OPPORTUNITIES AND CHALLENGES OF FOOD AND FUNCTIONAL FOOD USE OF INDIAN SEAWEED: FOODOMICS AND GREEN CHEMISTRY APPROACHES

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Introduction

With increased public awareness on the relation of diet and health; consumption of marine sourced functional food and nutraceuticals are on the rise (Granato et al., 2020). Particularly, seaweeds are being considered as novel foods of future, being promising sources of novel molecules like polyphenols, secondary metabolites, carotenoids, peptides and sulphated carbohydrates possessing nutraceutical properties (Lafarga et al, 2020). Seaweeds can be classified as red algae (Rhodophyta), brown algae (Phaeophyta) or green algae (Chlorophyta), which has long history of use as human food either in fresh or processed form. A total of 770 species of seaweeds have been reported from different parts of the Indian coasts which include 184 species of green, 166 species of brown and 420 species of red (D.Sahoo, 2010) seaweed. However, in India use of seaweeds as food, functional food, and nutraceuticals is not well established like in Japan, China, Korea, and western countries. In India, seaweeds are mostly being used as a raw material for the domestic hydrocolloids industry and tiny part of it is exported. However, there is huge potential of commercial cultivation of Indian seaweed for high value food, functional food, and nutraceutical application. This article outlines the opportunities and challenges of food and functional food use of Indian seaweed and explores how frontier technologies such as Green Chemistry and Foodomics can support the initiative.

Green chemistry approach for extraction of bioactives from seaweed biomass

The extraction of bioactive compounds in an economic and nature-friendly approach is a big dilemma. Traditional methods comprise Soxhlet extraction, hydro distillation, maceration, decoction, infusion, pressing, percolation, *etc.* Although they have been used since ages, they are very often time-consuming and require relatively large quantities of polluting solvents, which can lead to sample contamination, losses due to volatilization during concentration steps, and environmental pollution from solvent waste **(**Zhao et al, 2009**).** Most of the commonly used organic solvents are harmful to the living system on earth. Because of the known hazards associated with many solvents, the transition to green alternatives has already begun to be popular in the laboratories and caught the attention of industrial project managers.

Green chemistry aims to minimize the environmental impact of the chemical industry. This includes shifting away from oil to renewable sources where possible. Green chemistry also prioritizes safety, improving energy efficiency and, most importantly, minimizing (and ideally) eliminating toxic waste from the very beginning **(**Bissember, 2017**).** The principles of green chemistry are depicted in the following Figure 1. More recently, multiple green chemistry process is being used sequentially in a bio-refinery approach that produce several bioactive material in different steps from the same starting biomass.

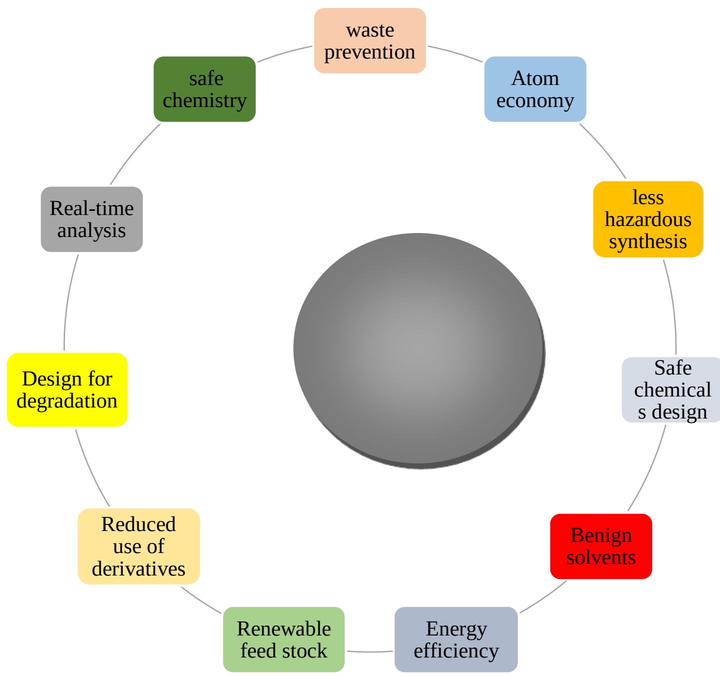

Figure. 1. Principles of Green Chemistry

There are several recent research that highlights the use of green chemistry extraction technologies for extraction of bioactives from seaweed biomass. A summary of major green chemistry extraction techniques used for seaweed extraction is presented in Table 1.

Table 1. Green extraction techniques used for extraction of bioactive compounds from seaweed

Sl.No.	Sample	Extraction aid adopted	Reference
1	*Eisenia bicyclis*	Pressurized liquid method	Shang et al. 2011
2	*Phaeodactylum tricornutum*	Pressurized liquid extraction and microwave assisted extraction	Gilbert et al. 2017

3	*Laminaria saccharina, Sargassum muticum, Fucus distichus*	Dimethyl Sulfoxide	Seely et al. 1972
4	*Phaeodactylum tricornutum*	Maceration, Ultrasound-assisted extraction, Soxhlet extraction, and Pressurized liquid extraction	Kim et al. 2012
5	*Eisenia bicyclis*	Pressurized liquid method	Shang et al. 2011
6	*Phaeodactylum tricornutum*	Ethyl acetate, Pressurized liquid method, Supercritical Fluid Extraction	Camargo et al. 2017
7	*Cylindrotheca closterium*	Microwave assisted Extraction	Pasquet et al. 2011
8	*Undaria pinnatifida*	Supercritical carbon dioxide Extraction	Roh et al. 2008
9	*Laminaria japonica, Undaria pinnatifida, Sargassum fusiforme*	Microwave assisted extraction	Xiao et al. 2012
10	*Isochrysis galbana*	Ethanol extraction	Kim et al. 2012
11	*Undaria pinnatifida*	Enzyme assisted extraction, Dimethyl ether and ethanol	Billakanti et al. 2013
12	*Saccharina japonica and Sargassum horneri*	Supercritical carbon dioxide Extraction	Sivagnanam 2015

13	*Sargassum muticum*	Supercritical carbon dioxide Extraction	Conde et al. 2015
14	*Undaria pinnatifida*	Supercritical carbon dioxide Extraction	Quitain et al. 2013
15	*Undaria pinnatifida*	Supercritical carbon dioxide Extraction and Subcritical Dimethyl ether	Goto et al. 2015
16	*Saccharina japonica*	Supercritical carbon dioxide Extraction	Saravana et al. 2017
17	*Undaria pinnatifida*	Dimethyl ether Extraction	Kanda et al. 2014

ICAR-CIFT, has established several green chemistry processes for extraction of bioactive compounds from seaweed in a biorefinery approach. Enzyme assisted extraction and supercritical carbon dioxide extraction in tandem, have been optimised for extraction of fucoidan and fucoxanthin from brown seaweed. Both, fucoidan and fucoxanthin are generally considered as GRASS or novel food by international regulatory agencies. Both of these phytochemicals from seaweed are being commercially exploited as high value nutraceuticals all over the world. The ICAR-CIFT technology for extraction and formulation of these bioactives have been commercialized to domestic industry who are successfully producing and marketing the products. Food products incorporating these seaweed extracts have been developed by ICAR-CIFT. For example, fucoidan incorporated curd, fucoidan incorporated bakery products, seaweed dietary fibre incorporated ready to eat products. All of these products are successfully transferred to different entrepreneurs. Some of the seaweed-based products from ICAR-CIFT are depicted in Figure 2.

Figure 2. Seaweed incorporated functional food and nutraceutical products from ICAR-CIFT

Foodomics approaches of high throughput identification of bioactives in seaweed

Foodomics has been defined as a new discipline that studies the food and nutrition domains through the application of advanced omics technologies to improve consumer's well-being, health, and confidence. The research and interest around this new discipline has grown exponentially during the last decade. Still, our understanding of how diet affects health is limited to 150 key nutritional components that are tracked and catalogued by the United States Department of Agriculture and other national databases. These nutritional components represent only a small fraction of the more than 26,000 distinct, definable biochemicals present in our food—many of which have documented effects on health but remain unexplored systematically. A 2020 publication in "Nature Food" termed these unknown metabolites as "Dark Matter of Food". The advances in high resolution mass spectrometry have made it possible to decode this dark matter and solve many issues of societal, health, and economic importance. The Foodomics approach has tremendous potential in high throughput identification several bioactive compounds in one go in seaweeds and can establish comprehensively the food value of the seaweed. In ICAR-CIFT, we are carrying out high throughput profiling of seaweed using high resolution mass spectrometry and chemoinformatics technology. This allows us to identify several bioactive compounds in one go and evaluate several seaweeds as potential source for those bioactives. For example, our study revealed that *Sargassum cinereum* is richer source of Fucoxanthin and isomers as compared to other brown seaweed species. Figure 3. Presents a comparative profile of fucoxanthin and isomers in several brown seaweed species.

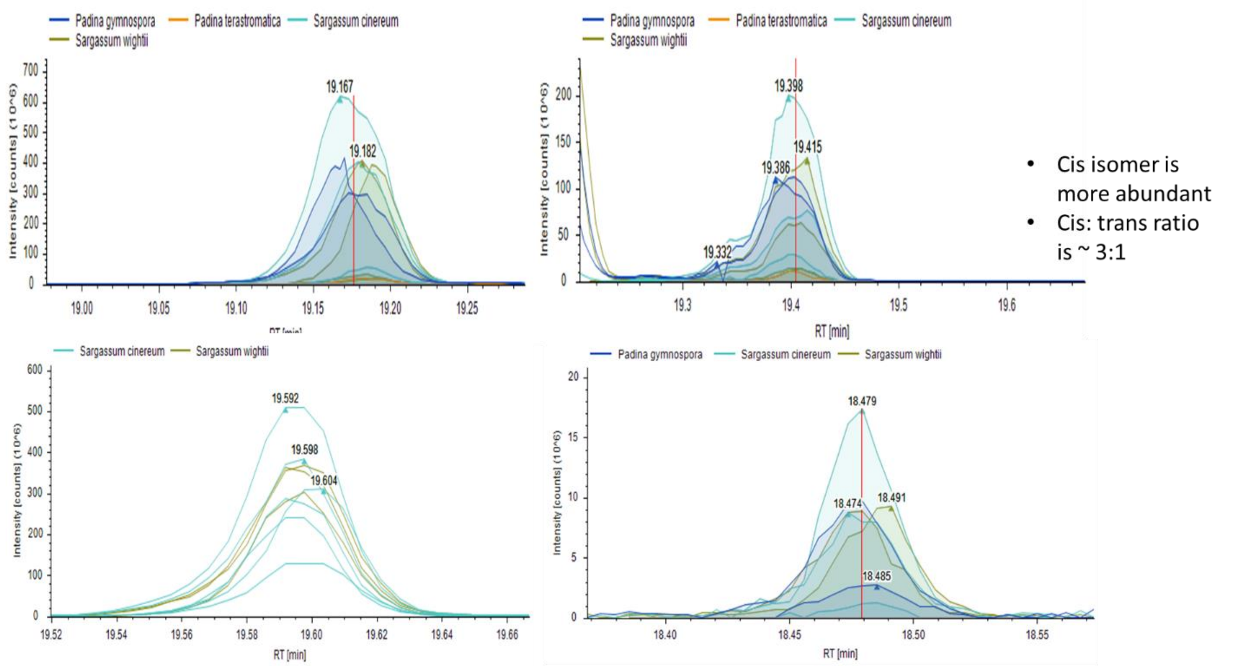

Figure 3. Comparative phytochemical profile brown seaweeds in high resolution mass spectrometer

Quality issues and regulatory challenges in food use of Indian seaweed

For successful use of Indian seaweed as food, and functional food a quality system has to be established, same as any other food/novel food product. Unfortunately, the ecosystem for seafood quality control regime is not well developed at the moment. The seaweed raw materials used for food and feed purposes need to be tested for Iodine and Mercury. In 2006, the European Union (EU) for Scientific Committee on Food (SCF) established an upper limit of 600 µg/day for iodine intake for adults and 200 µg/day for children of 1-3 years of age. For mercury in algae and prokaryotic organisms, a maximum residue level of 0.01 mg/kg is established according to Regulation (EC) No 396/2005. For arsenic, lead, cadmium, and mercury, the maximum levels in the feed are established under EU Directive 2002/32/EC of the European Parliament and the Council. As certain seaweed species are used as feed, the metal content of these species should also be investigated, both for animal health reasons and given the transfer of these metals to food products of animal origin. As per this EU directive 2002/32/EC Aldrin, Dieldrin, Toxaphene, Chlordane, DDT, Endosulfan, Endrin, Heptachlor, Hexachlorobenzene, and Hexachlorocyclohexane needs to be tested.

For polycyclic aromatic hydrocarbons and polychlorinated biphenyls such regulatory limits are not available. However, the presence of these organic pollutants is a possibility in seaweeds and should be monitored. In this case, a default regulatory limit of 0.01 ppm can be considered.

In India, as of now, there is no regulatory limit for heavy metals and persistent organic pollutants in seaweed for food supplement and feed purpose. The Food Safety and Standards (Contaminants, Toxins, and Residues) Regulations, 2011 mentions a regulatory limit for Mercury in non-specified food as 1 mg/kg and Methyl mercury in all food staff at 0.25 mg/kg. The same should be applied to seaweed-based food and supplements. More importantly, the Gazette of India Notification No. 465 on Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purpose, Functional Food and Novel Food) Regulations, 2016 mentions only "Kelp" as an approved nutraceutical or supplement ingredient in India. No other edible Indian seaweeds are listed. This Gazette notification is being amended and may include Indian edible seaweed species as they have long history of use as food.

Regulatory limits for heavy metals have been mentioned in European Commission Regulation (EU) No 231/2012 of 9 March 2012 for high-value food additives from seaweed. Formaldehyde (50 mg/kg), Arsenic (3 mg/kg), Lead (2 mg/kg), Mercury (1 mg/kg), and Cadmium (1 mg/kg) should be monitored. E. Coli should be absent in 5 g, and Salmonella sp. Should be absent in 10 g. In India, the Food Safety and Standards (Food Products Standards and Food Additives) Regulation, 2011 mentions regulatory limits for Agar, Alginates, and Carrageenan. For Agar and Alginate, the Lead and Arsenic content should be no more than 5 and 3 mg/Kg respectively. For Carrageenan, regulatory limits of Cadmium (1.5 mg/Kg), Mercury (1 mg/Kg), Arsenic (3 mg/Kg), and Lead (5 mg/Kg) have been specified. E. Coli and Salmonella sp. should be absent.

Conclusion

Considering the rich biodiversity of seaweed, there is immense potential for the use of Indian seaweed as food, functional food, and nutraceuticals. However, there are many challenges and the quality regime of seaweed-based product to enable export in international markets is not so well developed. ICAR-CIFT has developed several technologies for value added products and nutraceuticals from seaweed. Five of the technologies have been transferred to industries and commercial production has started for four of them. ICAR-CIFT is the national reference laboratory of FSSAI for fish and fisheries products and will have important role to play for quality control of seaweed and seaweed products. The institute has pilot plant facility for process demonstration and have transferred the technology for solar dryers for hygienic drying of fish and fish products. However, a conducive policy environment needs to be formulated for achieving the true potential of seaweed value addition and processing in India.

References

1. Granato, D., Barba, F. J., Kovačević, D. B., Lorenzo, J. M., Cruz, A. G. and Putnik, P. Functional foods: Product development, technological trends, efficacy testing, and safety. *Annual Review of Food Science and Technology*. **020**, 11, 93-118.
2. Lafargaa, T., Acién-Fernándeza, F.G., Garcia-Vaquerob, M. Bioactive peptides and carbohydrates from seaweed for food applications: Natural occurrence, isolation, purification, and identification. *Algal Research*. **2020**, 48:101909.
3. Sahoo, D. (2010). *Common seaweeds of India*. IK International Pvt Ltd.
4. Zhao, L., Chen, G., Zhao, G., & Hu, X. (2009). Optimization of microwave-assisted extraction of astaxanthin from Haematococcus pluvialis by response surface methodology and antioxidant activities of the extracts. Separation Science and Technology, 44(1), 243-262.
5. Bissember, A, (2017). Green chemistry is key to reducing waste and improving sustainability. The Conversation Media Group Ltd, Australia, [Magazine Article].
6. Shang, Y. F., Kim, S. M., Lee, W. J., & Um, B. H. (2011). Pressurized liquid method for fucoxanthin extraction from Eisenia bicyclis (Kjellman) Setchell. *Journal of bioscience and bioengineering*, *111*(2), 237-241.
7. Gilbert-López, B., Barranco, A., Herrero, M., Cifuentes, A., & Ibáñez, E. (2017). Development of new green processes for the recovery of bioactives from Phaeodactylum tricornutum. *Food Research International*, *99*, 1056-1065.
8. Seely, G. R., Duncan, M. J., & Vidaver, W. E. (1972). Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. Marine Biology, 12(2), 184-188.
9. Kim, S. M., Jung, Y. J., Kwon, O. N., Cha, K. H., Um, B. H., Chung, D., & Pan, C. H. (2012). A potential commercial source of fucoxanthin extracted from the microalga Phaeodactylum tricornutum. *Applied biochemistry and biotechnology*, *166*(7), 1843-1855.
10. del Pilar Sánchez-Camargo, A., Pleite, N., Herrero, M., Cifuentes, A., Ibanez, E., & Gilbert-López, B. (2017). New approaches for the selective extraction of bioactive compounds employing bio-based solvents and pressurized green processes. *The Journal of Supercritical Fluids*, *128*, 112-120

11. Pasquet, V., Chérouvrier, J. R., Farhat, F., Thiéry, V., Piot, J. M., Bérard, J. B.,& Picot, L. (2011). Study on the microalgal pigments extraction process: Performance of microwave assisted extraction. *Process Biochemistry*, *46*(1), 59-67.
12. Roh, M. K., Uddin, M. S., & Chun, B. S. (2008). Extraction of fucoxanthin and polyphenol from Undaria pinnatifida using supercritical carbon dioxide with co-solvent. *Biotechnology and Bioprocess Engineering*, *13*(6), 724-729.
13. Xiao, X., Si, X., Yuan, Z., Xu, X., & Li, G. (2012). Isolation of fucoxanthin from edible brown algae by microwave-assisted extraction coupled with high-speed countercurrent chromatography. *Journal of separation science*, *35*(17), 2313-2317.
14. Billakanti, J. M., Catchpole, O. J., Fenton, T. A., Mitchell, K. A., & MacKenzie, A. D. (2013). Enzyme-assisted extraction of fucoxanthin and lipids containing polyunsaturated fatty acids from Undaria pinnatifida using dimethyl ether and ethanol. *Process Biochemistry*, *48*(12), 1999-2008.
15. Sivagnanam, S., Yin, S., Choi, J., Park, Y., Woo, H., & Chun, B. (2015). Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO_2 extraction. *Marine drugs*, *13*(6), 3422-3442.
16. Conde, E., Moure, A., & Domínguez, H. (2015). Supercritical CO2 extraction of fatty acids, phenolics and fucoxanthin from freeze-dried Sargassum muticum. *Journal of applied phycology*, *27*(2), 957-964.
17. Quitain, A. T., Kai, T., Sasaki, M., & Goto, M. (2013). Supercritical carbon dioxide extraction of fucoxanthin from Undaria pinnatifida. *Journal of agricultural and food chemistry*, *61*(24), 5792-5797.
18. Goto, M., Kanda, H., & Machmudah, S. (2015). Extraction of carotenoids and lipids from algae by supercritical CO_2 and subcritical dimethyl ether. *The journal of supercritical Fluids*, *96*, 245-251.
19. Saravana, P. S., Getachew, A. T., Cho, Y. J., Choi, J. H., Park, Y. B., Woo, H. C., & Chun, B. S. (2017). Influence of co-solvents on fucoxanthin and phlorotannin recovery from brown seaweed using supercritical CO2. *The Journal of Supercritical Fluids*, *120*, 295-303.
20. Kanda, H., Kamo, Y., Machmudah, S., & Goto, M. (2014). Extraction of fucoxanthin from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether. *Marine drugs*, *12*(5), 2383-2396