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Review

Enzyme-assistant extraction (EAE) of bioactive components: A useful approach for recovery of industrially important metabolites from seaweeds: A review

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ABSTRACT

Over the years, the biological activities of seaweeds could have gained a considerable research interest because of their specific functional compounds, which may not be available in land plants. Thus, efforts at discovery of novel metabolites from seaweeds over the past years have yielded a considerable amount of new active compounds. In addition, studies about the extraction of active compounds from natural products have attracted special attention in the last recent years. Potent biologically active compounds of seaweeds have been demonstrated to play a significant role in prevention of certain degenerative diseases such as cancer, inflammation, arthritis, diabetes and hypertension. Therefore, seaweed derived active components, whose immense biochemical diversity looks like to become a rich source of novel chemical entities for the use as functional ingredients in many industrial applications such as functional foods, pharmaceuticals and cosmeceuticals. Thus, the interest in the extraction of active compounds from seaweeds is obvious. However, the physical and chemical barriers of the plant material become the key drawbacks of such extraction process. Therefore, enhanced release and recovery of active compounds attached to the cells have been addressed. Taken together, the aim of this communication is to discuss the potential use of enzyme treatment as a tool to improve the extraction efficiency of bioactive compounds from seaweeds.

Contents

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1. Introduction

Seaweeds are potentially excellent sources of highly bioactive secondary metabolites that could represent useful leads in the development of new functional ingredients [1]. They are a large and diverse group of simple, typically autotrophic organisms ranging from unicellular to multicellular forms. Macroalgae (seaweeds) can be classified into three broad groups as red algae, brown algae and green algae, based on their pigmentation [2]. These naturally growing seaweeds are an important source of food, especially in Asian countries such as China, Japan and Korea [3,4]. In addition, many reports have been published regarding isolated compounds from seaweeds with various biological activities, demonstrating their ability to produce important metabolites unlike those found in terrestrial species [5].

Seaweeds have been recognized to provide chemically and functionally novel metabolites. Those secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity including antioxidant, antiinflammatory, anticancer, antidiabetic and anti HIV activity [6]. Therefore, seaweeds can be considered as very interesting natural sources containing new compounds with numerous biological activities that could be used as functional ingredients in many industrial applications such as functional food, pharmaceutical and functional cosmetic industries [7]. Thus, the investigation of seaweed derived chemical compounds, a different source of natural products, has proven to be a promising area of functional ingredient study. An expanding market for natural products is a fact and is facing a new challenge of growing algae on a large-scale without harming any further the marine environment [5].

Various extractants were used to release soluble compounds from the algal matrix. The basic procedure for large-scale samples is to extract the algal powder with water or organic solvents. Under these conditions, the extraction yield varies from 8% to 30% of the algal dry yield [8]. The presence of various polysaccharides of large quantities in the cell wall strongly reduced the extraction efficiency during application of classical extraction methods. However, recently, new kinds of extraction techniques appeared, such as enzymolysis and microwave-assisted extraction. The former has impressive effects with characteristics of high catalytic efficiency, high specificity, mild reactive conditions and preserving the original efficacy of active compounds to the maximum [9]. The latter method also has many advantages, such as shorter time, less solvent, higher extraction rate and better products with lower cost [10,11]. In addition to the studies of the soluble compounds, there are compounds attached to the cell wall (cell-wall-bound compounds) which cannot be easily extracted using typical extraction methods with aqueous solvents. Further, this might limit the study and potential industrial applications of seaweed derived active components. Interestingly, enzymatic digestion of algae gains high bioactive yield and shows enhanced biological activity in comparison with water and organic extract counterparts [12]. In this point this review is a discussion about the use of enzyme-assistant or the enzyme-enhanced extraction as an alternative method to improve the recovery of industrially useful compounds from seaweeds.

2. Enzymatic degradation of algal cell walls and extraction of bioactive components

The discovery of new chemical entities has become the modern focus of much natural product works [13]. Recent trends in active compound study from natural sources have shown that seaweeds are promising materials to discover novel biologically active compounds. In addition, the search for bioactive compounds from seaweeds has been a very attractive research area and a number of recent research communications dealt with bioactive compounds isolated from them [6]. Extraction is the most important and starting step in isolating different types of bioactive compounds from plant materials. However, the extraction efficiency of active components from seaweeds is reduced due to the presence of complex cell wall polysaccharides such as alginates and carrageenans. Hence, different extraction methods have been employed in order to maximize the extraction efficiency of active compounds [14]. Further, the extraction efficiency of active compounds from any plant material can be affected by a number of factors such as an extraction solvent, particle size, temperature, time, pH *etc.* However, the ideal extraction method should be quantitative, non-destructive and time-saving.

The high contents of various cell wall polysaccharides in seaweeds limit the active compound accessibility. Plant cell wall mainly consists of interconnecting polysaccharides, the most abundant source of organic carbon in the biosphere. Generally cell walls are made of highly complex large biopolymers such as cellulose, hemicellulose, lignin and pectin. Cellulose, the most abundant carbohydrate polymer in nature is the main structural component of plant cell walls and it is extremely difficult to degrade, as it is insoluble and is present as hydrogen-bonded crystalline fibers [15]. However, seaweed cell walls and cuticles are chemically and structurally more complex and heterogenous than those of land plants. They are composed of mixtures of sulfated and branched polysaccharides which are associated with proteins and various bound ions including calcium and potassium [16]. Some of the red algae cell walls are made of cellulose, agars and carrageenans [17]. Therefore, it is clear that the physical barriers due to cell wall polysaccharides limit the efficiency of general extraction procedures of active compounds from seaweeds. Enzymatic degradation of cell wall polymers has received attention for many years and is becoming a more and more attractive alternative to chemical and mechanical processes. Therefore, the degradation of cell wall polysaccharide structures is a fundamental step in the release of active components. Hence, degradation of plant cell wall polysaccharides is of major importance in many applications. In contrast, use of appropriate enzyme treatment to improve the extraction efficiency of algal active components could be a useful step in classical extraction procedures.

3. Importance of enzyme treatment prior to extraction of bioactive compounds

This promising biotechnological procedure has been widely used to improve the extraction efficiency of bioactive components from land plants. In contrast, the successful use of this technique to enhance the recovery of polyphenols

from citrus peel [9], grape skin [18], apple skin [19], unripe apples [20], black currant [21] and *Ginkgo biloba* leaves [22] has been reported. In addition, the application of the enzyme-assistant extraction (EAE) method on seaweed materials was also reported (Table 1). Seaweeds are sustainable natural resource with industrial potential that is not fully utilized and there is a considerable interest in the use of seaweeds in commercial applications, especially in adding value to extracted components for a wide range of uses such as nutraceutical, cosmeceutical and functional food. In order to identify conditions improving algal extraction efficiency, their cell wall materials can be sequentially extracted by different solvents. The yields of extracts, the chemical compositions, the chemical structures and the macromolecular properties of soluble extracts and insoluble residues depend on the nature of interactions of cell wall components. Therefore, alternative extraction conditions and techniques such as EAE can be successfully employed in order to degrade seaweed tissues on the basis of recovery of biologically active compounds with a considerably high yield from seaweeds. In addition, the technique allowed successful production of water-soluble materials from seaweed materials as well. By contrast, the use of cell wall degrading enzymes as alternative extractive auxiliaries might facilitate the better access to the seaweed derived metabolites.

4. Selection of appropriate enzymes and optimum extraction conditions

Enzymes play a critical role in many commercial applications. They are biological catalysts. Therefore, the enzymatic hydrolysis of substances depends on several physicochemical factors. Selection of appropriate hydrolytic enzyme or optimal mixture of enzymes is vital to obtain expected output. Addressing the potency issue first has to select the suitable enzyme to digest specific polymer bonds present in the intact seaweed materials. After selection of the suitable enzymes various process conditions can be employed in order to obtain the maximum recovery of active components. There are several factors that directly influence the effect of enzymes in the degradation of cell wall polymers and release of the target active compounds. Therefore, the influencing factors can be adjusted to find the optimal reaction conditions. Thus, the optimization strategies in EAE are crucial.

Incubation time–temperature combination of the enzymatic treatment prior to extraction is possibly one of the most important factors to be considered. In the case of enzymatic reactions, many enzymes are adversely affected by high temperatures. Specially, when extracting polyphenols, temperature cannot be increased indefinitely due to their instability. Further, pH influences the rate of enzyme reaction to a large extent. The most favorable pH value, the point where the enzyme is most active is known as the optimum pH. Therefore, to perform the enzyme activity at maximum level, temperature and pH should be adjusted to their optimal conditions. The optimum reaction conditions (pH and temperature) of commonly used enzymes are shown in Table 2. The proportion of substrate to enzyme or the enzyme concentration is also one of the most considerable variables. The type of the extraction solvent used and the solvent to material ratio are also other important factors to be considered. In fact, the type of solvent is one of the most influencing variables in the extraction process. In addition, size of the interested molecules and agitation also play a critical role during the extraction process. Fig. 1 summarizes the critical steps involved in the EAE process.

5. Possible bioactive components from seaweeds

Seaweeds represent a valuable source of novel bioactive compounds. Over the years many active components have been isolated from seaweeds with diverse chemical nature.

5.1. Polyphenols and brown algal phlorotannins

The formation of excess reactive oxygen species (ROS) is an unavoidable consequence in aerobic organisms as byproducts during metabolic respiration [23,24]. These highly reactive ROS can be considered as strong oxidants and have been shown to induce damage in all cellular macromolecules, such

Table 1
Summary of enzyme-assistant extraction of bioactive components from seaweeds.

Seaweed	Enzyme/s treated	Bioactivity	Reference
Porphyra yezoensis (red)	Digestive enzymes obtained from the gut of abalone		[55]
Ulva pertusa (green)	Cellulase and macerozyme mixture		[56]
Laminaria japonica (brown)	An extract of gut from abalone in addition to the same enzymatic mixture used for the green seaweeds A mixture of digestive enzymes from the gut of abalone plus		[56]
Callymenia perforate (red)	Macerozyme		[56]
Chondrus crispus (red)	Carrageenase and cellulase		[57]
Garcilaria verrucosa (red)	Agarase and cellulase		[57]
Sargassum horneri (brown)	Commercial carbohydraces and proteases	Antioxidant	[68]
Scytosiphon lomentaria (brown)	Commercial carbohydraces and proteases	Antioxidant	[67]
Ecklonia cava (brown)	Food industrial carbohydrates	Antioxidant	[63]
Seven species (brown)	Commercial carbohydraces and proteases	Antioxidant	[12]
Ecklonia cava (brown)	Proteases	Antioxidant	[60]
Ecklonia cava (brown)	Kojizyme	Immunomodulatory	[64]
Hizikia fusiformis (brown)	Alcalase and ultraflo	Antioxidant	[59]
Ecklonia cava (brown)	Kojizyme	Immunomodulatory	[65]
Palmaria palmate (red)	Umamizyme	Antioxidant	[58]
Ecklonia cava (brown)	AMG	Antiinflammatory	[66]

Table 2
Optimum conditions for some commonly used enzymes.

Enzyme	Optimum conditions		Reference
	pH	Temperature (°C)	
Viscozyme	4.5	50	[12,60]
Celluclast	4.5	50	[12,60]
AMG	4.5	60	[12,60]
Termamyl	6.0	60	[12,60]
Ultraflo	7.0	60	[12,60]
Carrageenase	6.8	45	[57]
Agarase	6.0	55	[57]
Xylanase	5.0	55	[57]
Cellulose	3.8	50	[57]
Protamex	6.0	40	[12,60]
Kojizyme	6.0	40	[12,60]
Neutrase	6.0	50	[12,60]
Flavourzyme	7.0	50	[12,60]
Alcalase	8.0	50	[12,60]
Umamizyme	7.0	50	[58]

as lipids, proteins and DNA. Over the years, dietary polyphenols from seaweeds have been widely studied for their biological activities including antioxidant activity [25–28]. Polyphenols are naturally occurring compounds containing phenolic functionality and this large diverse group of secondary metabolites exists both in terrestrial and aquatic environments [29,30].

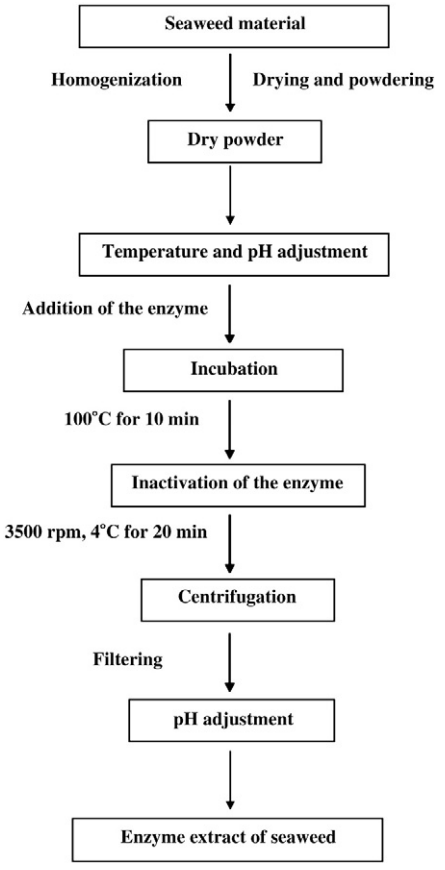

Fig. 1. Preparation of enzyme extracts from seaweeds.

Besides the strong antioxidant properties, these naturally occurring polyphenols are known to have numerous biological activities such as antiinflammation [31], antiallergic [32], antibacterial [33,34], antiplasmin inhibition [35], matrix metalloproteinase inhibition [36] and anticancer [37]. Therefore, the possibility of use of these active compounds in many industrial applications as functional ingredients is very clear. Phlorotannins (brown algal polyphenols) are polyphenolic compounds found exclusively in brown seaweeds. Phlorotannins, a subgroup of tannins, are produced entirely by polymerization of phloroglucinol units [38,39]. During the last two decades, the roles and functions of phlorotannins have been the subject of many studies [33].

Use of organic solvents such as ethanol or methanol is the common method for the extraction of polyphenolic compounds from plant materials. In addition, aqueous mixtures of the particular organic solvents also improve the extraction efficiencies. But, some plant polyphenols appear to be twisted together with cell wall polysaccharides *via* tight hydrophilic and hydrophobic bonds. Moreover, the distribution of polyphenols is not homogeneous inside the plant tissues. Therefore, the release of these polyphenolic compounds can be enhanced by enzyme-enhanced degradation of cell wall polysaccharides [18]. However, polyphenolic compounds that exist in plants are not always associated with the plant cell walls [18]. The ability of tannins to form strong complexes with proteins, either reversibly by hydrogen bonding through peptide or amide linkages, or irreversibly by covalent condensations is well-known [40]. This protein-binding activity depends mainly on the structure of the protein as well as the length and the structure of the tannin polymer [41,42]. Moreover, phlorotannins are found to form covalent bonds with proteins; further, the protein precipitation of phlorotannins varies in a pH-dependent as well as a concentration-dependent fashion [40]. Thus, this interaction between phlorotannins and proteins could be one of the most important factors to be considered in the extraction of either proteins or phlorotannins. The techniques used for extraction of tannins from plant materials are widely variable.

5.2. Bioactive polysaccharides from seaweeds

Seaweed derived polysaccharides are the most widely studied group of metabolites together with polyphenols [5]. Generally, the polysaccharide contents of seaweeds vary according to the species. Marine algae appear to be good sources of fibers presenting great chemical, physicochemical and rheological diversities [43]. Dietary fibers from edible seaweeds are little or non-digested by man [44]. In addition, sulfated polysaccharides of soluble fibers, found in cell walls of marine algae are not toxic for human. Fucans and alginic acid derivatives are known to exhibit different biological properties such as anticoagulant, antiinflammatory, antiviral and antitumoral activities [45,46]. Commonly these polysaccharides have been extracted using water or aqueous organic solvents. However, since the cell wall consists of complex polymers, it is not easy to extract active polysaccharides using solvent extraction process. The production of different bioactive polysaccharides with enzymatic digestion is required for the development of more functional ingredients for industrial use. Therefore, EAE can be employed as an

alternative method to improve the extraction efficiency of bioactive polysaccharides from seaweeds.

5.3. Carotenoids of seaweeds

The value of seaweed natural source of active metabolites is not only due to polyphenols and sulfated polysaccharides, but also because of the presence of useful pigments such as carotenoids. Carotenoids are tetraterpenoid organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus, some bacteria and aphids. People consuming diets rich in carotenoids from natural foods, such as fruits and vegetables, are healthier and have lower mortality from a number of chronic illnesses [47].

Carotenoids with molecules containing oxygen, such as lutein and zeaxanthin, are known as xanthophylls. Fucoxanthin is a xanthophyll, with formula $C_{42}H_{58}O_6$. It is found as an accessory pigment in the chloroplasts of brown algae and most other heterokonts, giving them a brown or olive-green color. In addition, fucoxanthin is one of the major xanthophyll variants in brown seaweeds with important biological functions such as anticancer, antihypertensive, antiinflammatory, antioxidant and antiobesity effects [48,49]. Currently, the most common way for extraction is by liquid solvent extraction using toluene, hexane, or petroleum ether [50]. Barzana et al. [51] reported that successful use of enzyme-mediated solvent extraction of carotenoids from marigold flower (*Tagetes erecta*). In contrast, 97% recovery yield of carotenoids was obtained under the optimal conditions. Generally, enzyme extraction prior to mechanical or solvent extraction has improved yields of target components. However, up-to-date there was no literature report found on extraction of carotenoids from seaweeds using the EAE process. The process could be employed to enhance the extraction efficiency of carotenoids from brown seaweeds.

5.4. Bioactive peptides from seaweeds

There is considerable evidence to suggest that seaweed derived proteins and peptide fragments can exert biological effects both *in vitro* and *in vivo*. Bioactive peptides can be generated by hydrolytic reactions using various proteases [52]. The role of proteins as physiologically active components in the diet is being increasingly acknowledged. The primary structure of natural proteins consists of certain amino acid sequences that have the ability to exert physiological benefits in human beings. Those kinds of peptides are inactive within the sequence of the parent protein and can be released in different ways such as enzyme hydrolysis by digestive enzymes and hydrolysis by proteolytic microorganisms.

Recently marine peptides have opened a new perspective for pharmaceutical developments. Biologically active peptides and proteins have been isolated not only from marine animals, but also from seaweeds [53]. With respect to nutraceuticals and pharmaceutical potentialities of seaweeds, different proteins and peptides with various bioactivities have been discovered. Some red or green seaweed species contain considerably high amount of proteins. However, extraction of protein from most seaweeds is difficult due to the presence of large amounts of cell wall polysaccharides, such as alginates of the Phaeophyta or the carrageenans of some Rhodophyta [54]. Amano and Noda [55] reported the use of algal cell wall degradation enzymes to facilitate the extraction of proteins from red alga *Porphyra yezoensis*. Further, they demonstrated the use of an enzymatic mixture, including digestive enzymes to improve the protein accessibility. In addition, the technique was tested on several algae including *Ulva pertusa*, *Laminaria japonica* and *Callymenia perforate* and significant differences were obtained in protein composition for all the treated seaweeds [56]. Fleurence et al. [57] reported that the simultaneous application of carrageenase and cellulase activities on red alga *Chondrus crispus* led to a 10-fold increase in extraction efficiency of the proteins. Further they demonstrated that coupled use of agarase and cellulase on another red alga *Gracilaria verrucosa*, led to a 3-fold increase in protein yield. Therefore the cooperation of polysaccharidases is an attractive and efficient alternative method which is facilitating access to the algal protein fraction. Moreover, in the case of enzymatic hydrolysis, the initial non-specific breakdown or the depolymerization of structural and storage polysaccharides could enhance the easy access of proteases to their respective substrates which are located inside the cells. However, the protein content of seaweeds is considerably low when compared to polysaccharides or polyphenols therefore; seaweeds are rarely promoted for the functional properties of their proteins.

6. Biological activities of enzyme-assistant extracts from seaweeds

Application of EAE for recovery of industrially important bioactive components from seaweeds is a promising technique. The potential antioxidant properties of enzymatic extracts from seven species of brown seaweeds were reported by Heo et al. [12]. Four different ROS scavenging assays were used to evaluate the antioxidant potentials. In this study they have employed commercially available five different carbohydrate degrading enzymes and five proteases. According to their results, the enzyme extracts showed remarkable antioxidant effect and the activity indicated a marked correlation with phenolic contents as well. One of the recent studies on EAE showed the enhanced recovery of polyphenols and other hydrophilic antioxidant compounds from the red algae *Palmaria palmata* [58]. The effect of various protease and carbohydrase treatments on the extraction of the active compounds from the red algae was also discussed in the same experiment. In addition, Siriwardhana et al. [59] reported that effective extraction of algal bioactive compounds can be achieved with treatments such as pH control, heat and enzymatic hydrolysis. They also demonstrated that integration of those optimized treatments in the extraction sequence of heat, enzymatic hydrolysis and pH control was the most effective sequence to extract antioxidants from the brown algae *Hizikia fusiformis*.

Ecklonia cava is a brown alga that is found abundantly in the sub tidal regions of Jeju Island, Korea and Japan [60,61] and it has long been utilized as a traditional food and also as a traditional folk herb [28,62]. In addition, this brown seaweed has a variety of compounds including peptides, carotenoids, fucoidans, and phlorotannins showing different biological activities [26]. Therefore, it is thought that *E. cava* would be a very useful

material in the production of functional foods. Thus, food grade extraction of *E. cava* is required. The brown seaweed *E. cava* was enzymatically hydrolyzed to prepare water-soluble extracts, using five carbohydrases and five proteases, and their potential antioxidant activities were reported [60]. Their results indicated that the > 30 kDa fraction of the Celluclast enzymatic extract possesses good antioxidant activity against H_2O_2 mediated cell damage *in vitro*. Heo et al. [63] suggested that the enzymatic extraction of seaweeds for the purpose of obtaining natural antioxidants would provide advantages of simple and large-scale production-process of perfect water-soluble antioxidant extracts. In addition, they have reported that the potential antioxidant activity of enzymatic extracts from *E. cava* was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl and alkyl radical scavenging using electron spin resonance (ESR). Further their results confirmed the carbohydrase extracts of *E. cava* as potent antioxidants. In addition, another two recent reports revealed the immunomodulatory effects of enzymatic extracts from *E. cava* [64,65]. Kang et al. [66] reported another successful use of EAE on purification of sulfated polysaccharide from *E. cava*. The study demonstrated the potential antiinflammatory activity of a sulfated polysaccharide purified from amyloglucosidase (AMG)-assistant extract of *E. cava*. The brown seaweed *E. cava* has been identified as a source of fucoidan, which is a useful bioactive sulfated polysaccharide. According to the results of the study, the purified polysaccharide significantly inhibited NO production, prostaglandin-E2 (PGE2) production and suppressed inducible nitricoxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression in LPS-stimulated RAW264.7 cells. Therefore demonstrated antiinflammatory activity of the isolated polysaccharide from *E. cava* might be attributable to the modulation of antiinflammatory agents.

Hydrolytic enzymes can convert water-insoluble seaweed materials into water-soluble materials. Hence, thought that different bioactive properties will be expected with the resulting bioactive materials from enzymatic extracts of seaweeds. Similarly Ahn et al. [67] reported the radical scavenging activities of the enzymatic extracts hydrolyzed from a brown seaweed *Scytosiphon lomentaria*. According to the results obtained they have reported the potent antioxidant properties of the enzymatic extracts of *S. lomentaria*.

The antioxidant activity of water-soluble natural extracts from edible brown seaweed, *Sargassum horneri*, was evaluated by examining the radical scavenging activities of the enzyme extracts of hydrolyzates from *S. horneri* [68]. The brown seaweed was enzymatically hydrolyzed to obtain water-soluble extracts using commercially available proteases and carbohydrases. Further they reported that Alcalase and Viscozyme extracts were more effective than the other extracts tested.

With these significant results it is clear that EAE affords big advantage over commonly used classical extraction technique. Taken together, enzyme treatment previous to extraction has resulted in improved yields in the case of bioactive components from seaweeds. In all instances mentioned above, hydrolytic enzymes have been used in different combinations as agents that interact on cell walls, breaking down the structural integrity rendering the intracellular materials more exposed for solvent extraction. Since EAE has attracted growing interest in the extraction of biologically active components, the technique could be explored as a means to enhance the extraction of particular metabolites from seaweeds.

7. Conclusion

Seaweeds represent a valuable source of new compounds. The biochemical diversity of seaweeds becomes a rich source of novel chemical entities for the discovery of more effective functional metabolites. Bioactive compounds discussed here are obtained from different seaweeds exhibiting different chemical structures and displaying a large variety of biological effects on specific targets. On the other hand, these components seem to be very useful and promising for biological research to clarify the mechanism of action in the human body as well as in the design of very specific and potent new functional ingredients for a wide variety of industrial applications such as functional foods, pharmaceuticals and functional cosmetics. Enzymes convert water insoluble materials into water-soluble materials and this method does not adapt any toxic chemicals. In addition, enzymatic digestion helps in removing mechanical barriers for both water soluble and non-soluble bioactive compounds. Therefore, cheap and food grade enzymes may be useful in the future to extract commercially important compounds from algal bio-mass. Hence, EAE of bioactive compounds from seaweeds could be a useful technique and the process may provide a valuable alternative to overcome physical and chemical extraction barriers. Therefore, from this point the potential use of EAE to improve value-added utilization of seaweed extracts as biologically active ingredients in appropriate industrial applications is suggested. Thus, enzyme treatment has been proposed as an alternate stage to solvent extraction processes to improve the extraction yields and the quality of several bioactive compounds.

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