The Analysis of Fatty Acid Composition and Heavy Metals in Edible Seaweeds *Kappaphycus alvarezii* and *Caulerpa lentillifera* Using Gas-Chromatography-Mass Spectrometry and Atomic Absorption Spectrometry

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Abstract: Seaweeds were collected from wet markets in Metro Manila during the months of August and September. Lipid extraction on the freeze-dried samples were done using a modified version of Bligh & Dyer. The fatty acid composition was analyzed by converting the lipid extracts into fatty acid methyl esters and analyzed using gas chromatography-mass spectrometry .For analysis of the concentration of cadmium and lead, the freeze-dried samples were subjected to nitric acid digestion and analyzed via atomic absorption spectroscopy together with prepared standard solutions. Results of fatty acid composition analysis show the presence of both saturated and unsaturated fatty acids ranging from C8-C24. Among the saturated fatty acids, myristic acid was found in all samples. Oleic acid, a monounsaturated fatty acid, and arachidonic acid, a polyunsaturated fatty acid, were also common among the samples. However, omega-3 fatty acids were only detected in the fish oil sample. Results of heavy metal analysis on the freeze-dried samples show that the concentration of cadmium and lead on the freeze-dried samples, which represent approximately 10% of the actual mass of seaweeds when consumed as ingredient in salads and other delicacies are within the safe levels set by the World Health Organization. Further studies are currently being undertaken to confirm this results. Results of the study can be used to evaluate the nutritional value and health hazards of seaweed consumption.

Key Words: polyunsaturated fatty acids (PUFA), heavy metals, atomic absorption spectroscopy, gas chromatography-mass spectrometry, Bligh and Dryer method

1. INTRODUCTION

Seaweeds have been known to have a variety of purposes such as for food, medicine, waste water treatment, cosmetics and many more. Seaweeds are commonly consumed and used as ingredients in various dishes especially in the Asian culture. These are known to have nutritional value because they are rich in antioxidants, minerals, vitamins and proteins (Benjama and Masniyom, 2011; 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; 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). These are also rich in essential fatty acids such as Omega-3 and Omega-6 fatty acids which have been shown to have many beneficial effects (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; Stone, 1994).

However, like other marine organisms, their consumption could lead to possible heavy metal accumulation in the body (Kwon, et al. 2009; Nordberg, 2006; Victoria-Besada, et al. 2009). Studies have shown that high exposures to lead can affect renal function, production of hemoglobin, neurological function, cardiovascular function, increases the risk of osteoporosis, and a possible carcinogen (Lidskya and Schneiderb, 2006; Trzeciakowski, et al., 2014; Wilhelma, et al., 2010;). Several studies have also demonstrated that health cadmium accumulation can results in kidney damage (Bernard, 2004).

This study aims to analyze the fatty acid composition of lipid extracts from *Caulerpa lentillifera* and *Kappaphycus alvarezii* . It also seeks to determine cadmium and lead content in these samples. This study will guide consumers on the possible benefits and risks of seaweed consumption.

2. METHODOLOGY

2.1 Analysis of Fatty Acid Composition

Seaweeds were collected from wet markets in Metro Manila during the months of August and September. Samples were washed, air-dried, and stored inside the freezer. A modified version of Bligh & Dyer (1959) for the lipid extraction was used. Two grams of samples were weighed and transferred to a 50 mL beaker. The following were added to the sample in exact order: 20 mL methanol, 20 mL of 1:1 methanol:chloroform, 20 mL chloroform. After each addition, the mixture was mixed thoroughly for 1 minute using a stirring rod. The mixture was filtered and the filtrate collected. An equal volume of distilled water was then added to the filtrate, mixed, and transferred to a separatory funnel. The chloroform layer was collected, dried with anhydrous sodium sulfate, and concentrated under vacuum at 60°C. Samples were redissolved in 1-2 mL chloroform.

For the preparation of FAME, the method of Moreda and Perez-Camino (2000) was used. Samples were transferred screw top test tubes. Hexane (2 mL) of was added, stirred, and then 2N methanolic potassium hydroxide solution was added. The test tubes were then sealed and mixed using the vortex mixer for 30 seconds. After the separation of layers, the hexane layer was collected using a Pastuer pipette.

For the GC-MS analysis, fish oil available from local pharmacy was used as standard. The GC-MS analysis was set to run for 41 minutes at an initial oven temperature of 80°C with an increase of 10 °C per minute until 280 °C is reached. The injection volume used was 1μL. The GC-MS Model used was the Perkin Elmer Clarus 500 GC-MS.

2.2 Analysis of Cadmium and Lead

For analysis of amount of cadmium and lead, the freeze-dried samples were subjected to nitric acid digestion and analyzed via atomic absorption spectroscopy together with prepared standard solutions.

3. RESULTS AND DISCUSSION

Table 1 presents the fatty acid composition of the lipid extracts from *Kappaphycus alvarezii* and *Caulerpa lentillifera.* Results show the presence of saturated and unsaturated fatty acids ranging from C8-C24. Among the saturated fatty acids, myristic acid (C14:0) was found in all samples. Oleic acid (C18:1 Δ9), an omega-9 monounsaturated fatty acid (MUFA), as well as arachidonic acid (C20:4 Δ 5, 8, 11, 14), an omega-6 polyunsaturated fatty acid (PUFA) were common among the samples. However, omega-3 fatty acids were only detected in the fish oil sample.

In comparison to a previous study done by Muralidhar et al. (2010) on *Kappaphycus alvarezii*, 7-hexadecenoic acid, lauric acid and oleic acid have similarly been detected. However instead of arachidonic acid (seen in this study), oleic acid was found instead. It is noted that both arachidonic acid and linoleic acid (C18:2 Δ9,12) are omega-6 fatty acids. On the other hand, Matanjun et al. (2009) also detected the presence of myristic and oleic acid in *Caulerpa lentillifera*. Likewise, the omega-6 fatty acid found in this study was arachidonic instead of linoleic acid.

Table 1. Fatty Acid Composition

Fatty Acid	Fish Oil	*Kappaphycus alvarezii*	*Caulerpa lentillifera*
C 8:0 caprylic acid, octanoic acid	✓		
C 10:0 capric acid, decanoic acid	✓		
C12:0 lauric acid, dodecanoic acid		✓	
C14:0 myristic acid, tetradecanoate	✓	✓	✓
C 9:1 Δ7 7-nonenoic acid			✓
C16:1 Δ2 2-hexadecenoic acid			✓
C 16:1 Δ7 7-hexadecenoic acid	✓	✓	
C 18:1 Δ9 oleic acid, 9-octadecenoic acid		✓	✓
C 19:1 Δ10 10-nonadecenoic acid			✓
C 16:2 Δ7,10 (ω-6) 7,10-hexadecadienoic acid			✓
C 16:2 Δ9,12 9,12-hexadecadienoic acid			✓
C 18:2 Δ9,12 (ω-6) linoleic acid, 9,12-octadecadienoic acid			✓
C 20:3 Δ8, 11, 14 (ω-6) 8,11,14-Eicosatrienoic acid			✓
C 20:4 Δ 5, 8, 11, 14 ω-6 arachidonic acid 5,8,11,14-eicosatetraenoic acid	✓	✓	✓
C 20:4 Δ 5, 11, 14, 17 (ω–3) 5,11,14,17-eicosatetraenoate	✓		
C20:5 Δ 5, 8, 11, 14, 17 (ω–3) eicosapentaenoic acid	✓		
C22:1 Δ 13 brassidic , 13-docosenoic acid	✓		
C22:3 Δ 8, 11, 14 docosatrienoic acid			□
C22:6 Δ 4,7,10,13,16,19 (ω–3) docosahexaenoic acid	✓		
C24:1 Δ 9 nervonic 15-tetracosaenoic acid	✓		

Results of heavy metal analysis on the freeze-dried samples are summarized in Table 2 It is noted that the August 2014 collection yielded relatively higher amounts of both lead and cadmium. *C. lentillifera* also consistently showed higher amounts of both cadmium and lead. Based on the results, the amount of cadmium and lead detected on the seaweed samples were within the safe level of cadmium and lead intake set by the World Health Organization (WHO): <30 μg/day and <25 μg/day, respectively. The WHO provisional tolerable weekly intake (PTWI) level for Cd at 7 μg/kg body weight and for Pb at 25 μg/kg body weight. The freeze-dried samples represent approximately 10% of the actual mass of the seaweeds when consumed as ingredient in salads and other delicacies.

Table 2. The Concentration of Cadmium and Lead in Freeze-Dried Samples (average of three trials)

Sample Description	Conc in Freeze-Dried Samples (ppm)	
	cadmium	lead
Caulerpa lentillifera (Aug 2014)	0.1190	1.3493
Caulerpa lentillifera (Sep 2015)	0.0427	1.3000
Kappaphycus alvarezii (Aug 2014)	0.08927	0.6841
Kappaphycus alvarezii (Sep 2015)	0.0492	1.0768

4. CONCLUSIONS

This study has shown the presence of both saturated and unsaturated fatty acid in edible seaweeds *Kappaphycus alvarezii* and *Caulerpa lentillifera*. Among these are omega-6 fatty acids. However omega-3 fatty acids were only detected in fish oil sample. Results also show that the amount of lead and cadmium in the samples are within the tolerable levels, considering that this represents only 10% of the mass of seaweeds. Further studies are currently being undertaken to confirm this results.

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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. and Masniyom, P (2011). Nutritional composition and physicochemical properties of two green seaweeds (*Ulva pertusa* and *U. intestinalis*) from the Pattani Bay in Southern Thailand Songklanakarin J. Sci. Technol. 33, 575-583.

Bernard, A. (2004) Renal Dysfunction Induced by Cadmium: Biomarkers of Critical Effects. *Biometals* 17: 519-523.

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

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

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.

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. and Paxman J. The potential health benefits of seaweed and seaweed extract (2012) In: POMIN Vitor H. (ed) Seaweed: ecology, nutrient composition, and medicinal uses. Marine Biology. Earth Sciences in the 21st Century. Hauppauge, NY Nova Science Publishers, 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.

Kwon, Y. M., Kim, D. S., Lee, Y. N., Kim, J. A., Kim, G. S., and Kim, C. H. (2009). Dietary Exposure and Risk Assessment of Mercury from the Korean Total Diet Study. J. Toxicol and Environ Health. 72, 1484-1492.

Lidskya, T.I., Schneiderb, J.S. (2006) Adverse effects of childhood lead poisoning: The clinical neuropsychological perspective. Environmental Research, 100, 284-293. doi:10.1016/j.envres.2005.03.002

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., Anantharaman, P., and Balasubramanian, T. (2009). Proximate composition of different groups of seaweeds from Vedalai coastal waters. Southeast Coast of India Middle-East J Scientific Res 4, 72-77.

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.

Mohammadi, M. Tajik H., Hajeb P. (2013) Nutritional composition of seaweeds from the Northern Persian Gulf. Iranian J Fisheries Sci 12, 232- 240.

Moreda, W., Pérez-Camino, M.C., Cert, A. (2000). Methods of preparation of fatty acid methyl esters (FAME). Statistical assessment of the precision characteristics from a collaborative trial. v51.i6.464.

Nordberg, G. F. (2006). Lung cancer and exposure to environmental Cd. The Lancet Oncology, 7, 99-101.

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.

Pattama Ratana-arporn, P. and Chirapart, A. (2006) Nutritional Evaluation of Tropical Green Seaweeds *Caulerpa lentillifera* and *Ulva reticulate* Kasetsart J. 40, 75 – 83.

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

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.

Trzeciakowski, J.P., Gardiner, L. & Parrish, A.R. (2014). Effects of environmental levels of cadmium, lead and mercury on human renal function evaluated by structural equation modeling. Toxicology Letters, 228. doi:10.1016/j.toxlet.2014.04.006

Victoria Besada, V., Andrade, J., Schultze, F., and González, J. (2009). Heavy metals in edible seaweeds commercialised for human consumption J Marine Systems 75, 305–313.

Wilhelma, M., Heinzowb, B., Angerer, J., & Schulz, C. (2010). Reassessment of critical lead effects by the German Human Biomonitoring Commission results in suspension of the human biomonitoring values (HBM I and HBM II) for lead in blood of children and adults. International Journal of Hygiene and Environmental Health, 213, 265-269. doi:10.1016/j.ijheh.2010.04.002