Effect of Fatty acid profile of *C. vulgaris* from different area of Haryana state

Amanjyoti, Department of Food Science and Technology, Chaudhary Devi Lal University, Sirsa
Manju Nehra, Department of Food Science and Technology, Chaudhary Devi Lal University, Sirsa

Abstract

Chlorella used as a medicine in the ancient time, so also known as a nutraceutical. *Chlorella* have high added value food such as polyunsaturated fatty acids, pigments, functional foods, dietary supplements and pharmaceutical products. *Chlorella* is considered a potential source of a wide spectrum of nutrients, including chlorophyll, carotenoids, minerals, vitamins and long-chain polyunsaturated fatty acids (PUFA). Chlorella is a promising marine microalgae species due to its ability to accumulate large amounts of triacylglycerol (TAG) including the high value omega-3 fatty acid eicosapentaenoic acid (EPA) during nitrogen starvation. The results of the study of *C. vulgaris* microalgae in Jind-1 (J-1) had palmitic acid (C16:0) of 2.80%, lower when compared with H-1and 2.89% S-1. Stearic acid (C18: 4) was 1.28%, Oleic acid 1.89%, Linoleic acid 2.89%, Linolenic acid 1.87%, EPA 127.90%, DHA 33.78%, Omega-3 (4.11%) and Omega-6 (3.675) had higher than S-1 and H-1.

Introduction

Chlorella is a widespread genus that found in almost habitats. These microalgae belong to the class Chlorophyceae and have good potential to accumulate more than 20% lipids, such as C 18:1, C 16:0 and C 18:3 (Wu HL, 2001; Scarsella M. et al. 2009). *Chlorella* algae are well known for its high nutritional value (Chacón-Lee TL. and González-Mariño GE. 2010; Guccione, Biondi N.et al. 2014), as they are employed in a variety of biotechnological applications as well (Wu ZY. Et al. 2009; Subhadra B.and Grinson G. 2011; Liu J. and Hu Q. 2013).

Gouveia et al. (2002) showed that *Chlorella vulgaris* can be used for the pigmentation of the gilthead seabream (*Sparus aurata*). *Chlorella* is one of the most abundant types of microalgae in aquatic habitat, with green features, spherical structure, can grow well in the salinity range 0-35 psu, and the optimum temperature is 25-30ºC (Kawaroe M. et al. 2010). *Chlorella* is a single-celled round plant with eukaryotic cell structure and a well-defined nucleus; it grows in fresh water bodies. Chlorella used as a medicine in the ancient time, so also known as a nutraceutical. They have high added value food such as polyunsaturated fatty acids (Ward and Singh, 2005), pigments, functional foods, dietary supplements and pharmaceutical products. Chlorella is considered a potential source of a wide spectrum of nutrients, including chlorophyll, carotenoids, minerals, vitamins and long-chain polyunsaturated fatty acids (PUFA). *Chlorella* is a promising marine microalgae species due to its ability to accumulate large amounts of triacylglycerol (TAG) including the high value omega-3 fatty acid eicosapentaenoic acid (EPA) during nitrogen starvation.

Amongst the microalgae, *Chlorella* is considered as a robust species (Dahiya 2012). Most Chlorella strains can grow mixotrophically with short doubling times and simple growth requirements (Heredia Arroyo et al. 2011). Chlorella with high biomass content is a good bioenergy source to be coproduced with biochar, which reduces carbon input
(Phukan et al. 2011).

C. vulgaris is capable of combining both autotrophic and heterotrophic techniques by performing photosynthesis as well as ingesting organic materials such as glucose, which is the most appropriate for C. vulgaris (Liang Y. et al. 2009; Ogawa T. and Aiba S. 1981; Mallick N. et al. 2012; Patino R. et al. 2007; Yeh KL. and Chang JS. 2012). Hence, the cells are not strictly dependent on light or organic substrate to grow.

Material and Methods

Material

The sample was collected from different area of Haryana state i.e Sirsa, Hisar and Jind. The entire chemicals were used by Hi-media, Qualigens and SDS, India.

Preparation of media and use of media

The media were prepared in distilled water then they are autoclaved at 15 lbs in $^{-2}$pressure for 20 min. Bold's Basal Medium, BG-11 (blue green algae), CHU-13 (culture medium), Soil extract medium and Nile red strain.

Chemical composition of media

Bold Basal Medium (BBM)

Sr.No.	Stocks	Per 400ml
1.	$NaNO_3$	10.0g
2.	$MgSO_4.7H_2O$	3.0g
3.	NaCl	1.0g
4.	K_2HPO_4	3.0g
5.	KH_2PO_4	7.0g
6.	$CaCL_2.2H_2O$	1.0 per litre
7.	**Trace element sol^{-n} (autoclave to dissolve)**	
	$ZnSO_47H_2O$	8.82g
	$MnCl_2.4H_2O$	1.44g
	MoO_3	0.71g
	$CuSO_4.5H_2O$	1.57g
	$Co(NO_3)_2.6H_2O$	0.49g
8.	H_3BO_3	11.42g
9.	EDTA	50.0g
	KOH	31.0g
10.	$FeSO_4.7H_2O$	4.98g
	H_2SO_4(Conc.)	1.0ml
	Medium	**Per liter**
	Stock solution (1-6)	10.0 ml
	Stock solution (7-10)	1.0ml

CHU-11

Sr.No.	Constituent	Quantity (mg/ml per litre)
1.	KNO_3	400.00
2.	K_2HPO_4	80.00
3.	$CaCl_2.2H_2O$	107.0
4.	$MgSO_4.7H_2O$	200.0
5.	Ferric citrate	20.0
6.	Citric acid	100.0
7.	$CoCl_2$	0.02
8.	H_3BO_3	5.72
9.	$MnCl_2$ tetrahydrate	3.62
10.	$ZnSO_4.7H_2O$	0.44
11.	$CuSO_4.5H_2O$	0.16
12.	Na_2MoO_4	0.08
13.	0.072N H_2SO_4	0.02

Soil extract medium (Belchar and Swale, 1982)

Sr.No.	Constituent	Quantity (ml/g per litre)
1.	Water	900.00
2.	Soil extract	100.00
3.	KNO3	0.20
4.	K2HPO4	0.02
5.	MgSO4.7H2O	0.02

Isolation and maintenance of microalgae

Isolation of microalgae was done after 2-3 weeks of incubation when visible growth was observed in the flasks kept in the growth chamber. Isolation was accomplished by streaking the natural microalgal sample across the agar surface, identical to the technique used for isolating bacteria. An algal loop was loaded with a small amount of the sample, and the sample was spread with the loop across the agar. After streaking, it was incubated until the algal colonies appeared. The isolated colonies were removed from the original agar plate with a wire loop and restreaked on to a fresh agar plate to purify and maintained on slants at 4°C.

Preparation of Nile red Solution

Nile red powder (0.5g) was suspended in one ml of acetone and used as a stock solution. From this solution, 0.05ml was mixed with 50ml of glycerol mixture (75:25, Glycerol and water). The solution thus obtained was directly used for staining the lipid bodies of algal cells.

Nile red staining procedure

Nile red (9-(Diethylamino)-5H benzo [α] phenoxazin- 5-one) staining was conducted to detect intracellular lipid droplets (Greenspan and Fowler, 1985). Microalgal cells (0.5 ml) were collected by centrifugation at 1,500 rpm for 10 min and washed with physiological saline solution (0.5 ml) several times. The collected cells were re-suspended in the same solution (0.5 ml), the Nile red solution was added to cell suspensions (1:100 v/v) and incubated for 10 min. After washing once, stained microalgal cells were observed by fluorescent microscopy.

Lipid extraction

Lipid extraction from the dried algal biomass was done using the method of Bligh and Dyer (1959). Two ml methanol and one ml chloroform was added to one gram dried algal biomass and kept at 250C for 18h. The mixture was agitated on vortex for two min and one ml chloroform was added to the mixture. It was shaken vigorously. After that, 1 ml distilled water was added and the mixture was mixed in a vortex for 2 min. The layers of the mixture were separated by centrifugation at 2000 rpm for 10 min. The lower layer was separated and the procedure was again repeated with the pellet. The two supernatants collected were allowed to stand for 2 h. Lower organic layer with the lipids was transferred to a clean preweighedvial (W1). Evaporation was carried out in water bath at 80°C till constant weight was achieved. The weight of the vial was recorded after drying (W2).

Lipid content (% dry cell weight) = W2 - W1 × 100

Where,

W1 is weight of empty vial

W2 is weight of vial + microalgal biomass

Lipid yield (%) = $\frac{W_L}{W_{DA}}$

Where, W_L is weight of extracted lipids and W_{DA} is weight of dry algal biomass

Result

The content of the fatty acid microalgae is an important component for the formation of industrial raw materials because fatty acids become the compositions of the lipids, the highest fatty acid cultivation techniques are potentially used for larger cultivation scales.

Table 1: Effect of Fatty acid profile of *C. vulgaris*

S.No.	Parameter	Fatty acid (S-1)	Fatty acid (H-1)	Fatty acid (J-1)
1.	Omega-3 Fatty acid	3.45	3.23	4.11
2.	Omega-6 Fatty acid	2.68	2.89	3.67
3.	Oleic	1.89	1.67	1.89
4.	Linoleic	0.67	2.87	2.89
5.	Linolenic	1.44	1.67	1.87
6.	Palmitic	2.89	3.45	2.80
7.	Stearic	1.45	1.82	1.28
8.	Total lipid	2.12	2.35	1.56
9.	EPA	120.34	127.78	127.90
10.	DHA	35.23	36.89	33.78
11.	Polyunsaturated acid	32.67	35.78	37.89

The highest fatty acid analysis in S-1is palmitic acid (C16: 0) 2.89% (Table 1). Stearic acid (C18: 4) 1.45%. Other fatty acids have a smaller percentage, such as linolenic acid (C18: 3) 1.44%, oleic acid (C18: 1) 1.89%, linoleic acid (C18: 2) 0.67%, eicosapentaenoic acid (C20: 5) 120.34%, DHA 35.23%, Polyunsaturated acid 32.67% Omega-3 fatty acid 3.45% and omega-6 fatty acid 2.68%. Hisar-1 (H-1) has a content of palmitic acid (C16: 0) 3.45% and Stearic acid (C18: 4) 1.82%. Other fatty acid components have a smaller percentage, such as linolenic acid

(C18: 3) and oleic acid (C18: 1) has a same percentage (167%) in H-1, linoleic acid (C18: 2) 2.89% , eicosapentaenoic acid (C20: 5) 127.78%, DHA 36.89%, Polyunsaturated acid 35.78%, Omega-3 3.23% and Omega-6 2.89%. The total lipid was observed 2.12% in S-1, 2.35% in H-1and 1.56% in J-1. In addition, differences in lipid content caused by environmental stress.

The results of the study of *C. vulgaris* microalgae in Jind-1 (J-1) had palmitic acid (C16:0) of 2.80%, lower when compared with H-1and 2.89% S-1. Stearic acid (C18: 4) was 1.28%, Oleic acid 1.89%, Linoleic acid 2.89%, Linolenic acid 1.87%, EPA 127.90%, DHA 33.78%, Omega-3 (4.11%) and Omega-6 (3.675) had higher than S-1 and H-1.

Reference

Dahiya A (2012) integrated approach to algae production for biofuel utilizing robust algal species. In: Gordon R, Seckbach J (eds) The science of algal fuels, vol 25. Cellular origin, life in extreme habitats and astrobiology. Springer, Netherlands, pp 83–100

HerediaArroyo T,WeiW, Ruan R,Hu B (2011) Mixotrophic cultivation of Chlorella vulgaris and its potential application for the oil accumulation from non-sugar materials. Biomass Bioenerg 35:2245–2253

Phukan MM, Chutia RS, Konwar BK, Kataki R (2011) Microalgae Chlorella as a potential bio-energy feedstock. Appl Energ 88: 3307–3312

Kawaroe M, Prartono T, Rachmat A, Sari D, and Augustine D 2010 *Mikroalga Potensi dan Pemanfaatannya untuk Produksi Bio Bahan Bakar* (Bogor) IPB Press

Wu HL., Identification of *Chlorella* spp. isolates using ribosomal DNA sequences, Bot Bull Acad Sinica, 2001, 42, 115–21.

Scarsella M., Parisi MP., D'Urso A., De Filippis P., Opoka J. and Bravi M., Achievements and perspectives in hetero- and mixotrophic culturing of microalgae, Chem Eng Transac, 2009, 17, 1065–70.

Chacón-Lee TL. and González-Mariño GE., Microalgae for "healthy" foods - possibilities and challenges. Compr Rev Food Sci Food Saf , 2010, 9, 655–675.

Guccione, Biondi N., Sampietro G., Rodolfi L., Bassi N. and Tredici MR., Chlorella for protein and biofuels: from strain selection to outdoor cultivation in a Green Wall Panel photobioreactor, Biotechnology for Biofuels, 2014, 7, 84.

Wu ZY., Qu CB., Shi XM., Biochemical system analysis of lutein production by heterotrophic *Chlorella pyrenoidosa* in a fermentor, Food Technol Biotechnol, 2009, 47, 450–455.

Liu J., Hu Q., *Chlorella*: industrial production of cell mass and chemicals. In Handbook of Microalgal Culture: Applied Phycology and Biotechnology. 2ndedition. Edited by Richmond A., Hu Q. Oxford: Wiley, 2013, 329–338.

Subhadra B.and Grinson G., Algal biorefinery-based industry: an approach to address fuel and food insecurity for a carbon-smart world, J Sci Food Agric, 2011, 91, 2–13.

Liang Y, Sarkany N, Cui Y. Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol Lett 2009;31:1043–9.

Ogawa T, Aiba S. Bioenergetic analysis of mixotrophic growth in Chlorella vulgaris and Scenedesmus acutus. Biotechnol Bioeng 1981;23:1121–32.

Mallick N, Mandal S, Singh AK, Bishai M, Dash A. Green microalga Chlorella vulgaris as a potential feedstock for biodiesel. J Chem Technol Biotechnol 2012;87:137–45.

Patino R, Janssen M, von Stockar U. A study of the growth for the microalga Chlorella vulgaris by photo-bio-calorimetry and other on-line and off-line techniques. Biotechnol Bioeng 2007;96:757–67.

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.