartaj K, Pruthi PA, Pruthi V. Bioremediation of domestic and industrial wastewaters integrated with enhanced biodiesel production using novel oleaginous microalgae. Environ. Sci. Pollut. Res. Int. 2016;23:20997–21007.
10. Draaisma RB, Wijffels RH, (Ellen) Slegers P, Brentner LB, Roy A, Barbosa MJ. Food commodities from microalgae. Curr. Opin. Biotechnol. 2013;24:169–77.
11. Skjånes K, Rebours C, Lindblad P. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Crit. Rev. Biotechnol. 2013;33:172–215.
12. Wang HMD, Chen CC, Huynh P, Chang JS. Exploring the potential of using algae in cosmetics. Bioresour. Technol. 2015;184:355–62.
13. Feng D, Chen Z, Xue S, Zhang W. Increased lipid production of the marine oleaginous microalgae *Isochrysis zhangjiangensis* (Chrysophyta) by nitrogen supplement. Bioresour. Technol. 2011;102:6710–6.
14. Cheirsilp B, Torpee S. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. Bioresour. Technol. 2012;110:510–6.

15. Cuellar-Bermudez SP, Romero-Ogawa MA, Vannela R, Lai YS, Rittmann BE, Parra-Saldivar R. Effects of light intensity and carbon dioxide on lipids and fatty acids produced by *Synechocystis* sp. PCC6803 during continuous flow. Algal Res. 2015;12:10–6.
16. Bohnenberger JE, Crossetti LO. Influence of temperature and nutrient content on lipid production in freshwater microalgae cultures. An. Acad. Bras. Ciênc. 2014;86:1239–48.
17. Lei A, Chen H, Shen G, Hu Z, Chen L, Wang J. Expression of fatty acid synthesis genes and fatty acid accumulation in *Haematococcus pluvialis* under different stressors. Biotechnol. Biofuels. 2012;5:18.
18. Zeng M, Hao W, Zou Y, Shi M, Jiang Y, Xiao P, et al. Fatty acid and metabolomic profiling approaches differentiate heterotrophic and mixotrophic culture conditions in a microalgal food supplement "Euglena." BMC Biotechnol. 2016;16:49.
19. Karpagam R, Preeti R, Ashokkumar B, Varalakshmi P. Enhancement of lipid production and fatty acid profiling in *Chlamydomonas reinhardtii*, CC1010 for biodiesel production. Ecotoxicol. Environ. Saf. 2015;121:253–7.
20. Karpagam R, Raj KJ, Ashokkumar B, Varalakshmi P. Characterization and fatty acid profiling in two fresh water microalgae for biodiesel production: Lipid enhancement methods and media optimization using response surface methodology. Bioresour. Technol; 2015.
21. Kaur S, Sarkar M, Srivastava RB, Gogoi HK, Kalita MC. Fatty acid profiling and molecular characterization of some freshwater microalgae from India with potential for biodiesel production. New Biotechnol. 2012;29:332–44.
22. Reitan KI, Rainuzzo JR, Olsen Y. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. J. Phycol. 1994;30:972–9.
23. Khozin-Goldberg I, Leu S, Boussiba S. Microalgae as a source for VLC-PUFA production. Subcell. Biochem. 2016;86:471–510.
24. Barclay W, Johansen J, Chelf P, Nagle N, Roessler P, Lemke P. Microalgae culture collection, 1986-1987 [Internet]. Solar Energy Research Inst., Golden, CO (USA). Report No.: SERI/SP-232-3079; 1986. Available:http://www.osti.gov/scitech/biblio/6953341
25. Cobos M, Rodríguez JDP, Castro JC. Inducción de la producción de lípidos totales en microalgas sometidas a estrés nutritivo. Acta Biológica Colomb. 2015;21:17–26.
26. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 1956;28:350–6.
27. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 1959;37:911–7.
28. Huang ZZJ, Zhou JLC, He CZP, Yu X. Isolation of a novel strain of *Monoraphidium* sp. and characterization of its potential application as biodiesel feedstock. Bioresource Technology. 2012;121:256–262.
29. Hartree EF. Determination of protein: A modification of the lowry method that gives a linear photometric response. Anal. Biochem. 1972;48:422–7.
30. Ichihara K'ichi, Fukubayashi Y. Preparation of fatty acid methyl esters for gas-liquid chromatography. J. Lipid Res. 2010;51:635–40.
31. Behzadi S, Farid MM. Review: Examining the use of different feedstock for the production of biodiesel. Asia-Pac. J. Chem. Eng. 2007;2: 480–6.
32. Chisti Y. Biodiesel from microalgae. Biotechnol. Adv. 2007;25:294–306.
33. Zhou W, Li Y, Min M, Hu B, Chen P, Ruan R. Local bioprospecting for high-lipid producing microalgal strains to be grown on concentrated municipal wastewater for biofuel production. Bioresour. Technol. 2011;102:6909–19.
34. Bayona KCD, Garcés LA. Effect of different media on exopolysaccharide and biomass production by the green microalga *Botryococcus braunii*. J. Appl. Phycol. 2014;26:2087–95.
35. George B, Pancha I, Desai C, Chokshi K, Paliwal C, Ghosh T, et al. Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus* - a potential strain for bio-fuel production. Bioresour. Technol. 2014;171:367–74.
36. Li-Beisson Y, Beisson F, Riekhof W. Metabolism of acyl-lipids in *Chlamydomonas reinhardtii*. Plant J. 2015;82:504–22.
37. Khozin-Goldberg I, Cohen Z. Unraveling algal lipid metabolism: Recent advances in gene identification. Biochimie. 2011;93:91–100.
38. Bellou S, Baeshen MN, Elazzazy AM, Aggeli D, Sayegh F, Aggelis G. Microalgal lipids biochemistry and biotechnological perspectives. Biotechnol. Adv. 2014;32:1476–93.

39. Chen CY, Yeh KL, Aisyah R, Lee DJ, Chang JS. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. Bioresour. Technol. 2011;102:71–81.
40. Isleten-Hosoglu M, Gultepe I, Elibol M. Optimization of carbon and nitrogen sources for biomass and lipid production by *Chlorella saccharophila* under heterotrophic conditions and development of Nile red fluorescence based method for quantification of its neutral lipid content. Biochem. Eng. J. 2012;61:11–9.
41. Mandotra SK, Kumar P, Suseela MR, Ramteke PW. Fresh water green microalga *Scenedesmus abundans*: A potential feedstock for high quality biodiesel production. Bioresour. Technol. 2014;156:42–7.
42. Becker EW. Micro-algae as a source of protein. Biotechnol. Adv. 2007;25:207–10.
43. Ho SH, Huang SW, Chen CY, Hasunuma T, Kondo A, Chang JS. Characterization and optimization of carbohydrate production from an indigenous microalga *Chlorella vulgaris* FSP-E. Bioresour. Technol. 2013;135:157–65.
44. Piligaev AV, Sorokina KN, Bryanskaya AV, Peltek SE, Kolchanov NA, Parmon VN. Isolation of prospective microalgal strains with high saturated fatty acid content for biofuel production. Algal Res. 2015;12:368–76.
45. Tokuşoglu Ö, üUnal MK. Biomass nutrient profiles of three microalgae: *Spirulina platensis*, *Chlorella vulgaris*, and *Isochrisis galbana*. J. Food Sci. 2003;68:1144–8.
46. Yen HW, Hu IC, Chen CY, Ho SH, Lee DJ, Chang JS. Microalgae-based biorefinery--from biofuels to natural products. Bioresour. Technol. 2013;135:166–74.
47. Li Y, Horsman M, Wang B, Wu N, Lan CQ. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Appl. Microbiol. Biotechnol. 2008;81:629–36.
48. Knothe G. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process. Technol. 2005;86:1059–70.
49. Hashimoto K, Yoshizawa AC, Okuda S, Kuma K, Goto S, Kanehisa M. The repertoire of desaturases and elongases reveals fatty acid variations in 56 eukaryotic genomes. J. Lipid Res. 2008;49:183–91.
50. Yongmanitchai W, Ward OP. Growth of and omega-3 fatty acid production by *Phaeodactylum tricornutum* under different culture conditions. Appl. Environ. Microbiol. 1991;57:419–25.
51. Sánchez Null, Martínez Null, Espinola Null. Biomass production and biochemical variability of the marine microalga *Isochrysis galbana* in relation to culture medium. Biochem. Eng. J. 2000;6:13–8.
52. Huerlimann R, de Nys R, Heimann K. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnol. Bioeng. 2010;107: 245–57.
53. Yeh KL, Chang JS. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31. Bioresour Technol. 2012;105:120–7.
54. Anand J, Arumugam M. Enhanced lipid accumulation and biomass yield of *Scenedesmus quadricauda* under nitrogen starved condition. Bioresour. Technol. 2015;188:190–4.
55. Talebi AF, Mohtashami SK, Tabatabaei M, Tohidfar M, Bagheri A, Zeinalabedini M, et al. Fatty acids profiling: A selective criterion for screening microalgae strains for biodiesel production. Algal Res. 2013;2:258–67.
56. Knothe G. Improving biodiesel fuel properties by modifying fatty ester composition. Energy Environ. Sci. 2009;2:759–66.
57. Mahmoud EA, Farahat LA, Abdel Aziz ZK, Fatthallah NA, Salah El Din RA. Evaluation of the potential for some isolated microalgae to produce biodiesel. Egypt. J. Pet. 2015;24:97–101.
58. Pandian Prabakaran, Ravindran AD. Scenedesmus as a potential source of biodiesel among selected microalgae. Curr. Sci. 2012;102:616–20.