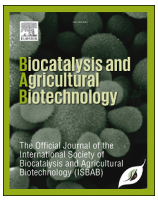
Biocatalysis and Agricultural Biotechnology 56 (2024) 103042

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab



Enzymatic processing of algae for food applications

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ARTICLE INFO

Handling Editor: Dr. Ching Hou

Keywords:
Algae
Enzymatic treatment
Food application

ABSTRACT

Today, almost all human health problems are related to malnutrition. Algae (macro- and micro-algae) are potent to provide the necessary nutrients for our bodies (i.e., starch, lipids, protein, dietary fiber, minerals, and vitamins). Nevertheless, there are limitations due to the extraction efficiency, size, compatibility, and hydrocolloid nature of some algal compounds of interest to the food industry. As a result, enzyme-assisted extraction of algal biomass under optimal conditions will be a safer and more sustainable approach than using hazardous organic solvents. Also, enzymes can be used to modify molecular structures and introduce new biomolecules that exhibit higher stability of interest to food industries. Throughout this review, we explore the most recent research on enzymes used to process algal biomass for use as food.

1. Introduction

In response to climate extremes and population growth, the yield of our production systems needs to be doubled, and alternative food resources need to be developed. In this regard, algae are exceptionally attractive for producing various a wide variety of biomolecules, including carbohydrates, lipids, proteins, and amino acids, owing to their diversity of metabolites. However, there are limitations due to (1) extraction efficiency of algal biomolecules and (2) the size, compatibility, and hydrocolloid nature of some algal compounds (Naseri et al., 2020a,b; RU et al., 2020; Kim et al., 2022). As a practical solution, a wide range of biological and chemical agents, such as enzymes, can be used (Fig. 1). Over centuries, humans have unintentionally used enzymes to improve food processes, but Wilhelm Kühne coined the word enzyme in 1877 (Heckmann and Paradisi, 2020).

Nowadays, enzyme applications in food science have become industry standards and commercially available since they have advantages, including high catalytic efficiency, specificity, and mild condition of action during the extraction process. Enzymes utilized in commercial applications across various sectors encompass amylases, proteases, lipases, cellulases, xylanases, catalases, and more. Notably, α-amylases stand out as particularly versatile within the industrial enzyme sector (Li et al., 2012). These commercially available enzymes can hydrolyze most peptide bonds in a protein molecule (i.e., Alcalase-Protease), degrade cell walls, and break down pectin-like compounds as well as β-glucans and arabinoxylans (i.e., Viscozyme and Ultraflo), breaks down cellulose to glucose, cellobiose, and longer glucose polymers (i.e., Celluclast), hydrolyzes malt sugars into glucose, hydrolyzes the 1, 4, and 1, 6 linkages in starch (i.e., Amyloglucosidase), hydrolyzes the alpha-1, 4 glycosidic linkages in polysaccharides (i.e., Termamyl), etc. (Fig. 2) (Naseri et al., 2020a,b; Heckmann and Paradisi, 2020; Li et al., 2022). Enzyme-assisted extraction (EAE) is based on the principle of destructing algal cells through hydrolysis by an enzyme under optimal conditions. In addition to producing superior extraction results, EAE minimizes the use of hazardous organic solvents. Furthermore, EAE of the cell wall during algal extraction presents a significant alternative to mechanical treatment because of its mildness and specificity (Kim et al., 2022; Zhao et al., 2019). This review delves into the utilization of enzymes in processing algae for food applications. We explore the factors influencing algae extraction, discuss optimiza-

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https://doi.org/10.1016/j.bcab.2024.103042
Received 31 August 2023; Received in revised form 20 December 2023; Accepted 22 January 2024
Available online 26 January 2024

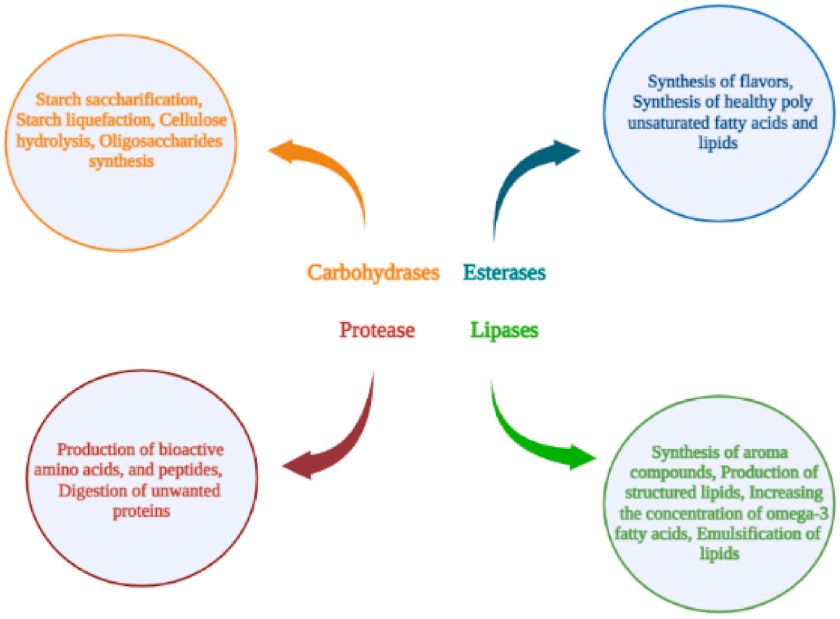

Fig. 1. Overview of enzymes commonly used in processing algae in the food industry. Note: Enzymes identification for converting the algal-specific macro-metabolites into the desired compounds for use in food processing is a major challenge in enzymatic processing.

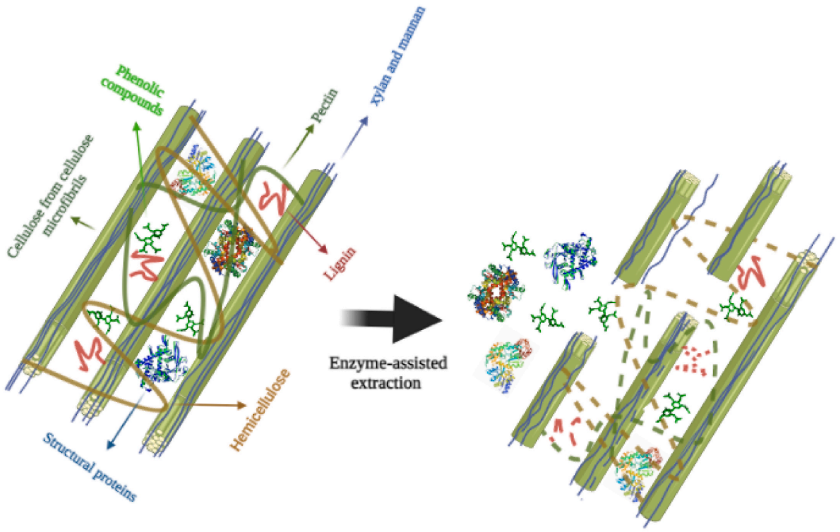

Fig. 2. Illustration of the enzyme-assisted extraction of algae. Note: The presence of cellulose, hemicellulose, lignin, pectin, xylan, and mannan hinders the extraction of high value metabolites from algae biomass. Thus, during the extraction processes enzymes such as cellulose, hemicellulases, and pectinases can be used for disruption of the algal cell walls and to improve the availability of the high value metabolites.

tion parameters, and overview strategies to reduce the cost associated with enzymatic processing for food applications involving algae.

2. Parameters influencing the enzyme-assisted extraction of algal biomolecules

Biomolecules mainly exist in the forms of soluble and insoluble forms. Although free forms of bioactive molecules are easily extracted using polar aqueous solvents, bound or non-extractable molecules remain insoluble in the solvent used to extract them. For example, most phenolics are linked by hydrogen bonds with the cell walls polysaccharides (i.e., pectin, cellulose, hemicelluloses) through hydrogen bonding, hydrophobic interaction, or encapsulation within hydrophobic pockets (Li et al., 2022; Otoni et al., 2021). However, other phenolic acids form ether bonds with lignin via hydroxyl groups in the aromatic ring. As a result, the enzymes, such as cellulose (i.e., endoglucanases, cellobiohydrolases, β-glucosidases), hemicellulases (i.e., endoxylanases and β-xylosidases), and pectinases (i.e., pectintranseliminase, polygalacturonase, and pectinesterase) can be employed in the isolation of bioactive compounds (Table 1). During the EAE process, the efficiency of the enzyme will be influenced by the key parameters, such as concentra-

Table 1
Enzymes utilized in the extraction of bioactive compounds from algal biomass for the food industry.

Source	Enzyme	Extracted compound	Enzyme concentration	Yield	Reference
Fucus vesiculosus	Viscozyme	Fucoxanthin	Enzyme-to-water ratio 0.52 %, seaweed-to-water ratio 5.37 %, and incubation time 3.05 h (40 °C)	0.657 mg/g dry weight	(Shannon and Abu-Ghannam, 2018)
Haematococcus pluvialis	Cellulose, and Pectinase	Astaxanthin	A cocktail of Cellulose (1.5 % v/v), and Pectinase (0.8 %), incubation time 1 h (40 °C)	60.93 ± 1.27 %	(Zhao et al., 2019)
Sargassum dupplicatum	Termamyl, cellulose, and viscozyme	Phlorotannin	Cellulase enzyme (7.5 % v/v), and incubation of 3 h (Room temperature)	4.45 ± 0.11 mg phloroglucinol equivalent/g dry weight	(Boi et al., 2020)
Undaria pinnatifida	Celluclast	Fucoidan	0.5 mL of Celluclast enzyme mixed with 2 L of dH_2O) per 100 gr dry weight for 24 h (50 °C)	97 % of the total dry carbohydrate content	(Oh et al., 2020)
Gracilaria lemaneiformis	Cellulose and arylsulfatase	Agar	Water with 8 U/mL cellulase and 26.6 U/mL arylsulfatase, incubation time 3 h (50 °C)	12.52 ± 0.06 %, with a gel strength of 1521 ± 54.5 g/cm^2	(Xiao et al., 2019)
Ulva sp.	Protamex	Ulvan	6 % v/v, incubation time 3 h (50 °C)	58.4 ± 7.3 % dry weight	(Fournière et al., 2019)
Nizamuddinia zanardinii	Alcalase, Flavourzyme, Celluclast, and Viscozyme	Fucoidans	Alcalase (5 % v/v, pH 8, 50 °C, 24 h), Celluclast (5 % w/v, pH 4.5, 50 °C, 24 h), Viscozyme (5 % v/v, pH 4.5, 50 °C, 24 h), or Flavourzyme (5 % v/v, pH 7, 50 °C, 24 h)	4.28–5.58 % dry weight	Alboofetileh et al. (2019)
Macrocystis pyrifera, and *Chondracanthus chamissoi*	α-amylase, cellulose, and pectinase	Protein	1/10 v/w of enzyme substrate (1.64 UI/mg dried weight), incubation time 10 min (50 °C)	7.39 g/100 g dry weight in *M. pyrifera* and 6.35 g/100 g dry weight in *C. chamissoi*	(Vásquez et al., 2019)

tion, pH, temperature, incubation time, cofactors, substrates concentration, and enzymes mode of action (i.e., active site, isoelectric point, enzyme/substrate complexes, cooperativity, and specificity) (Bashir et al., 2020). Noticeably, the cost and the lack of knowledge about the optimal enzyme formulations for cell disruption are potential limitations during the EAE process (Nadar et al., 2018). Multi-enzymatic assist extraction of biomolecules was seen to show higher recovery of high-value biomolecules as an effective approach (Table 1). However other factors, such as enzymes concentration, temperature, hydrolysis time, and solid/liquid ratio, should be optimized (Wang et al., 2020). EAE could be potentially combined with the other extraction methods, such as ultrasonication and high hydrostatic pressure. For example, the green-blue microalgae *Arthrospira platensis* (Microcoleaceae) is an essential source of Allophycocyanin (A-PC) and C-Phycocyanin (C-PC). However, 50–80 % of the A-PC is not extracted from the biomass during the extraction process. In this regard, the combination of EAE with ultrasonication is an ideal approach since the highest yield of 44.08 mg/g dry biomass with a purity of 1.09 (80 % yield) was obtainable from the *A. platensis* biomass (Tavanandi et al., 2019). In another study, the combination of an extra alkaline extraction process after EAE optimization extraction resulted in the extraction of a higher level of total amino acids in red seaweed *Palmaria palmate* (Rhodophyt-macroalgae) as a rich protein source (Naseri et al., 2020a,b).

The production cost of the EAE process can be considerably reduced by process optimization. In this regard, response surface methodology (RSM) is introduced as a useful technique for assessing and predicting of complex methods, such as EAE, in which the condition of the key parameter should be evaluated. The RSM method was used to optimize the temperature, pH, and enzyme/dry biomass ratio during EAE of R-PE from red seaweeds *Mastocarpus stellatus* (macroalgae) using xylanase. The RSM results showed that each analyzed parameter has a certain influence on the responses; for example, the pH has a considerable effect on R-PE extraction in comparison to the other factors, and temperature and enzyme/substrate ratio were the most important factor for the extraction of water-soluble proteins. The results showed that a 1.8-time increase in R-PE extraction is achievable using the RSM methodology (Nguyen et al., 2017).

3. Enzymatic treatment of algal cells for enhanced extraction of high-value metabolites

During the extraction process of bioactive molecules, the organic solvents (i.e., hexanes, diethyl ether, dichloromethane, and toluene) are generally used, which are undesirable since they have destructive effects on the human health. Therefore, the aqueous-based extraction (ABE) system is an ideal approach. However, this system requires support from a mixture of additional techniques to obtain maximum yield in a short extraction period. As a result, the EAE completes the ABE system. The algal cells have dense and firm cell walls made of complex, heterogeneous, soluble, and insoluble molecules such as glycoproteins, fibrillar matrixes, sulfated and branched polysaccharides (i.e., cellulose, uronic acid, mannose, xylan, algaenan), and lipids linked with various bound ions that are key drawbacks through extraction processes (Fig. 2) (Ghassemi et al., 2021). Thus, breaking down these complex molecules is necessary for an efficient recovery of intracellular bioactive molecules. In this regard, EAE is considered a high bioactive yielding technology that removes the undesired complex molecules from the cell walls and removes the obstacles of water solubility and insolubility for bioactive molecules.

During the extraction process, the specificity of each of the enzymes could be used efficiently to (1) prevent co-extraction of interfering bioactive compounds with similar solubility and (2) disrupt cell walls and improve the mass transfer of APS into extraction solvent. In this regard, the influence of different carbohydrases and proteases enzymes on the scavenging activity of algal extracts was studied by Habeebullah et al. (2021). The results revealed the specify of each enzyme on each of the tested macro algae, as follows, Termamyl on *Fucus serratus,* Viscozyme on *Fucus vesiculosus*, Alcalase on *Fucus vesiculosus*, and Termamyl on *Polysiphonia fucoides*. Further studies showed that, the antioxidant activity of the enzymes fractions was mainly based on phenolic-rich fractions. With in contrast, the iron-chelating activity was mainly due to protein and polysaccharide-rich fractions of seaweeds. Gallic acid, gentisic acid, chlorogenic acids were the main free phenolics in the phenolic fractions. Fucose-rich polysaccharides were obtained when *F. vesiculosus* and *F. serratus* were hydrolyzed with Viscozyme and Termamyl.

Rhodophyceae (red algae) class are the most widely cultivated macroalgae and they are often directly consumed in humans' daily diet and used as raw material in food hydrocolloid extraction (Cotas et al., 2022). The usage of enzymes commonly led to the discovery of new valuable products of interest for the food industry. For example, in the family of Rhodophyta, the *Gelidiella acerosa* is identified as a source for the production of Galactan, a dietary polysaccharide mainly composed of galactose with significant roles in gut microbiota regulation. The enzymatic processing of *G. acerosa* with papain (papaya proteinase I) at 60 °C for 6 h resulted in the discovery of a new polysaccharide molecule mainly consisting of galactose with an antithrombotic effect. The discovered novel molecule could prolong the coagulation time and inhibit the platelet aggregation without hemorrhage (da Silva Chagas et al., 2020).

Xylooligosaccharides (XOS) are potent bioactive molecules as prebiotics in food industries. XOS is xylanic oligosaccharides that are made up of 2–10 xylose units linked by β-(1 → 4) bonds. During the XOS extraction, the Cellulose, lignin, and pectin concentrations are the major barriers to the EAE, even though they can be hydrolyzed by using cellulase, β-glucosidase, and pectinase enzymes (Pinales-Márquez et al., 2021). To overcome this problem, one strategy is the identification of algal species with a low concentration of Cellulose and lignin. In this regard, the *Palmaria* sp., (Rhodophyta) is identified as an ideal candidate for xylan production. The influence of two available commercial enzymes Hemicellulase amano 90, and DXRF during XOS extraction from *Palmaria* sp. showed 66.6 % of XOS from the *Palmaria* sp. biomass was extracted (hydrolysis rate = 82 %) using a mixture of Hemicellulase amano 90 (54 U), and DXRF (10 mg/mL) (Yamamoto et al., 2019). In the Palmaria genus, the *Palmaria palmata* species is identified as high in protein, and essential amino acids, however it cell wall consists mainly of β-(1 → 4)/β-(1 → 3)-D-xylans and it must be disrupted during protein extraction. An optimized EAE method with higher than 90 % efficiency was reported by Naseri et al. (2020) using the Alcalase, Celluclast, and Shearzyme commercial enzymes. Further analysis revealed that the total amount of essential amino acids was higher in the post-extraction solid residue and pellet samples, and a higher ratio of up to 0.44 of essential amino acids to total amino acids is achievable using Alcalase.

EAE methods were also used for the extraction of natural flavors and colorants of interest for food industries due to the potential toxicity of some synthetic antioxidants (i.e., butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, tert-butylhydroquinone) (Chandra et al., 2020). Algae are a valuable source of pigments, such as phycobiliproteins (i.e., phycoerythrins, C-PC, and A-PC), carotenes (i.e., carotenoids and xanthophylls), and chlorophyll *a* with considerable food industry potentials (Fig. 3) (Manzoor et al., 2021). For example, C-PC has various applications in the pharmaceutical and nutraceutical industries as antioxidants, anti-inflammatory, neuroprotectors, hepatoprotectors, and food colorants, and statistics show that the PC market will reach 1 Billion $ (US) by 2028 (Markou et al., 2019). However, the efficiency of conventional solid-liquid extraction methods to extract C-PC is very low from their sources (i.e., *A. platensis, Aphanothece* sp., *Pyropia yezoensis*) because of the resistance of the cell membrane to disruption during the extraction process. For example, the cell wall of *P. yezoensis* (macroalgae) consists of different polysaccharides

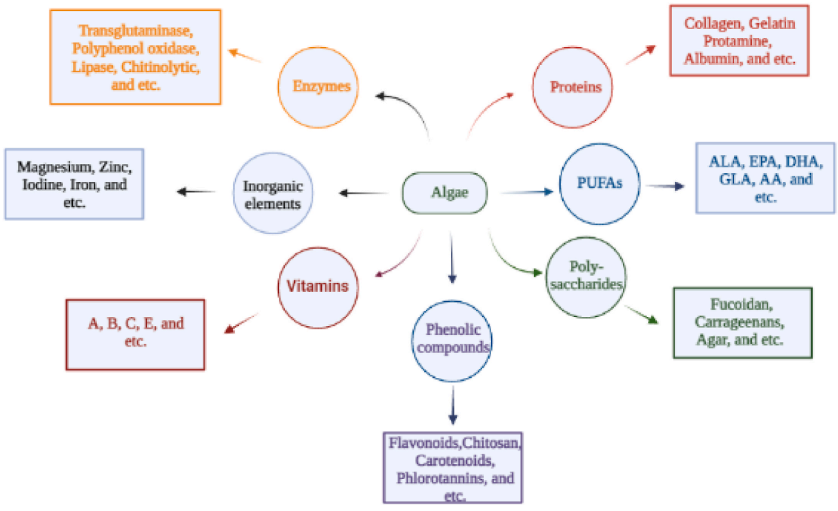

Fig. 3. A schematic representation of high value products from algae that are of interest to the food industry. Note: Enzymes can be used to extract and modify molecular structures, and introduce biomolecules with higher stability, which have applications in the food industry. PUFAs: polyunsaturated fatty acids, ALA: Alpha-linolenic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, GLA: Gamma-linolenic acid, and AA: Arachidonic acid.

(i.e., agar, carrageenan, and xylans) and a matrix primarily made of a cellulosic network, which are strong barriers for extraction of R-PE. EAE of R-PE using agarase and cellulose at the concentration of 104 and 100 U g^{-1} biomass, respectively (temperature = 29 °C, hydrolysis time = 5 h) were indicated to be 1.85 and 2.82 times more effective (purity index = 1.95-fold) than costly freeze-thaw and maceration extraction methods (Wang et al., 2020).

Astaxanthin (ASX, keto-carotenoid) is another strong natural antioxidant with a market growth rate of 16.8 % from 2021 to 2028 (Revenue forecast in 2028 = 4.75 billion USD) (Martins et al., 2022). The microalgae *Haematococcus pluvialis* (Chlorophyceae) is a main source of ASX, however it forms a ~2 μm complex thick acetolysis-resistant cyst-like cell wall during ASX accumulation. Cellulase and pectinase were reported to be efficient in ASX extraction from *H. pluvialis* while pectinase was more efficient than cellulose since the ASX yield and enzyme hydrolysis time were shorter (Kim et al., 2022; Zhao et al., 2019). Fucoxanthin (Fuco) is another marine carotenoid and a potential antioxidant molecule. The global total Fuco market is estimated (2020) to increase from $88 million in 2019 to more than $100 million over the next five years (Arora and Philippidis, 2021). Fuco can be isolated from different macroalgae (i.e., *Undaria pinnatifida*, *Laminaria japonica*, *Phaeodactylum tricornutum*, and *Sargassum fluitans*, *Cylindrotheca closterium*), however, some of these macroalgae like *Sargassum fluitans* (Phaeophyceae) are recognized as an invasive species in Tropical Atlantic Ocean and Caribbean region; nevertheless it is clear that many opportunities exist around the valorization of *S. fluitans* biomass as a source of Fuco, however, the extraction cost of the system plays an important factor. In this regard, a one-step EAE using an enzyme cocktail of cellulase (CellicCTec2) and alginate lyase from *Sphingomonas* sp. (SALy) (pH 6.0, 40 °C) is considered an eco-friendly and cost-effective approach for extraction of Fuco (0.05 % of the biomass DW) (Machado et al., 2022). The same method has been used for the extraction of Fucoidans from the macroalga *Fucus distichus* and *Saccharina latissima,* resulting in fucoidan yields comparable to chemically extracted with significantly larger molecular sizes of the fucoidans (300–800 kDa).

During the EAE process the polyphenols are often covalently joined to glucose as glucosides through glucosidic linkages. To release phenolic compounds β-glucosidase possesses the ability to break the β-1, 4 glucosidic linkages in glucosides. For example, in the macroalgae *Eucheuma denticulatum* (Solieriaceae) as a source for carrageenan, protein, and amino acid the enzymatic extraction with Alcalase and Viscozyme significantly improves the protein recovery and resulted in the highest extraction efficiency up to 59.4 % (Naseri et al., 2020a,b).

Enzymes can be used to extract and modify the molecular structures and introduce the new biomolecules with higher stability that is of interest to food industries (Fig. 2). For example, the quality of algal proteins is significantly related to the digestibility and availability of essential amino acids, which are the key factors in algal protein usage in the food industry. To increase the bioavailability and effective usages of proteins, proteolytic enzymes can be useful by fractional denaturation of proteins to peptides and increase the bio accessibility. In this context, the hydrolysis of proteins extracted from the macroalgae *Porphyra dioica* (protein content ≈ 45 % dry weight) using Prolyve, and a combination of Prolyve and Flavourzyme, facilitated the production of various peptides and low molecular mass components (<1 kDa) exhibiting significantly increased antioxidant activity. The antioxidant activity rose from 610 to 3054 μmol Trolox equivalents per gram of freeze-dried sample, representing a notable five-fold enhancement (Pimentel et al., 2022). Also, the enzymatic treatment of microalgae *Chlorella vulgaris* with chitinase, rhamnohydrolase, and galactanase caused the highest release of protein bio-accessibility. The study on the post-extraction oxidation of nutrient value compounds showed less off-flavor formation and stability of nutrients such as ploy unsaturated fatty acids over 3 months (Canelli et al., 2021). The enzymatic treatment was used to increase the added value in Rhodophyceae family (macroalgae), such as *Gracilariopsis lemaneiformis* by converting homogenized biomass through one-step enzymatic process to neoagarooligosaccharides (NAOs), and the dietary fiber using a cocktail of two agarases enzymes (endo-agarase GH16-1 from *Gilvimarinus chinensi* and exo-agarase GH50D from *Saccharophagus degradans* 2–40). Further studies on the enzymatic obtained dietary fiber indicated that, the great potential in the food industry because of its physiological properties, including its thermostability at 30–1150 °C, water holding capacity (24.20 g/g), and oil holding capacity (8.26 g/g). NAOs intake is proven to be safe (toxicity = 5 g/kg body weight/day) and due to multiple bioactivities (intestinal microorganisms' regulation, antioxidant activity, anti-obesity activity, and cholesterol reduction), NAOs became a target in food sciences studies (Song et al., 2022).

4. Parameters to optimize and strategies for reducing cost of using enzymatic processing of algae for food applications

Minimizing the costs associated with enzymatic processing requires strategic optimization across various process elements. Precise adjustments to these parameters are not only crucial for the cost-effectiveness of enzymatic processing in algal biomass but are also critical in achieving a high yield of algal biomolecules. In this regard, optimization methods, including selecting the appropriate enzyme, adjusting enzyme concentrations, managing extraction time, controlling temperature, optimizing pH levels, ensuring enzyme stability, implementing pre-treatment, choosing solvents, and making informed decisions about algal species and strains, play essential roles.

4.1. Enzyme selection, optimization, and immobilization

The effectiveness of enzymatic processing of algal biomass depends on the careful selection of enzymes with both high activity and specificity, balancing efficiency with cost-effectiveness. Prioritizing heightened enzyme activity is crucial, especially during time-sensitive downstream processing of algal biomass. Simultaneously, the specificity of enzymes, their ability to recognize and target specific substrates or reactions, is critical for achieving precise transformations without unwanted side reactions (Li et al., 2012). Meantime, the concentration of enzymes in a reaction mixture significantly influences the reaction rate, and it's essential to recognize that increasing enzyme concentration doesn't always linearly boost the reaction rate. In this context, there is an optimal concentration range where the reaction operates at its maximum efficiency. Beyond this range, additional enzymes may not contribute significantly

and potentially leading to unnecessary costs, undoubtedly given the potential expenses tied to enzymes, striking the right balance between concentration and reaction efficiency is important (Wang et al., 2020). In this context, researchers must accurately optimize the enzyme concentration through experiments or simulations, pinpointing the concentration that maximizes the reaction rate while factoring in cost considerations, for example, in the optimization process for laminarin production from the seaweed *Laminaria saccharina*, a unitary gross profit of ~ $ 1.15 per kilogram of biomass was achieved. This substantial difference in profitability is attributed to optimizing the enzyme extraction process, contrasting with the traditional alkaline extraction method that yields a profit of $ 0.15 per kilogram of *L. saccharina* (Herrera Barragán et al., 2022).

The enzyme immobilization technique is also introduced as an effective method to enhance the cost-effectiveness of enzymatic bioprocessing of the algal biomass. This process involves attaching or confining enzymes to a solid support or matrix, preventing them from freely diffusing in a solution, and maintaining their catalytic activities over an extended period. Immobilization boosts enzyme stability under diverse temperature and pH conditions, protecting them from degradation caused by chemicals in the reaction mixture, and allows for reusability across the multiple reaction cycles. Researchers have successfully applied immobilization technology to conduct the transesterification of *Chlorella protothecoides* lipids using the immobilized lipase enzyme from *Candidia* sp. This approach resulted in significant lipid contents ranging from 44 % to 49 % per dry cell weight and facilitated the transesterification of lipids obtained from *C. protothecoides* (Li et al., 2007). Currently different immobilized enzymes (i.e., lipase, protease, amylase, and cellulase) have been utilized in many foods related industrial sectors (Table 2), for example the use of D-glucose/xylose isomerase was used to produce high fructose corn syrup (HFCS) with a purity over 99 % (w/w) can also be obtained starting from HFCS-42 which further will be used for production of fructose in food products.

Another case to highlight in this point is the enzyme "Lipases" that catalyze the hydrolysis of triacylglycerols into glycerol and free fatty acids. Candida antarctica lipase B (CalB) possesses wide substrate specificity and has shown to be an effective catalyst for the synthesis of esters of ethyl D-glucopyranoside from fatty acids larger than octanoic acid ($C_8H_{16}O_2$). A study conducted by López et al., in 2023 demonstrated a notable increase in both lipid and eicosapentaenoic acid (EPA) contents within saponifiable lipids (SLs) derived from the microalga *Nannochloropsis* sp. using immobilized Lipases. Specifically, the research achieved an impressive enhancement, reaching up to 70 % EPA within polar lipids with a recovery rate of 92 %. This was accomplished using lipase QLM immobilized on the Accurel MP 1000, employing an immobilized lipase/SL ratio of 2.5:1, and conducting a 24-h hydrolysis time. This optimized approach not only led to a significant boost in EPA content but also contributed to increased profitability of the system. The higher recovery yield of EPA resulted in a more efficient utilization of resources, mitigating the elevated costs associated with enzyme use. These findings emphasize the economic advantages of employing immobilized lipase in enhancing EPA recovery, thereby promoting sustainability and economic viability in the process.

Another notable economic advantage of utilizing enzyme immobilization is the capacity to regenerate the immobilized matrices (i.e., ion exchange resins) in food applications, once they have been exhausted. In this connection, when the enzyme becomes depleted and its activity diminishes, the carrier can be regenerated *in situ* using NaOH and HCl. These reagents effectively remove the enzyme and cleanse the resin from fouling matter. Subsequently, a fresh batch of enzyme is usually introduced directly into the column containing the ion exchange resin, facilitating the immobilization process.

Table 2
A list of immobilized Enzymes Utilized in Industrial Food Processing.

Immobilized enzyme	Type of Enzyme Reaction	Application	Product	Reference
Lipase B from *Candida antarctica*	Transesterification	Antioxidant	Vitamin C ester (S)-phenylethylamine	(Cipolatti et al., 2020; Shu et al., 2023)
d-Glucose/xylose isomerase	Transamidation	Chiral amines	Organosilicone polyamide	(Ferreira, 2023)
Epimerase	Amidation	Polymers	Omega-3 ethyl esters	(Chen, 2020)
Inulinase	Esterification	Omega'3	HFCS	(Singh et al., 2019)
β-Galactosidase	Isomerization	Dietary sweetener	d-psicose or pseudofructose	(Gorbunova et al., 2022)
Lipase B from *Candida antarctica*	Isomerization	Dietary sweetener	HFS and FOS	(Knez and Lütge, 2023)
Lipase from *Thermomyces lanuginosus*	Exo-inulinase	Dietary sweetener	Tagatose	(Zhang et al., 2023)
Cellulase from *Aspergillus niger*	Hydrolysis of lactose	Dietary sweetener	Organosilicone polyamide	(Yasir, 2009)
	Amidation	Polymers	Triglycerides	
	Transesterification	CBEs	Extraction of κ-Carrageenan from the red alga *Kappaphycus alvarezii*	
	Hydrolysis	Cellulose hydrolysis, the fundamental component within lignocellulosic plant biomass		

Note: HFCS: High-fructose corn syrup, CBES: cocoa butter equivalent, HFS: High fructose syrup, FOS: Fructooligosaccharides.

4.2. Strain selection and technological advances

Choosing algae strains with high nutritional value and fast growth rates is a strategic decision within the food bioprocessing sector. Meantime, the composition relies on different parameters including phyla and species, growth phase, growth, and cultivation condition. In relation to this, differences can also be observed in the term of the composition (i.e., carotenoids, lipids, fatty acids, carbohydrates, fiber, and protein). For instance, a study by Tolpeznikaite et al. (2021) evaluated the macroalgae *Coryphaenoides rupestris*, *Furcellaria lumbricalis*, *Ulva intestinalis* and the microalgae *A. platensis,* and *C. vulgaris* for macro and microelement transitions, antioxidant, and antimicrobial properties, chlorophyll, and total carotenoid composition. The findings revealed that *C. rupestris* exhibited superior total carotenoid and chlorophyll composition compared to other tested macroalgae. Additionally, the highest levels of total phenolic compounds were detected in *C. rupestris* and *F. lumbricalis*. Concurrently, extracts from *A. platensis* demonstrated heightened antimicrobial activity against *Staphylococcus haemolyticus*. In contrast, extracts from *C. rupestris*, *F. lumbricalis*, and *U. intestinalis* exhibited inhibitory effects on *Bacillus subtilis*. Notably, the extracts from *U. intestinalis* were the sole inhibitors against *Streptococcus*.

It is undeniable that, the profound impact of high nutritional content is evident in the derived products, contributing significantly to the overall nutritional value of the final food product. This holds particular importance in addressing global food security challenges and meeting the dietary requirements of a growing global population. Algae varieties enriched with high levels of proteins, healthy fats, and essential nutrients emerge as valuable ingredients in food processing, actively contributing to the creation of functional and nutritious food items. Concurrently, the emphasis on the rapid growth rates is necessary for enhancing overall productivity in food bioprocessing. Algae characterized by accelerated growth rates facilitate increased biomass production within compressed timeframes. This proves advantageous in efficiently scaling up production processes, meeting market demands promptly, and sustaining a robust supply chain. Moreover, the rapid growth rates contribute significantly to the economic viability of algae-based food bioprocessing by reducing production time and associated costs. Evidence shows that, the commercially cultivated microalgae sector for food applications has matured significantly, with a pronounced focus on cultivating a diverse array of species. Specifically, industry leaders concentrate their efforts on *Spirulina*, *Chlorella*, *Dunaliella*, *Nannochloris*, *Nitzschia*, *Crypthecodinium*, *Schizochytrium*, *Tetraselmins*, and *Skeletonema*. This targeted emphasis highlights the pivotal roles these particular microalgae play in meeting the dynamic demands of the food industry, showcasing their established significance within the broader landscape of commercial microalgae cultivation (Lafarga et al., 2020).

Additionally, monitoring techniques, specifically those that are linked to monitoring enzyme activity and stability, are essential components of this optimization process. These techniques significantly contribute to maintaining efficiency and reliability throughout enzymatic processing, aligning with the overarching goal of cost reduction, and enhancing biomolecule yield. Research underscores that products with lower market value, such as protein concentrates and whole-cell protein, face challenges in terms of cost-effectiveness, making their production relatively expensive. Notably, a study by Soto-Sierra et al. (2021) identifies the ball milling technique as a pivotal factor that enhances enzymatic reaction rates, addressing limitations associated with enzyme concentration when extracting high-quality proteins from the microalgae *Nannochloropsis* sp. lipid-extracted algae (LEA). Intriguingly, preprocessing LEA into protein concentrates before hydrolysis does not yield discernible benefits. However, subsequent purification steps, involving acidic precipitation, centrifugation, and depth filtration, demonstrate remarkable efficiency, leading to impressive recovery rates and efficient chlorophyll removal. The application of an ion exchange (IEX) resin further refines protein purity. A novel aspect is the integration of hydrolysis and fractionation technique, that resulting in chlorophyll-free LEA protein with recovery rates exceeding 60 % and purity surpassing 70 %.

Biomass pretreatment is the pivotal first step in optimizing bioprocessing efficiency by improving the accessibility of crucial biomolecules like carbohydrates, lipids, and proteins. The primary aim is to alleviate biomass recalcitrance through unraveling complex matrices and expanding surface area. This process substantially enhances overall conversion yields, resulting in reduced system costs. In this context, hydrothermal liquefaction (HTL) stands out as one of the most promising methods for converting algal biomass into bio-oil and high-value molecules.

The exploration of residues from food and feed processing, along with organic waste streams, has gained significant attention as potential nutrient sources in biotechnological processes. Significantly, there is a heightened emphasis on recovering nitrogen compounds, specifically proteins and amino acids, driven by environmental concerns related to the uncontrolled decomposition of these compounds if not managed properly. Statistics indicates a predicted 14 % increase in global fish production, reaching 200 metric tons by 2029. Nevertheless, the fish industry currently processes only 30–40 % of the raw material into high-value products suitable for direct human consumption. In this regard, an innovative approach involves converting digestate through the heterotrophic cultivation of the microalga *Galdieria sulphuraria*, generating biomass that can be further refined into high-value food products, including carbohydrates and proteins. This strategy not only addresses environmental concerns linked to uncontrolled waste decomposition but also contributes to the sustainable production of valuable food resources (Pleissner et al., 2023).

Statistics show as of 2022, the worldwide market for enzymes reached a value of USD 12.27 billion, and it is projected to experience a compound annual growth rate (CAGR) of 6.5 % from 2023 to 2030. The increasing consciousness among consumers regarding health, specifically concerning food and beverage items, is poised to play a crucial role in driving the demand for enzymes in the upcoming years. To date, nearly 4000 enzymes have been identified, with approximately 200 microbial variants actively employed in commercial applications. However, a restricted subset, approximately 20 enzymes, currently dominates substantive usage within industrial contexts.

To enhance both efficiency and recovery, the integration of more effective enzymes is imperative. Fortunately, the accomplishments of recent genome sequencing programs have yielded abundant information within sequence databases. This presents a compelling opportunity to examine into the potential identification of superior enzymes through database mining. By scrutinizing the ge-

netic content of entire microbial communities, researchers can pinpoint enzymes that exhibit novel functions, unique substrate specificities, or enhanced catalytic efficiencies. In this regard, Metagenomic tools contribute to the discovery of novel enzymes with unique properties, and by analyzing the genetic content of entire microbial communities, researchers can identify enzymes that may have novel functions, substrate specificities, or catalytic efficiencies. In this regard, some of the available metagenomic tools are QIIME (https://qiime2.org/), MG-RAST (https://www.mg-rast.org/), MetaPhlAn (https://huttenhower.sph.harvard.edu/metaphlan), Kraken (https://ccb.jhu.edu/software/kraken/), Anvi'o (https://anvio.org/), and BFD (https://bfd.mmseqs.com/) that can be used as a platform that allows users to explore the biocatalytic functions directly from the sequencing data. To date, the primary sources of *de novo* enzyme discoveries have been culturable bacteria and fungi, rather than environmental DNA (eDNA) and uncultivated microbes (Robinson et al., 2021). Nevertheless, it is evident that a wealth of unexplored diversity within protein families can be unveiled through metagenomic analyses, for example, the recent discovery of non-ribosomal peptides (NRPs) incorporating multiple ρ-aminobenzoic acids (PABAs) prompted scientists to explore soil metagenomes for biosynthetic gene clusters (BGCs) responsible for PABA polymerization. The metagenomic analysis of BGC showed encode a unique N-acylated PABA and thiazole containing structure (Wang et al., 2022). Meanwhile, employing advanced metagenomic analyses provides a valuable avenue for characterizing novel reaction types within the framework of previously identified proteins. As an illustration, a comprehensive high-throughput metagenomic analysis focused on Carbohydrate-active enzymes (CAZy) is accessible at https://www.cazy.org. Researchers have utilized this resource to identify glycoside hydrolases displaying simultaneous activities, such as glucosidase, arabinase, and xylosidase (Tong et al., 2021). Another illustrative example of using metagenomic analysis involves sulfated fucans a class of polysaccharides that initially isolated in 1913, commonly known as fucoidans, which constitute highly diverse cell wall polysaccharides in brown algae, including species like *Pelvetia canaliculata*, *Himanthalia elongata*, *Ascophyllum nodosum*, and *Mariniflexile fucanivorans*. Fucoidans exhibits wide range of bioactive properties, indicating potential applications in the pharmaceutical field. Despite this, limited research has been conducted on the identification of enzymes capable of hydrolyzing sulfated fucans a critical step for the targeted release of specific bioactive oligosaccharides. To address this gap, a metagenomic analysis of available enzymes, utilizing paired-end sequencing of a short-insert library on an Illumina HiSeq platform, revealed hydrolytic activity against α-1,4-fucosidic linkages in sulfated fucans, specifically within the glycoside hydrolase family 107 (Schultz-Johansen et al., 2018).

Metagenomic analysis was employed to engineer recombinant industrial enzymes for producing agaro-oligosaccharides, offering a fresh avenue for crafting natural anti-inflammatory molecules. Researchers identified an agarase gene (aga 1904), encoding a 640-amino-acid protein, from the metagenomic library of bacteria associated with the macroalgae. This gene was then expressed and purified in *Escherichia coli* BL21 (DE3). The enzyme's breakdown of agarose substrates primarily yielded polysaccharides, such as neoagarobiose, neoagarotetraose, and neoagarohexaose. Notably, agaro-oligosaccharides, particularly those rich in neoagarobiose, exhibited significant inhibition of key pro-inflammatory markers, including nitric oxide (NO), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) (Schultz-Johansen et al., 2018).

In the contemporary research landscape, metagenomics data has become a keystone across diverse domains in the past decade. Metagenomic approaches stand out as powerful and efficient tools for enzyme discovery, tapping into the genetic diversity within intricate microbial communities.

5. Conclusion

Enzymes are necessary in diverse sectors, such as technology, food production, animal nutrition, cosmetics, medication, and as invaluable tools for research and development. Notably, algae are gaining prominence as an alternative food resource for the food industry due to their nutritional and functional attributes. Enzymatic treatment of algae offers a safer and more eco-friendly approach for the isolation, modification, and discovery of new biomolecules with the higher stability of interest for food industries. However, further developments are required for process optimization, operational cost reduction, consumer acceptance, and fulfillment of food regulations. In this regard, optimization of the system, enzymes immobilization, new enzymes discovery, and a combination of newly available technologies (i.e., supercritical fluid enzyme-assisted, and microwave-assisted enzymatic hydrolysis) will be a great support.

CRediT authorship contribution statement

Ali Parsaeimehr: Writing – original draft, Writing – review & editing. **Gulnihal Ozbay:** Writing – review & editing.

Declaration of competing interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Data availability

No data was used for the research described in the article.

Acknowledgments

This review article is funded by USDA NIFA Capacity Building Grant Program Award# #2017-38821-26439 and National Science Foundation EPSCoR Grant No. 1757353 and the State of Delaware.

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