



Recent Innovations of Microalgae Single Cell Phytochemical Studies

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Abstract

Global interest of new consumption, nutrition trends, and market for dietary phytochemical functional supplements have greatly increased over recent decades. In which, commercially available food supplements based on microalgae such as Spirulina (Cyanobacteria) are becoming increasingly popular. Microalgae are rich in important nutritional phytochemicals such as phycocyanin novel protein in Spirulina, polyphenols, flavonoids, and vitamins, all which are known to positively impact human health. This review discusses the beneficial impacts of using microalgae and their phytochemicals in the different fields with their production limitation. Also, different recent analytical methods are highlighted to emphasize the trending of this very important approach which are directly related to the human health and the global food consumption.

Keywords: Microalgae Phytochemistry; organic molecules; analytical methods; single cell Raman; novel bioactive molecules

Introduction

Microalgae is considered as a potential new food sources, due to their composition and nutritional content. In which the organic chemical composition among algal species is affected with the growth environment (light, temperature) and the media composition. The important microalgae nutritional components that affected by the surrounded environment are protein and lipid content, as well as vitamins, mineral, and bioactive contents, all which are known to positively impact human health. There are only a few microalgae that Generally Recognized as Safe (GRAS) by the FDA like (Arthrospira platensis, Auxenochlorella protothecoides, Chlorella vulgaris, Dunaliella bardawil, and Euglena gracilis) Gouda, M.; Huang, Z.; Liu, Y.; He, Y.; Li, X., Physicochemical impact of bioactive terpenes on the microalgae biomass structural characteristics. Bioresource technology 2021, 334, 125232. [1]. Lipids and proteins are an indispensable components of microalgae cells and are precursors of many essential molecules and are a novel source of these bioactive molecules. For instance, Phaeodactylum tricornutum can accumulate up to 30% to 40% of total the fatty acids produced as eicosapentaenoic acid (EPA) of its dry biomass as lipids. Also, these lipids contain essential fatty acids include linolenic acid, linoleic acid, docosahexaenoic acid (DHA), all known

as omega-3 fatty acids [2]. Also, microalgae are a novel sources of novel proteins. These kind proteins include commercially available phycocyanin novel food protein supplement produced from Spirulina (Arthrospira platensis; or Cyanobacteria) [3]. Microalgae are rich in important nutritional phytochemicals such as protein in Spirulina, polyphenols, flavonoids, and vitamins, all which are known to positively impact human health. On the other hand, as an effective alternative to non-biodegradable plastics, edible films which are biopolymer-based materials created from microalgae as a natural safe source for polysaccharides offer numerous points of interest nowadays in food science and technology field. One of these biopolymers, alginate is one of the foremost versatile biodegradable polymers which are algal extracted polysaccharide [4]. On the other hand, however, these approved microalgae have long history of safety a serious concerns have been raised against some products manufactured from these microalgae due to their contamination with cyanotoxins, toxic metals, or inorganic arsenic. In which, one of the common reasons for serious contamination in microalgal products are coming from the improper culture media purity and the presence of the toxic cyanobacteria species. For instance, microcystins are the most common cyclic protein peptide toxins

which mainly affect microalgae's safety. They are considered as a powerful hepato-toxins, and they are treated as very dangerous for causing liver cancers to mammals as it inhibits acetylcholinesterase activity needed for cell regulation [5]. Thus, there are a high need to establish and present comprehensive overview of microalgae organic composition advanced nondestructive and instant measurements which could be applied during its cultivation process. In which, scientists have been working hard to explore the scientific essence of phytochemicals principle in pharmaceutical and food science fields [6-8]. The physical and chemical effects of the different industrial process on the functional phytochemicals could change its complicated chemical composition and desired complex mechanism Gouda, M.; El-Din Bekhit, A.; Tang, Y.; Huang, Y.; Huang, L.; He, Y.; Li, X., Recent innovations of ultrasound green technology in herbal phytochemistry: A review. *Ultrasonics sonochemistry* 2021, 73, 105538. [9]. Thus, too many beneficial impacts could be reached by enhancing this field biochemical investigation. A multidisciplinary approach to evaluate their phytochemistry and correlate them with their safety could be one of the novel ways. In which, several analytical methods could be combined like spectroscopic, chromatographic, electrochemical, immune, and molecular techniques to discuss and enhance this field. All

of these methods are confirmed their novelty when they mixed together as a multi-combined approach for microalgae sensation and analysis. Also, the potential uses of microalgae extracts for treating of different health diseases will add to this field. Several sources, phytochemistry, and the risks of the different alternative and edible microalgae is very important to be further investigated. Also, the common techniques and the fast used ones in this field are mentioned with their innovations and limitations.

Methods for Identification of Microalgae Macromolecules

Microalgae macromolecules assessment has been established for several decades. Of particular interest is lipids, and carbohydrates reported for certain uses obtained from insects and plant sources. A promising strategy for microalgae chemical assessment is using bioinformatics that provide a fast predication tool of its lipids, proteins and even carbohydrates. Nowadays, a potential limitation is the lack of reference structures of some microalgae proteins and thus wild range of analytical methods should be used for building a strong database based on these different analytical destructive or nondestructive techniques combined with chemometrics and other informatics methods (Figure 1).

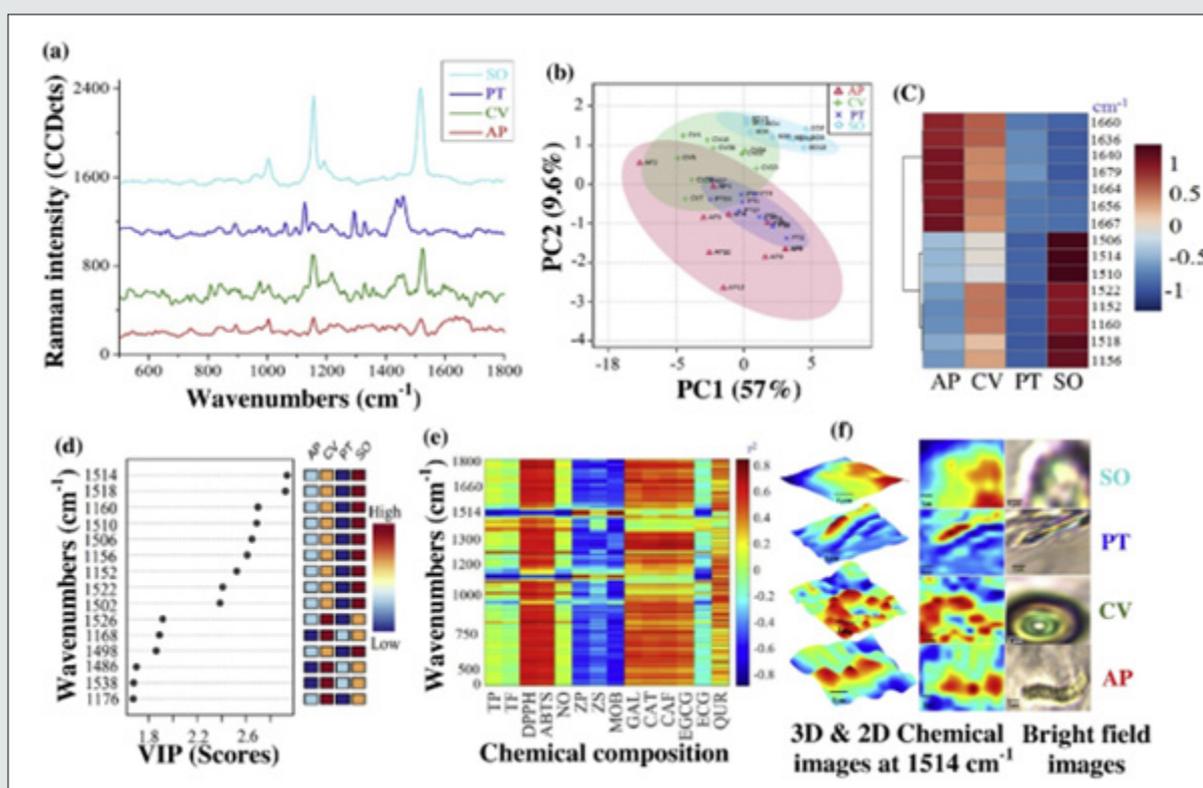


Figure 1: Single-cell Raman micro spectrometry (SCMR) chemical composition of four different microalgae species; (a) Full spectra of the four studied species; (b) Principle component analysis; (c) Clustering heatmap; (d) Important features identity classification; (e) Correlation (r^2) summarization between the spectral bands and the chemical composition; (f) 3D & 2D chemical images at wavenumber of 1514 cm^{-1} ($1\text{ }\mu\text{m}$), and the bright-field images. *Arthrospira platensis* (AP), *Chlorella vulgaris* (CV), *Phaeodactylum tricornutum* (PT), and *Scenedesmus obliquus* (SO). (Copyright permission: 5015110738674) [1].

Chemical dependent destructive methods

The analysis of microalgae proteins, lipids and other different hydrocarbons using mass spectrometry approach is a very promising and fast method for multi-detection and in-depth characterization of the physicochemical properties of these kinds of molecules [10]. Also, chromatography enables researchers to separate their components, identifying their properties, and determining their amounts [11].

Chemical free non-destructive methods

Spectroscopic methods for microalgae chemical composition become a very popular techniques. For instance, Raman micro-spectroscopy is a rapid, chemical-free, and non-invasive tool that is used for characterization of single-cell molecules and their activities through detection information of functional groups frequency vibration by these molecules' laser light inelastic scattering [1,12]. Also, Raman spectroscopy has been used in many aspects of single microalgae research, such as chemical imaging of microalgae biochemical molecules like antioxidants phytochemicals [1] (Figure 1), astaxanthin [13], and carotenoids [14]. On the other hand, single-cell electrochemical current by microelectrode has emerged as an increasingly important technique for fundamental studies of single-cell activities and functionality [15]. It is known that the combination of biomolecules, especially antioxidants, with nanoparticles creates interesting features for the development of nano sensors [16]. Gouda Chen [1] fabricated a new method for detecting four species of microalgae single cell current (SCC) by using gold nanoprobe. However, specific care is needed to maintain a very small current response (10^{-10} Amp) relation with the actual biochemistry of cells. Therefore, continuous development of intracellular electrochemical detection and their relationship with the chemical invasive methods and the other non-invasive methods like micro-Raman spectroscopy should be more solidify.

Conclusion

In conclusion, the chemical analytical measurement of microalgae species could enhance its applicability in the fields of pharmaceutical, food and all related science. In which, the development of their application in microalgal strain screening, and studying of the real-time functional activity of its phytochemicals and bioactive compounds during microalgal cultivation, as well as evaluation of its physiological status are a very important approach to be developed.

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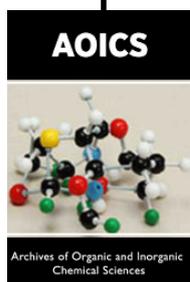


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