Original Research Article

FATTY ACID COMPOSITIONS OF MIXED MICROALGAE FROM TILAPIA FISH PONDS

.

ABSTRACT

Microalgae has been getting broad attention of researchers and investors lately, especially when discussing on healthy food and energy sources for the future. In this study, twelve samples of mixed microalgae from outdoor ponds were analyzed for their fatty acid compositions. The potential of microalgae to solve variety of world's problems was not realized because of bottleneck in microalgal supplies at reasonable cost. Therefore, the objective of this study is to determine fatty acid profiles of mixed microalgae from tilapia fish ponds. The study was conducted in Tapak Ternakan Ikan, Taman Pertanian Universiti and Department of Biology, Faculty of Science, Universiti Putra Malaysia. Mixed microalgae were extracted for their lipids with methanol: chloroform mixture and after transesterification, the fatty acid methyl ester were analyzed using gas chromatography equipped with flame ionization detector. Results showed that saturated was the major constituent fatty acids. The average percentages of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids obtained were 45.62 ± 1.37%, 20.05 ± 1.14%, and 34.33 ± 3.17% respectively. The most dominant fatty acid profiles were C18:3n3 (*α*-linolenic acid) and C16:0 (palmitic acid), with the overall percentages of 19.97% and 19.40% respectively. The fatty acid profiles of mixed microalgae was good with a decent balance of saturated, monounsaturated and polyunsaturated fatty acids.

Keywords: mixed microalgae, lipids, fatty acids, gas chromatography

1. INTRODUCTION

Microalgae have become an alternative, with great efficiency of renewable green source, thanks to the ability of microalgae to convert solar energy into many useful chemical compounds at a faster rate. Studies have shown that microalgae offer promising potential in providing world population for food [1], feed [2], fuel [3] and other essential chemicals [4]. Most of that past and present studies of microalgae focused more on finding the potential species with high productivity and lipid content [5, 6, 7] culture conditions and culture systems [8, 9]. One aspect of microalgae that is of interest lately is lipid content and fatty acid profiles. This is because microalgae can produce lipid many folds compared to terrestrial plant. Fatty acids are one of the metabolites produced by microalgae, that enrich their utility both in the form of food and fuels. Microalgae are widely used as a suitable source of fatty acids traditionally for many years [10, 11]. The accumulation of fatty acids by microalgae is well studied and discussed by many researchers [7,12]. Among of the researches that have been carried out are on the compositions of fatty acids in *Spirulina platensis* [13] and the triglycerol content in microalgae [14]. Most research has been focusing on the use of microalgal oils containing long-chain polyunsaturated fatty acid as nutraceuticals products. Omega-3 fatty acids are commonly processed from fish oil. But, due to decreasing of fish oil supplies in recent years, also because of the unpleasant taste and odour of fish oils as well as poor oxidative stability, the potential of fish oils is less promising [15]. Compared to oils from fish, microalgae are considered as self-producing omega-3 and the process is straightforward and economical.

Lipids from microalgae are also being studied as feedstock for the sustainable supply of biodiesel [2, 16]. The choice of feedstock for biodiesel production has developed from the edible vegetable oils to the non-edible oils, and now emphases on microalgae. The interest on the potential use of microalgae as feedstock for biodiesel production has been increasing in recent years. Dependency on petroleum-based fuels is not sustainable because of the increase of fuel price, lessening of crude oil supply as well as the environmental impact of fossil fuel usage [17]. Earlier studies demonstrated that under selected conditions, microalgae have potential to produce oil for biodiesel 40 times compared to the oil seed crops per unit land area [18]. But, in the scarcity of publicly available data, it is still unclear whether such gains can be realized for a mass commercial scale production. Hence, the economically potential of microalgal based biofuels to significantly affect present and future needs remains in doubt. To study the practicality of microalgae oil as biodiesel substitute, it is necessary to study the fatty acid profile as only lipid with certain carbon chain are suitable for biodiesel conversion. The properties of biodiesel are mainly determined by the structure of its component fatty acid esters. Among the range of the fatty acids found, the saturated medium-chain fatty acids (C8 to C14) are ideal for biodiesel [19]. On the other hand, biodiesel from PUFAs showed good cold-flow characteristics but it is particularly susceptible to oxidation [14].

In recent years, fatty acids compositions of microalgae in large scale for commercial production have created interests among researchers. Most of the present and past studies on microalgal lipids are done in laboratory conditions using single species. The potential of lipid production in microalgae cells is species-specific and this can also be applied to the ability to produce PUFA [20]. Efforts are being focused on the establishment of optimal conditions for mass production of microalgae with high quality of lipid content. Large-scale of microalgae propagation activity in Malaysia is very limited compared to its market potential and its wide use for both domestic and international market. Among the targeted products from the algal resources in Malaysia is microalgal lipids, especially polyunsaturated fatty acid and carotenoids [21]. To make the process of producing microalgae in large-scale available, the production has to depend on free sunlight available, atmospheric carbon dioxide and nutrients present in wastewater. The high cost of microalgal culture systems relates to the need for a stable culture strain and a practical system. The system not only must be productive, but also exhibit cost and energy efficient. Among the new prospects, there are possibility to produce microalgal biomass in large-scale outdoor production by the application of mixed microalgae to substitute the monospecies microalgae farming that prone to crash. In this regard, we try to determine the potential of mixed microalgae cultured in tilapia pond to replace the practice of monoculture and high-cost photobioreactor system. The principal objective was to determine the fatty acid compositions of mixed microalgae from tilapia fish ponds.

2. MATERIAL AND METHODS

2.1 Mixed microalgae samples collection

Twelve samples of mixed microalgae were collected from different tilapia fish ponds located in Taman Pertanian Universiti, Universiti Putra Malaysia (UPM). The mixed microalgae were harvested using flocculation agent, ferric chloride ($FeCl_3$). Then, the collected samples were filtered using nylon clothes, washed three times, and kept in -80 º C freezer for at least three days. After that, the samples were freeze-dried and ground into powder for further analysis.

2.2 Extraction of lipid

In this study, the Folch method [22] was employed to extract the lipid from mixed microalgae samples. The powder of mixed microalgae samples was weighed and homogenized with chloroform:methanol (2:1 by volume) to a final volume 20 times the volume of mixed microalgae samples (1g in 10ml of solvent mixture). After that, the mixtures were sonicated for five minutes in ice bath before the whole mixtures were agitated for 24 hours at room temperature in an orbital shaker. Next, the liquid phase was recovered by centrifuging at 5000 rpm for 30 minutes. After centrifuged, the bottom layer was discarded. The recovered solvent was washed with 0.9% sodium chloride (NaCl) solutions. The mixtures were centrifuged again at 2000 rpm for 30 minutes to separate the phases. The upper layer was discarded by siphoning and the lower layer was evaporated under vacuum using rotary evaporator at 40ºC.

2.3 Fatty acid methyl ester preparation

0.5g of samples oils were weighed and transferred into a vial with a tight sealing cap using a pasteur pipette. One milliliter of hexane was added into the vial and vortexed briefly to dissolve the lipids. 0.5ml of sodium methoxide was added and vortexed again for one minute. After that, the clear upper layer of methyl ester was pipetted off prior to analysis.

2.4 Gas chromatography analysis

The analyses were performed on a SHIMADZU GC2010 equipped with a flame ionization detector (FID) system. Helium was utilized as the carrier gas. To assist in the confirming identification, the standard Supelco 37- component FAME Mix (47885-U) contained methyl esters of fatty acids ranging from C4 to C24 was used. Based on the chromatogram, the compositions of fatty acid profile were evaluated by comparing the retention time of each peak and its area with standard.

3. RESULTS AND DISCUSSION

Commercial productions of microalgae in open pond system have been fully established by different microalgae ventures to produce valuable products from microalgal biomass. The main advantages of open systems are the lower capital costs together with low maintenance and reduced energy needs [23]. Most microalgal cultivation nowadays are grown as monocultures. The main reason for this is due to the specific strain of microalgae containing the high value by-product desired for harvest. The main drawback of monocultures in open systems is they are prone to contamination since cultures are exposed to the environment directly [24]. For this reason, mixed culture microalgae were used to substitute the monocultures cultivation. A culture of mixed microalgae composed of various strains with various optimal growing conditions would be less susceptible to environmental shifts compared to a monoculture. In this study, the samples of lipids from mixed microalgae collected from tilapia fish ponds were extracted to determine the fatty acid compositions.

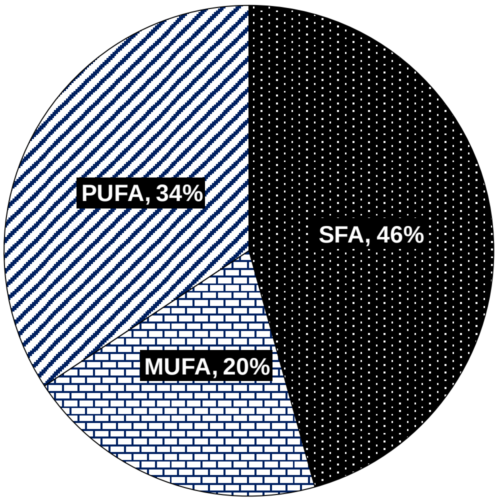

Figure 1: Overall percentage of fatty acid compositions in mixed microalgae obtained from present study.

From the study, the fatty acid compositions varied among the samples, with relatively high percentage of saturated fatty acid (45.62 ± 1.37%). The second major constituent was polyunsaturated fatty acid with the percentage of 34.33 ± 3.17% and the least percentage was monounsaturated fatty acid with the percentage of 20.05 ± 1.14%. Figure 1 showed the average percentage of fatty acid compositions in 12 samples of mixed microalgae obtained from this study.

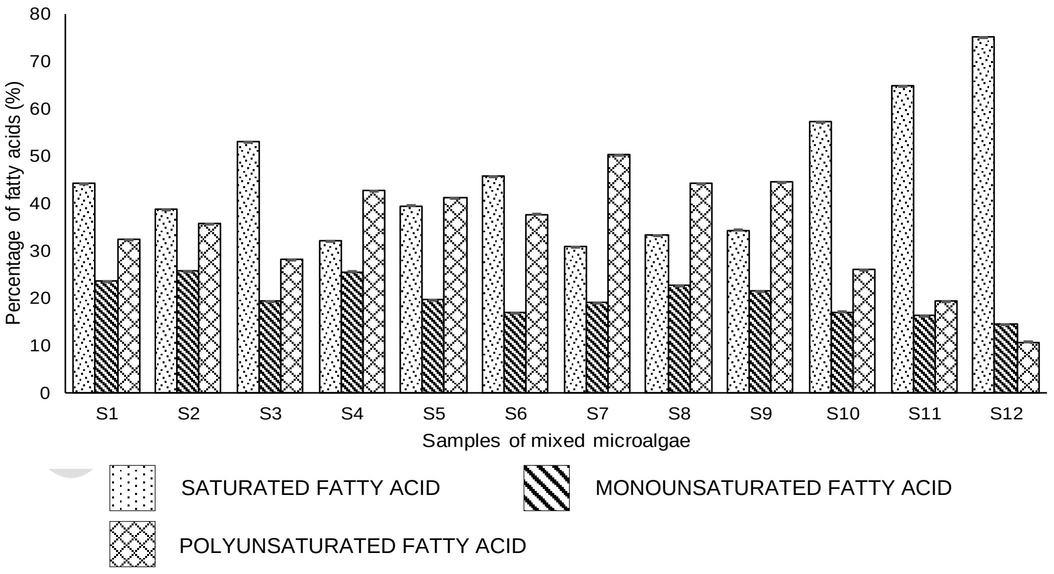

Figure 2: Percentage of fatty acid compositions in mixed microalgae in twelve different tilapia ponds obtained from present study.

The total cellular composition of fatty acid, the lipid class and the length of fatty acid chain as well as the degree of saturation are highly varied among microalgae species and the culture conditions. As shown in Figure 2, the saturated fatty acid (SFA) content were in contrary with polyunsaturated fatty acid (PUFA) content. This might be due to the differences in the culture conditions, growth phase and also the variation of microalgae available in the culture.

Table 1 represented the five most prominent fatty acids of mixed microalgae identified in this study. From previous studies, the most common fatty acid profiles of microalgae were palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic acids (C18:3) [25, 26]. From present study, all of the dominant fatty acids were comparable to [25]. These results also suggested that mixed microalgae culture produces fatty acid compositions comparable to those of pure microalgal species in many literatures [27, 28]. Previously, a study was conducted on biodiesel production using mixed microalgal culture grown in domestic wastewater [9]. In the study, the palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid methyl esters were found to be the predominant fatty acid constituents. All of these fatty acids were also predominantly found in present study. Most of the microalgae investigated by many literatures have comparable fatty acid profile, but the proportion of fatty acid for each microalgae is different. This is largely depending on the strain used and culture conditions [29].

As proposed by a study [11], microalgae are considered as a good source of polyunsaturated fatty acids and the data collected from present study agreed with the statement. From 29 classes of fatty acid compositions determined in this study, the polyunsaturated fatty acid, *α*-linolenic acid (C18:3n3) was the most abundant in mixed microalgae in this study. In present study, *α*-linolenic acid was the most abundant and present in all samples of mixed microalgae. The third major fatty acids determined in this study was also polyunsaturated fatty acid which is linoleic acid (C18:2n6). *α*-linolenic acid is an omega-3 type of PUFA while linoleic acid is an omega-6 polyunsaturated fatty acid. This suggested that mixed microalgae mass production have potential to be developed as the main source of polyunsaturated fatty acid mainly omega-3 to substitute the present source of omega-3 that mainly comes from fish. The high content of palmitic acid of mixed microalgae found in this study make it possible to be utilized in the production of biodiesel.

Table 1: The major fatty acids composition found in the mixed microalgae samples in this study.

Fatty acid	Common name	Average percentage (%)
C18:3n3	*α*-linolenic acid	19.97
C16:0	Palmitic acid	19.40
C18:2n6	Linoleic acid	13.80
C18:1n9	Oleic acid	8.42
C11:0	Undecylenic acid	8.30

Table 2: Comparisons of fatty acid composition in several vegetables oil and microalgae oils.

	SAF	GRA	SUN	WHE	PUM	SES	ALM	RAP	COC	NAN	ISO	MIX	MIX	MIX
SFA	9.3	10.4	9.4	18.2	19.6	16.9	9.3	6.3	92.1	37.1	20.4	43.7	55	46
MUFA	11.6	14.8	28.3	20.9	26.1	42	67.9	72.8	6.2	22.8	17.0	32.4	35.3	20
PUFA	79.1	74.9	62.4	61	54.3	41.2	22.8	20.9	1.6	37.8	39.9	23.9	9.7	34
Omega-3	0.2	0.2	0.2	1.2	0.1	0.2	0	1.2	0	*uk*	*uk*	*uk*	2.1	20.1
Omega-6	79	74.7	62.2	59.7	54.2	40.9	22.8	19.6	1.6	*uk*	*uk*	*uk*	7.6	14.1
	[30]									[31]		[9]	[32]	Present study

Data are expressed as percentages of total fatty acid methyl esters (FAMEs); *uk* means that FAs was unknown. Abbreviations of the samples mean: SAF- Safflower, GRA- Grape, SUN- Sunflower, WHE- Wheat germ, PUM- Pumpkin seed, SES- Sesame, ALM- Almond, RAP- Rapeseed, COC- Coconut oils, NAN- *Nannochloropsis oceania*, ISO- *Isochrysis galbana*, MIX- Mixed microalgae.

4. CONCLUSION

In present study, twelve samples of mixed microalgae collected from tilapia ponds were used to extract lipids and determine the fatty acid compositions. The study revealed that the compositions of fatty acid from mixed microalgae exhibited balance proportion of saturated and unsaturated fatty acids. The mixed microalgae cultivated in outdoor production have a comparable fatty acid compositions as high-maintenance monoculture microalgae. Besides, in view of economics and practicality of the production of biomass, the mixed microalgae cultivation has a very high potential as substitute for current monoculture system. This result can be improved by optimizing few parameters depend on targeted by-products.

REFERENCES

1. Pulz, O., & Gross, W. (2004). Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*, 65: 635-648.

2. Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, 25: 294-306.

3. Borowitzka, M. A., & Moheimani, N. R. (2013). Sustainable biofuels from algae. Mitigation and Adaptation Strategies for Global Change, 18(1): 13-25.

4. Dominguez, H. (2013). Functional ingredients from Algae forfoods and nutraceuticals. In: Woodhead Publishing Series in Food Science, Technology and Nutrition. Sawston, Cambridge: Woodhead Publishing Limited.

5. Griffiths, M. J., & Harrison, S. T. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*, 21(5): 493-507.

6. Liu, B., & Benning, C. (2013). Lipid metabolism in microalgae distinguishes itself. *Current Opinion in Biotechnology*, 24: 300-9.

7. Tan, K. W., & Lee, Y.K. (2016). The dilemma for lipid productivity in green microalgae: Importance of substrate provision in improving oil yield without sacrificing growth. *Biotechnology for Biofuels*, 9: 255.

8. Soydemir, G., Keris-Sen, U. D., Sen, U., & Gurol, M. D. (2016). Biodiesel production potential of mixed microalgal culture grown in domestic wastewater. *Bioprocess and Biosystems Engineering*, 39(1): 45-51.

9. Stemmler, K., Massimi, R., & Kirkwood, A.E. (2016). Growth and fatty acid characterization of microalgae isolated from municipal waste-treatment systems and the potential role of algal-associated bacteria in feedstocks production. PeerJ, 4: 1780.

10. Benemann, J. R. (1992). Microalgae aquaculture feeds. *Journal of Applied Phycology*, 4(3): 233-245.

11. Borowitzka, M.A. (2013). High-value products from microalgae—their development and commercialisation. *Journal of Applied Phycology,* 25(3): 743–756.

12. Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., et al. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, 102(1): 100-112.

13. Yasar, D., & Sevket, G. (2006). Á tocopherol and fatty acids of *Spirulina platensis* biomass in glass panel bioreactor. *Pakistan Journal of Biological Sciences*, 9: 2901-2904.

14. Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M.,Posewitz, M., & Seibert, M. (2008). Microalgal Triacylglycerolsas Fe (Eds.). Stocks for Biofuel production: Perspectivesand advances. *The Plant Journal*, 54: 621-639.

15. Luiten, E. E. M., Akkerman, I., Koulman, A., Kamermans, P., Reith, H., Barbosa, M. J., et al. (2003). Realizing the promises of marine biotechnology. *Biomolecular Engineering*, 20; 429– 439.

16. Schenk, P., Thomas-Hall, S., Stephens, E., Marx, U., Mussgnug, J., Posten, C., et al. (2008). Second generation biofuel: High efficiency microalgae for biodiesel production. *Bioenergy Research*, 1: 20-43.

17. Srivastava, A., & Prasad, R. (2000). Triglycerides-based diesel fuels. *Renewable and Sustainable Energy Reviews*, 4(2): 111-133.

18. Sheehan, J., Dunahay, T., Benemann, & J., Roessler, P. (1998). A Look Back at The U. S. Department of Energy's Aquatic Species Program: Biodiesel from Algae. Golden, CO, USA: National Renewable Energy Laboratory.

19. Durrett, T. P., Benning, C., & Ohlrogge, J. (2008). Plant triacylglycerols as feedstocks for the production of biofuels. *The Plant Journal*, 54: 593-607.

20. Chauton, M. S., Reitana, K. I., Norsker, N. H., Tveterås, R., & Kleivdal H.T. (2015). A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: research challenges and possibilities. *Aquaculture*, 436: 95-103.

21. Chu, W. L., Phang, S. M., & Goh, S. H. (1992). Microalgal lipids. *Malaysian Oils Science and Technolog*y, 1: 37- 40.

22. Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, 226: 497-509.

23. Lee, Y. K. (2001). Microalgal mass culture systems and methods: Their limitation and potential. *Journal of Applied Phycology,*13: 307-315.

24. Pulz, O. (2001). Photobioreactors: Production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology*, 57(3): 287-293.

25. Klass, D. L. (1998). Biomass For Renewable Energy, Fuels, and Chemicals (pp. 333-344). San Diego, CA: Academic Press.

26. Knothe, G. (2009). Improving biodiesel fuel properties by modifying fatty esters composition. *The Journal of Energy and Environmental Science,* 10;1039-1054.

27. Gouveia, L., & Oliveira, A.C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology*, 36: 269-274.

28. Halim, R., Gladman, B., Danquah, M. K., & Webley, P. A. (2011). Oil extraction from microalgae for biodiesel production. *Bioresource Technology*, 102: 178-185.

29. Tzovenis, N., De Pauw, & Sorgeloss, P. (2003). Optimation of T-ISO biomass production rich in essential fatty acids, II. Effect of different light regimes on growth and biomass production. *Aquaculture*, 216(1-4): 223-242.

30. Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contributionto dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International Journal of Molecular Sciences*, 16: 12871-12890.

31. Patil, V., Källqvist, T., Olsen, E., Vogt, G., & Gislerød, H. R. (2007). Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquaculture International*, 15(1): 1- 9.

32. Selvakumar, P., & Umadevi, K. (2016). Biomass production of multipopulation microalgae in open-air pond for biofuel potential. *Indian Journal of Experimental Biology*, 54(4): 271-9.