Presented at the DLSU Research Congress 2017
De La Salle University, Manila, Philippines
June 20 to 22, 2017

Analysis of the Fatty Acid Composition of *C. lentillifera, C. racemosa, E. cottoni,* and *G. tenuistiptata* using Gas Chromatography-Mass Spectrometry

Clark Ian Miguel A. Hormigos, Brian Aldrin V. Lagrosas, Tedd Isaac B. Ecaruan, Diane Keely V. Razon, and Nancy Lazaro-Llanos
Chemistry Department, De La Salle University
2401 Taft Avenue Manila 1004
**Corresponding Author: nancy.lazaro-llanos@dlsu.edu.ph*

Abstract: The monounsaturated and polyunsaturated fatty acid composition of four local seaweeds *Caulerpa lentillifera, Caulerpa lentillifera, Eucheuma cottonni,* and *Gracilaria tenuistipitata* was determined. Lipid extraction on the freeze-dried samples were done using a modified version of Bligh & Dyer. The lipid extracts were derivatized into fatty acid methyl esters using based catalysed transesterfication via methanolic potassium hydroxide solution and analyzed using gas chromatography mass spectrometry. Results revealed the presence of monounsaturated and polyunsaturated fatty acids. Omega-3- fatty acids 7,10,13-hexadecatrienoic acid (HTA), alpha-linoleic acid (ALA), 5,8,11,14,17-eicosapentaenoic acid (EPA) were shown to be present in the seaweed samples. The presence of another omega-3 fatty acid Δ 4,7,10,13,16,19 docosahexaenoic acid (DHA) was only detected in the commercial fish oil sample which was used for comparison. Several omega-6 and omega-9 fatty acids were also found, namely: oleic acid, gamma-linolenic acid (GLA), and eicosatetraenoic acid (ETA). Similar results were shown in previous studies done on other seaweed samples in other locations. Results also reveal *C. lentillifera* and *C. racemosa*, contain the most number of omega-3 fatty acids. Considering the many benefits of omega-3-fatty acids, *Caulerpa* species therefore is a good potential dietary source of this fatty acid. Locally known as *lato, Caulerpa* is used as a main ingredient in many seafood delicacies. This study is expected to contribute significantly in establishing the benefits of seaweed consumption.

Key Words: polyunsaturated fatty acids (PUFA); fatty acid methyl esters (FAME); gas chromatography-mass spectrometry; Bligh and Dryer method

Presented at the DLSU Research Congress 2017
De La Salle University, Manila, Philippines
June 20 to 22, 2017

1. INTRODUCTION

Marine macroalgae or seaweeds are plant-like organisms that are naturally abundant in the Philippines and are among the major marine products for export. The use of seaweeds as raw material has a wide range of application: as main ingredient in various dishes; as thickening agent; as component of herbal products; used in animal feeds, as fertilizer, as filtering agent in waste water treatment, as ingredient in personal care products, and many more. As food and dietary supplement, seaweeds are known to have high nutritional value. Studies have revealed that seaweeds are a rich in minerals, vitamins, proteins, fiber, and antioxidants (Benjama and Masniyom, 2011; Brownlee et al 2012; Boonchum, et al. 2011; Brownlee, et al. 2012; Dawczynski, et al. 2007; Fleurence, 1999; Ismail and Hong, 2002; Lou, et al. 2010; Manivannan, et al. 2008; Manivannan, et al. 2009; Mohammadi, et al. 2013; Nadia, 2013; Norziah and Ching, 2000; Ortiz, et. al 2006; Pattama Ratana-arporn and Chirapart, 2006; Polat, S., & Ozogul, Y. 2013; Suresh, et al. 2012; Shanab, 2007; Seenivasan, et al. 2012). Studies have also shown that seaweeds are also sources of many essential fatty acids associated with many beneficial effects such as reduce fat absorption, improve immune system, and reduce risks for cardiovascular diseases (Agree et al.1997; Bonaa, et al. 1990; Connor, 1994; Harris, 2005; Ismail, 1997; Horricks, 1999; Kinsella, et al., 1990; Kris-Etherton, et al., 2003; Oomen, et. al. 2000; Schmidt, 1997). This research aims to contribute valuable data regarding the monounsaturated and polyunsaturated fatty acid composition of this marine product.

2. METHODOLOGY

2.1 Seaweed Collection and Sample Preparation

Seaweeds were collected during the month of October from wet markets along the Metro Manila and Cavite area. Seaweed samples were washed repeatedly using running water in order to remove contaminants and then twice with distilled water. The samples were lyophilized and stored in zipper storage bags inside the freezer at -20°C.

In addition to the four seaweed samples, commercial fish oil products, claimed to be a rich source of omega-3 fatty acids, were also analysed for comparison. Two different commercial fish oil brands were purchased from a local drugstore.

2.2 Lipid Extraction via Modified Bligh & Dyer Method

A modified version of Bligh & Dyer method (1959) was used to extract the lipids from the freeze-dried samples. Exactly 2 grams of each sample was successively soaked in the following 20 mL of the following solvents: methanol, 1:1 methanol:chloroform, and chloroform. After each addition, the mixture is stirred for two minutes and filtered. The filtrates were collected, pooled, and transferred to a separatory funnel. An equal volume of water was added and the mixture was allowed to stand for about three hours. The denser chloroform layer was collected, dried using anhydrous sodium sulphate, and concentrated under vacuum. The crude extract was re-dissolved in 2 mL hexane.

2.3 Preparation of Fatty Acid Methyl Esters (FAME)

A 2.0 mL of 2M methanolic potassium hydroxide solution were added to each sample. The test tubes containing the reaction mixture was covered by a rubber stopper and mixed for 1 minute using a vortex mixer. Afterwards, the upper hexane layer was collected using a glass syringe. Extracts were filtered into its respective glass vials, using a Whattman filter, and then subjected to GC-MS analysis

2.4 Gas Chromatography - Mass Spectrometry Analysis

GCMS model used was the Agilent Technologies 7890A GC System and 5977A MS System. There were two parameters used. The first parameter was designed for optimizing peaks for EPA and DHA. For this method, total run time was set to 35 minutes with an injection volume of 1 µL. Initial temperature was set to 80^{0}C with an increase of 10^{0}C per minute until 300^{0}C was reached. The second parameter was designed to optimize peaks relating to HTA and ALA. The total run time was set to 58 minutes with an injection volume 1 µL Initial temperature was set to 80^{0}C with an increase of 10^{0}C per minute until 300^{0}C was reached.

3. RESULTS AND DISCUSSION

There were two methods used for analysis of fatty acid composition. The short method was used for detecting compounds that elute at a longer retention time since it narrows peak width and improves peak height. On the contrary, the long method allows compounds that elutes at a shorter retention time to have better separation.

Results show (Table 1) the presence of monounsaturated and polyunsaturated fatty acids in the seaweed samples as well as the commercial fish oil.

Table 1. Fatty Acid Composition
(1) C. lentillifera; (2) C. racemosa; (3) E. cottonni; (4) G. tenuispitata; (5) Commercial fish Oil 1; (6) Connercial Fish Oil 2

Fatty Acid	SAMPLE					
	1	2	3	4	5	6
C 16:2 Δ7,10 (ω-6) 7,10-hexadecadienoic acid	x	x	x			
C 16:3 Δ7, 10, 13 (ω-3) 7,10,13-hexadecatrienoic acid (HTA)	x	x	x			
C 18:1 Δ9 (ω-9) 9-octadecenoic acid oleic acid	x	x	x	x	x	x
C 18:2 Δ9, 12 (ω-6) 9,12-octadecadienoic acid linolenic acid	x	x	x	x	x	x
C 18:3 Δ9, 12, 15 (ω-3) 9,12,15-octadecatrienoic acid Alpha-linoleic acid (ALA)	x	x				
C 18:3 Δ6, 9, 12 (ω-6) 9,12,15-octadecatrienoic acid gamma-linoleic acid	x	x	x	x	x	x
C 20:3 Δ8, 11, 14 (ω-6) 8,11,14-Eicosatrienoic acid	x	x	x	x	x	x
C 20:4 Δ 5, 8, 11, 14 (ω-6) 5,8,11,14-eicosatetraenoic acid arachidonic acid (ETA)	x	x	x	x	x	x
C 20:4 Δ 5, 8, 11, 14, 17 (ω–3) 5,11,14,17-eicosapentaenoic acid (EPA)	x	x	x	x	x	x
C22:6 Δ 4,7,10,13,16,19 (ω–3) docosahexaenoic acid					x	x

Based on the data gathered, *C. lentillifera* and C. racemosa contain the following omega-3-fatty acids namely: 7,10,13-hexadecatrienoic acid (HTA), alpha-linoleic acid (ALA), 5,8, 11,14,17-eicosapentaenoic acid (EPA). Omega-3 fatty acids were also detected in *E. cottonni* similar to the two *Caulerpa* species except ALA, while *G. tenuispitata* only has EPA. The two commercial fish oil samples have similar omega-3-fatty acid composition as *Caulerapa* except that HTA is absent. The presence of another omega-3 fatty acid Δ 4,7,10,13,16,19 docosahexaenoic acid (DHA) was only detected in the commercial fish oil samples. Several omega-6 and omega 9 fatty acids were also found namely: oleic acid, gamma-linolenic acid (GLA), and eicosatetraenoic acid (ETA). Similar results were in seen in the studies published by: Benjama (2011); Bhaskar (2004); Dawczynski (2007); Manivannan et al. (2008); Manivannan et al. (2009); Matanjun (2009); Mohammadi (2013). Muralidhar et al. (2010); Norziah, et al. (2000); Ortiz et al. (2006); Ratana-Arpon (2006); Sanchez-Machado et al. (2004); Majority of the results of these studies have also shown the presence of EPA but not DHA in seaweed samples.

4. CONCLUSIONS

This study has demonstrated the presence of mono- and polyunsaturated fatty acids in selected seaweeds commonly found in Philippines. Analysis have shown the presence of omega-3 fatty acids HTA, ALA, and EPA,

however DHA was not detected. Identical results were found in similar studies done on other seaweed samples in other locations. Results also show that among the seaweed samples, *C. lentillifera* and *C. racemosa*, contain the most number of omega-3 fatty acid. Considering the many benefits of omega-3-fatty acids, *Caulerpa* species therefore is a good potential dietary source of this fatty acid. Locally known as *lato, Caulerpa* is used as a main ingredient in many seafood delicacies. This study is expected to contribute significantly in establishing the benefits of seaweed consumption.

5. ACKNOWLEDGMENT

The authors would like to express sincere gratitude to the DLSU University Research and Coordination Office and DLSU Chemistry Department.

6. REFERENCES

Agree, J.J., Vaisanen, S., Hannien, O., Muller, A.D. and Hornstra, G. (1997). Hemostatic factors and platelet aggregation after fish-enriched diet or fish oil or docosahexaenoic acid supplementation. Prostag. Leukot. Essent. Fatty Acids 57, 419-421.

Benjama, O., & Masniyo,, P. (2011). Nutritional composition and physiochemical properties of two green seaweeds (Ulva pertusa and U. intestinalis) from the Pattani Bay in Southern Thailand. Songklanakarin Journal of Science and Technology, 33(5), 575-583.

Berry, E.M. (1997). Dietary fatty acids in the management of diabetes mellitus. Am. J. Clin. Nutr. 66 (Suppl.), 991s-1007s.

Bhaskar, N., Kinami, T., Miyashita, K., Park, S., Endo, Y., and Fujimoto, K. (2004) Occurrence of Conjugated Fatty Acids in Seaweeds from the Indian Ocean. Z. Naturforsch 59: 5-6

Bonaa, K.H., Bjerve, K.S., Straume, B., Gram, I.T. and Thelle, D. (1990). Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromso Study. N. Engl. J. Med. 322: 795-801.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. Can.J.Biochem.Physiol. 37:911-917.

Boonchum, W., Y. Peerapornpisal, P. Vacharapiyasophon, J. Pekkoh, C. Pumas, U. Jamjai, D. Amornlerdpison, T. Noiraksar and Kanjanapothi, D. (2011). Antioxidant activity of some seaweed from the gulf of Thailand. Int. J. Agric. Biol., 13, 95–99.

Brownlee, I., Fairclough, A., Hall, A., & Paxman, J. (2012). The potential health benefits of seaweed and seaweed extract. Seaweed: ecology, nutrient composition and medicinal uses, 119-136.

Connor, W.E. (1994). Omega-3 fatty acids and heart disease. In: Kritchevsky, D., Caroll, K.K. (Eds.), Nutrition and Disease Update: Heart Disease. Am. Oil Chem. Soc., Champaign, Il.pp 7-42.

Dawczynski, C., Schurbert, R. and Jahreis, R. (2007). Amino acids, fatty acids, and dietary fiber in edible seaweed products. Food Chem 103: 891-899

Fleurence, J. (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends in Food Science and Technology 10, 25-28.

Harris, W.S. (1997). N-3 fatty acids and serum lipoproteins: human studies. Am. J. Clin. Nutr. 65, 16455-16545.

Horrocks, L.A. (1999). Health benefits of docosahexaenoic acid (DHA). Pharmacological Res 40, 211-225.

Ismail, A. and Hong, T. (2002). Antioxidant Activity of selected commercial seaweeds. Mal. J. Nutrition 8, 67-177.

Ismail, H.M. (2005). The role of omega-3 fatty acids in cardiac protection: on overview. Front Biosci, 10, 1079-1088.

Kinsella, J.E., Lokesh, B. and Stone, R.A., (1990). Dietary n-3 polyunsaturated fatty acids in amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr. 52, 1-28.

Kris-Etherton, P.M., Harris, W.S. and Appel, L.J. (2003). Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. Arterioscler Thromb Vasc Biol 23, 151-152.

Lou, H., Want, B. Yu C., Qu, Y, Su, C. (2010). Evaluation of antoxidant activities of five selected brown seaweeds from China. J. Medicinal Plant Research 4, 2557-2565.

Manivannan, K., Thirumaran, G., Karthikai Devi, G., Hemalatha, A., and Anantharaman, P. (2008). Biochemical composition of seaweeds from Mandapam coastal regions along Southeast Coast of India. American-Eurasian J Bot, 1, 32-37.

Manivannan, K., Thirumaran, G., Karthikai Devi, G., Anantharaman, P., & Balasubramanian, B. (2009). Proximate Composition of Different Group of Seaweeds from Vedalai Coastal Waters (Gulf of Mannar): Southeast Coast of India. Middle-East Journal of Scientific Research, 4(2), 72-77.

Matanjun, P., Mohamed, S., Mustapha, N. M., and Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, Eucheuma cottoni, Caulerpa lentillifera and Sargassum polycysum. J. Appl. Phycol. 21, 75–80.

Mohammadi, M. (2013). Nutritional composition of seaweeds from the Northern Persian Gulf. Iranian Journal of Fisheries Sciences, 12(1), 232-240.

Muralidhar, A. P.,Syamala, K.,Prakash, C.,Kalidas, C., & Prasad Naik, R. (2010). Comparative Studies on Fatty Acid Composition of Three Marine Macroalgae Collected from Mandapan Region: South East Coast of India. *World Applied Sciences Journal*,*11*(8), 958-965.

Nadia, H. (2013). What are the Health Benefits of Eating Seaweed.

Norziah, M. and Ching, C. (2000). Nutritional composition of edible seaweed *Gracilaria changgi*. Food Chem 68: 69-76.

Oomen, C.M., Freskens, E.J., Rasanen, L., Fidanza, F., Nissinem, A.M., Menotti, A., Kok, F.J. and Kromhout, D. (2000). Fish consumption and coronary heart disease mortality in Finland, Italy and The Netherlands. Am. J. Epidemiol. 151, 999-1006.

Ortiz, J. Romero, N. Robert, P., Araya, J., Lopez-Hernandez, J., Bozzo, C., Navarette, E., Osorio, A., and Rios, A. (2006) Dietary fiber, amino acid, fatty acid and tocopherol contents of edible seeweeds *Ulva lactuca* and *Durvillaea antartica*. Food Chem, 99, 8-104.

Polat, S., & Ozogul, Y. (2013). Seasonal Proximate and Fatty Acid Variations of Seaweeds from the Norther Mediterranean Coast. Oceanologia, 55(2): 375-391. doi:10.5697/oc.55-2.375

Ratana-Arporn, P. and Chirapart, A. (2006) Nutritional Evaluation of Tropical Green Seaweeds *Caulerpa lentillifera* and *Ulva reticulate* Kasetsart J. 40, 75 – 83. Sanchez-Machado D.I., Lopez-Cervantes, J., Lopez-Hernandez, J. and Paseiro-Losada, P. (2004) Food Chemistry. 85: 439-444.

Schmidt, E.B. (1997). N-3 fatty acids and the risk of coronary heart disease. Danish Med. Bull. 44, 1-22.

Seenivasan. R, Rekha. M, Indu. H, and Geetha.S (2012) Antibacterial activity and phytochemical analysis of selected seaweeds from Mandapam Coast, Indian J of App Pharmaceutical Sci 2, 159-169.

Shanab, S. (2007). Antioxidant and antibiotic activities of some seaweeds. Intl J Agr Biol 9, 220-225.

Stone, N.J. (1996). Fish consumption , fish oil, lipids, and coronary heart disease. Circulation 94:2337-2340.

Suresh, V., Kumar, N,. Murugan, P., , Palani3, P., Rengasamy3, R., Anbazhagan, C. (2012). Antioxidant properties of sequential extracts from brown seaweed, Sargassum plagiophyllum, C. Agardh. Asian Pacific Journal of Tropical Disease S937-S939.