The Chemical Analysis of Omega-3 Fatty Acid and Cadmium in *Caulerpa lentillifera* and *Euchema denticulatum* using High Performance Liquid Chromatography and Atomic Absorption Spectroscopy

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Abstract: Seaweeds like *Caulerpa lentillifera* (lato) and *Eucheuma denticulatum* (guso) are used as main ingredient in Filipino delicacies popular here and abroad. They have been branded as healthy food being rich in dietary fiber, minerals, vitamins, proteins, phytochemicals, with low lipid content but rich in omega-3 fatty acids. Studies have shown that consumption of food rich in omega-3 fatty acids are essential for proper brain functioning and normal growth, and is associated with reduced risk of cardiovascular diseases. However like all marine organisms, there is also the risk of accumulation of heavy metals like cadmium which may lead to renal failure and bone demineralization. In this study, seaweeds samples collected from wet markets in Metro Manila during the months of June and October will be analyzed for their omega-3 fatty acid and cadmium content. Prior to extraction and analysis, samples were air-dried and then grounded. Lipids were extracted using a modified Folch method and analyzed via high performance liquid chromatography (HPLC) using fish oil available in local drugstores as standard. In the heavy metal analysis, samples were subjected to nitric acid digestion and cadmium concentration was analyzed using atomic absorption spectroscopy. HPLC chromatogram demonstrated the presence of a dominant peak in all samples that is similar to the fish oil standard, which could possibly correspond to a triacylglycerol with omega-3 fatty acids. To better assess the risks and benefits of seaweed consumption, further studies that will quantify the amount of omega-3 fatty acids and heavy metals in the seaweed samples are necessary.

Key Words: omega-3 fatty acids, heavy metals, atomic absorption spectroscopy, high performance liquid chromatography, Folch method

1. Introduction

Omega-3 fatty, polyunsaturated fatty acids (PUFA) like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been implicated in the lowering cholesterol and blood pressure (Bonaa, et al. 1990; Horricks, 1999; Connor, 1994; Stone, 1994; Schmidt, 1997). Omega-3 fatty acids have been proven to have protective effects in reducing arrhythmias and thrombosis (Kinsella, et al., 1990; Kris-Etherton, et al., 2003; Oomen, et. al. 2000), lowering plasma triglycerides (Ismail, 1997; Harris, 2005), reducing blood clotting tendency (Agree, 1997), and improve many metabolic consequences of insulin resistance in humans (Berry, 1997). The American Heart Association has affirmed that omega-3 fatty acids benefit the heart and lower risk of cardiovascular disease (Stone, 1996).

Marine macroalgae like *Caulerpa* (lato) and *Eucheuma* (guso), commonly known as seaweeds, are a popular delicacy in the Philippines. They have been branded as healthy food being rich in dietary fiber, vitamins, proteins, low in lipids but rich in omega-3 fatty acids; moreover they have demonstrated antimicrobial and antioxidant properties (Brownlee, et al. 2012; Ismail and Hong, 2002; Lou, et al. 2010; Boonchum, et al. 2011; Suresh, et al. 2012; Shanab, 2007; Seenivasan, et al. 2012; Manivannan, et al. 2008; Manivannan, et al. 2009; Mohammadi, et al. 2013; Benjama and Masniyom, 2011; Norziah and Ching, 2000; Fleurence, 1999; Dawczynski, et al. 2007; Ortiz, et. al 2006; Pattama Ratana-arporn and Chirapart, 2006). In addition, seaweed industry in the Philippines has also found its application in cosmetic and pharmaceutical industry as well as in agriculture.

Regardless of the benefits of seaweeds as a food product, consumption of different marine species like fish, shellfish, and seaweeds are a possible route of heavy metal exposure that may cause health risks if taken in high dosage. Heavy metals poisoning is a global public health issue linked to life threatening illness including cancer (Kwon, et al. 2009; Nordberg, 2006; Victoria-Besada, et al. 2009).

This study aims to analyze lipid extracts from *Caulerpa* and *Eucheuma* for the presence of omega-3 fatty acids. It also seeks to determine cadmium content in these samples. This study will guide consumers on the possible benefits and risks of seaweed consumption.

2. METHODOLOGY

Seaweeds were collected from wet markets in Metro Manila during the months of July and September. Samples were washed, air-dried, and stored inside the freezer. Total lipid was extracted using a modified procedure by Folch, Lees, and Sloane-Stanley, according to Christie (Folch, et al. 1957, Christie, 2011). The powdered samples were soaked in $CHCl_3$:methanol mixture overnight in the absence of light. The samples were filtered, the filtrate collected, and extracted with $CHCl_3$:H_2O mixture. The chloroform layer was evaporated under the hood and the weight of the residue was noted. The residues were dissolved in 1ml hexane and analyzed via HPLC using 100% using acetonitrile as the solvent system (10.0 μL injection volume, flow rate of 1.0 mL/min., retention time of 25 minutes, UV detector λ=210). Fish oil purchased from local drugstores were used as standard. For analysis of cadmium, the dried seaweeds were subjected to nitric acid digestion and analyzed via atomic absorption spectroscopy (AAS).

3. RESULTS AND DISCUSSION

Table 1 is a summary of the major peak (retention time) in the HPLC chromatograms obtained. Results show similarity between the fish oil standards to that the lipid extracts (Range: fish oil standard = 2.980-3294; *Caulerpa* lipid extract = 3012-3385; and *Euchema* lipid extract 3.250-3.264). This implicates similarity in the composition of fish oil available in the local drugstore and to those isolated from the seaweeds.

Table 1. Data from HPLC

Sample Description	Major Peak (Retention Time)
Fish Oil (Trial 1)	2.980
Fish Oil (Trial 1)	3.213
Fish Oil (Trial 1)	3.284
Samples Collected in June	

Caulerpa from Market 1 (Trial 1)	3.151
Caulerpa from Market 1 (Trial 2)	3.012
Caulerpa from Market 2 (Trial 1)	3.378
Caulerpa from Market 2 (Trial 2)	3.134
Caulerpa from Market 3 (Trial 1)	3.386
Caulerpa from Market 3 (Trial 2)	3.385
Euchema from Market 1 (Trial 1)	3.254
Euchema from Market 1 (Trial 2)	3.256
Euchema from Market 2 (Trial 1)	3.264
Euchema from Market 2 (Trial 2)	3.258
Euchema from Market 3 (Trial 1)	3.258
Euchema from Market 3 (Trial 2)	3.250
Samples Collected in October	
Caulerpa from Market 1 (Trial 1)	3.266
Caulerpa from Market 1 (Trial 2)	3.274
Caulerpa from Market 2 (Trial 1)	3.319
Caulerpa from Market 2 (Trial 2)	3.279
Caulerpa from Market 3 (Trial 1)	3.337
Caulerpa from Market 3 (Trial 2)	3.333
Euchema from Market 1 (Trial 1)	3.261
Euchema from Market 1 (Trial 2)	3.262
Euchema from Market 2 (Trial 1)	3.270
Euchema from Market 2 (Trial 2)	3.263
Euchema from Market 3 (Trial 1)	3.256
Euchema from Market 3 (Trial 2)	3.267

Data for Cd analysis are shown in Table 2. Results of AAS show that the amount of Cd in the samples are higher than the World Health Organization provisional tolerable weekly intake (PTWI) level for Cd at 7 μg/kg. It should also be noted that the levels of cadmium in *Euchema* are much higher than in *Caulerpa*.

Table 2. Amount of Cadmium in the Samples

Sample Description	Concentration (ppm)
Samples Collected in June	
Caulerpa from Market 1 (Trial 1)	2.3108
Caulerpa from Market 2 (Trial 1)	3.0862
Caulerpa from Market 3 (Trial 1)	8.4387
Euchema from Market 1 (Trial 1)	19.8191
Euchema from Market 2 (Trial 1)	14.5666
Euchema from Market 3 (Trial 1)	15.3420
Samples Collected in October	
Caulerpa from Market 1 (Trial 1)	6.3878
Caulerpa from Market 2 (Trial 1)	2.3859
Caulerpa from Market 3 (Trial 1)	4.4118
Euchema from Market 1 (Trial 1)	14.9668
Euchema from Market 2 (Trial 1)	19.8441
Euchema from Market 3 (Trial 1)	15.4420

4. CONCLUSIONS

This study has shown similarity in the composition of seaweed lipid extracts to commercially available fish oil. However alongside with this is the risk of consuming appreciable amounts of the cadmium. Further studies are warranted. It is recommended that the fatty acid composition be verified by converting the triglycerides to the corresponding fatty acid methyl esters and analyzed by gas chromatography. The presence of other heavy metals like Pb should also be considered.

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6. REFERENCES

Acworth, I., Plante, M., Crafts, C., and Bailey, B. (2011). Quantitation of underivatized omega-3 and omega-6 fatty acids in foods by HPLC and charged aerosol detection. Planta Medica, 77, 1-5.

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.

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

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.

Christie, W. (2011). Preparation of Lipid Extracts from Tissues. The Scottish Crop Research Inst, *2,* 195-213.

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.

Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957). A simple method for isolation and purification of lipids from animal tissue. J. Biol Chem 226, 527-532.
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.

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.

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.

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.

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.