A review of the fermenting microalgae to improve their bioactive potential

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Abstract

Fermentation is the oldest strategy to grow and maintain food by boosting its nutritional value. Owing to increasing attention to renewable, nutritious meals, that emphasize cutting-edge processes in food manufacture, functioning for social health and ecological stability, fermented components made from algae have lately been manufactured and evaluated. Numerous composites, such as polyphenols and polyunsaturated fatty acids and polyphenols, which are valued meant for the production of food and pharmaceuticals, are the focus of current applications. As a maximal and acceptable base for bioactive composites, macro and microalgae take expanded attention as fermentation substrates. Microalgae fermentation mainly produces biofuel or composite materials with bioactive properties like antioxidant, antibacterial, and anti-inflammatory potential. The objective of this review is to present a note on microalgae fermentation to acquire diverse valued composites with bioactive abilities. Furthermore, the methods of fermentation, species of algae employed in fermentation, fermentation circumstances, and bioactive properties of the derived bioactive composites have conversed.

Keywords: Bioactives, Fermentation, Microalgae, Polyphenols, Sustainability.

Introduction:

Fermentation technology has developed as a substitution for the more traditional techniques, to attain required composites without causing toxic remains and no use of solvents which can alter the activity of these complexes (Larios-Cruz et al.,2019). Microorganisms are used in fermentation for the generation of enriched foods (Erkmen and Bozoglu, 2016) and fermentation is utilised to speed up the disintegration of raw materials into polymers, produce bioactive molecules or destroy toxic factors (Verardo et al.,2020). Initially, microorganisms yield huge quantities of primary and secondary substances, enhancing their nutritive worth (Verardo et al.,2020). However, microorganisms discharge acids and enzymes which reduce the complex molecules to operational minor components, enhancing their accessibility and assimilation (Ryu et al.,2013).

Micrometres for microalgae and metres for macroalgae or seaweeds are the different sizes of algae. Microalgae are single-celled organisms that belong to several systematic assemblages, including the Rhodophyta (red algae), Chlorophyta (green algae), Charophyta, Glaucophyta, Chlorarachniophyta, Euglenozoa (Euglenoids), Bacillariophyceae (Diatoms), Dinophyta (Dinoflagellate), Eustigmatophy (Keeling, 2004). Besides, oxygenic

photosynthesis, several microalgae execute heterotrophic or mixotrophic metabolism. This exceptional capability, which cannot be seen in plants (Perez-Garcia et al.,2011), permits them to flourish in several exciting ecological environments. Therefore, microalgae are scattered worldwide in different kinds of ecosystems (Varshney et al.,2015). Owing to their metabolic flexibility and multiplicity concerning genetics, appearance, and ecology, the usual macromolecular conformation (concerning lipids, proteins, and carbohydrates) differs extensively between different classes. In nutritive environments, cyanobacteria typically collect more quantity of proteins compared to diverse phyla, whereas Rhodophyta and Euglenophyta accumulate additional carbohydrates and lipids, correspondingly. Algae yield a boundless quantity of secondary metabolic substances with organic effects such as (Keeling, 2004) sulfated polysaccharides, polyunsaturated fatty acids (PUFA), carotenoids, phycobiliproteins, and trace minerals like iron, calcium, iodine, potassium, selenium, and vitamins A, C, and B-12. Different microalgal groups contain varying amounts of pigments: carotenoids, phycobiliproteins, and chlorophylls. (Demirbas, 2009). Normally, microalgae have 0.2% of carotenoids, 0.5–1.0% of chlorophylls, and up to 8.0% of phycobiliproteins (Lyon-Colbert et al., 2018). All of these substances are employed for food and beverage, animal food, cosmetics, and pharmacological productions. (Gilroy et al.,2000).

Fermentation Ideologies

The construction of fermentation with microalgae in has been recognized to be a profitable pathway to exploitation. This is due to the sterilized condition of the fermentation method, and whole control of the algaculture circumstances permitting better yield and produce development. The maximum microalgae concentrations could be attained helping harvest the cells, and the accessible volume of fitted fermentation vessels that are available across the globe. Commercially optimistic manufacture in fermenters has focused on the utility of strains from a restricted amount of microalgal groups: the dinoflagellates, the green algae, and the thraustochytrids. The current marketable microalgal fermentations are precisely illustrated and the future of commercial development using heterotrophic technology has conversed. Even though mixotrophic production—a combination of heterotrophic and photosynthetic production—has recently attracted interest as a means of boosting output in photobioreactors and fermenters. Despite the misconception that heterotrophic production of microalgae is more expensive, interest in this method of production has been sparked by the fact that it solves a number of the issues that have prevented the successful and widespread adoption of photosynthetic systems for the cultivation of microalgae.

In terms of equipment design and process operation, fermentation denotes a long-recognized type of production process. Algal technology developers can take advantage of excess fermentation capacity at both the pilot plant and manufacturing levels globally. Fermentation also signifies an extremely skilful manufacturing procedure, causing it easier to control the parameters that enhance cell development and product formation. It is also simpler to maintain a monoculture of the predicted strain in fermentors because of their closed nature, especially in axenic variants that allow for the use of algae or algae generated in the nutrition industry. Fermentation also produces suggestively greater cell concentrations (>100 g L^{-1}) in these fermenters/culture vessels, minimising several glitches associated with recovering cells from the diluted cell densities (0.5–2 g L^{-1}).

Fermentation is designated as the breakdown of organic compounds by microbes aerobically or anaerobically which produces energy (ATP molecules) along with valued profitable products or enables significant chemical conversions (Ryu et al.,2013). These chemical conversions due to their safety and financial advantage, are used in the industries of energy production, pharmaceutical, chemical, and food. The resultant organic molecule from this process acts as an electron acceptor during fermentation, with the formation of end products following. During fermentation, the organic substrate and carbohydrates act as electron donors (Erkmen and Bozoglu, 2016). In fermentation, the production of end products depends on the microorganism involved, and the nomenclature of several fermentation pathways is due to their primary products (Muller, 2008). Acids, alcohols, and gases are the primary products of fermentation procedures. The number of active bacteria, the number of fermentable sugars, and environmental factors including pH, temperature, pressure, salinity, and the composition of the fermented medium are all factors that affect fermentation.

There are five main sets of important fermentations: fermentations that yield biomass as the product. Those that generate microbial enzymes • Those that generate primary microbial metabolites (ethanol, lactic acid, etc.)• Creation of secondary metabolites (enzyme and vitamin synthesis) • Conversion of the substrate into products with additional value (Berenjian, 2009).

This review primarily focuses on the creation of both primary and secondary metabolites, as well as the conversion of substrate into products with added value. The species of microalgae mentioned in this review are included in Table I, along with the various bacteria that are employed to ferment the microalgae and the compounds having bioactive activity. The compounds are separated into groups based on the type of algae they came from, and it is stated whether they were released by the algae or created during fermentation.

Strategies for Fermentation

Based on the amount of water castoff throughout the fermentation process, fermentation can be classified as liquid/submerged fermentation and solid-state fermentation (Berenjian, 2019). Solid-state fermentation, sometimes called the earliest form of fermentation, occurs in a solid matrix, inert support, or support/substrate in the absence or near lack of free water (Singhania et al. 2016). Yet, the substrate needs humidity to facilitate microbes' development and enzymatic operations. (Thomas et al.) like bran, bagasse, and paper pulp (Berenjian, 2019. Solid-state fermentation is frequently exploited to produce foods like sauerkraut, pickles, kimchi, miso, tempeh, and sausages (Terefe and Augustin,2019). Submerged fermentation operated in a liquid medium/broths (Ramos and Malcata,2011), Which is appropriate for microbes that need more moistness content, such as Saccharomyces cerevisiae and Bacillus sp. (Singhania et al. 2016). This method is exploited for the manufacture of beverages, vinegar, citric acid, and enzymes (Terefe and Augustin,2019). According to the method of functioning submerged fermentation processes are classified as batch, fed-batch, or continuous.

Types of fermentation

The common industrial fermentation types produce acid, alcohol or gas as the primary industrial products. Composites that are generally generated through fermentation are ethanol,

lactic acid (homofermentation and heterofermentation), propionic acid, butyric acid, and acetic acid.

Lactic acid fermentation

Lactic acid fermentation exploits bacteria of the genus Lactobacillus, Lactococcus, Enterococcus, Streptococcus and Leuconostoc, with lactic acid as the chief derivative (Terefe and Augustin,2019). The food, pharmaceutical, textile, cosmetic, and chemical sectors all use lactic acid (Overbeck et al.,2016). Lactic acid bacteria (LAB) are vital microorganisms employed in food fermentation (Scieszka and Klewicka,2020), they can break down plant and cyanobacterial cell walls by hydrolysis, degrade Polysaccharides, lipids, and proteins are broken down into fragments with improved antioxidant, immunomodulatory and anti-inflammatory properties (Castro et al.,2019).

Lactic acid fermentation of algae was initially observed in 1998 (Scieszka and Klewicka, 2018). Uchida & Murata, 2004, informed that algae were successfully fermented utilizing a microbial association together with LAB Lactobacillus brevis. The LAB Lactobacillus plantarum ATCC 8014 is widely used in the fermentation of microalgae (Castro et al.,2019). Other LABs identified comprise L. brevis (Scieszka and Klewicka, 2020), *Lactobacillus paracasei* (Nguyen et al., 2012), Lactobacillus casei (Overbeck et al., 2016), *Lactobacillus pentosus* (Talukder et al.,2012), *Lactobacillus rhamnosus* (Hwang et al.,2012), *Lactobacillus zeae, Lactobacillus acidophilus, Lactobacillus kefir, Lactobacillus fermentum, Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus, Leuconostoc mesenteroides* (Uchida et al.,2007) and *Lactobacillus salivarius* (Hwang et al.,2012). The bioactive profile of *Arthrospira platensis* (Spirulina) has increased through fermentation with L. plantarum by increasing total phenolic content, phycocyanin, and antioxidant activity, besides discharging free amino acids and bioactive peptides from the cell wall (Castro et al.,2019).

Some strains of algae have the potential to be used as prebiotics, and they have also been used to boost LAB growth (Parada et al.,1998). *A. platensis* microalgae have shown the ability to sustain the growth of *Lactococcus lactis* subsp. lactis C2, L. acidophilus, L. casei, ,*Lactobacillus* sp. JL2, *L. delbrueckii* subsp (Parada et al.,1998), *L. casei, L. acidophilus* and *S. thermophilus Bhowmik et al.,* 2009. A concentration of 10 mg/mL of *A. platensis* amended the progress of *L. casei, L. acidophilus,* and *S. thermophilus* by 150, 169.7, and 190%, respectively (Bhowmik et al.,2009).

Propionic acid fermentation

U.S. Food and Drug Administration (FDA) approved that propionic acid is generally regarded as safe (GRAS) with advantages in numerous industries (Gonzalez-Garcia et al.,2011). Propionic acid is a colourless three-carbon carboxylic acid with a pungent odour, that can be produced by fermentation (Vivek et al.,2011). Propionic acid is primarily used for its antimicrobial properties. As a castoff in thermoplastics, anti-arthritic medications, fragrances, solvents, cellulose plastics, and the synthesis of vitamin E, it is also utilized as a food preservation agent or herbicide. (Gonzalez-Garcia et al.,2017). Propionibacterium freudenreichii, Propionibacterium thoenii, Propionibacterium jensenii, and Propionibacterium acidipropionici are the microorganisms responsible for propionic fermentation (Ciani et al.,

2013). To make acetic acid and CO2 (Ciani et al., 2013), propionic bacteria can employ a variety of carbon sources, including hexoses, pentoses, glycerol, whey lactose, sorbitol, and lactate as the starting substrate (Vivek et al., 2017). As far as we are aware, no studies have utilised Propionibacterium bacteria for the fermentation of microalgae.

Butyric acid fermentation

The food, beverage, cosmetic, plastic, and textile fibre sectors can benefit from butyric acid, a four-carbon short-chain fatty acid. Additionally, it is employed as a bioactive and therapeutic substance (Wei et al., 2013). Butyric acid is typically obtained by the use of petroleum feedstocks, which raises environmental issues (Xiao et al., 2018). According to Ciani et al. (2013), the replacement is achieved through fermentation employing obligate anaerobic bacteria such Butyribacterium, Butyrivibrio, Eubacterium, and Fusobacterium. Since members of the genus Clostridium can use a variety of substrates from different biomass feedstocks, including hexoses, pentoses, oligosaccharides, and polysaccharides, they are used to produce butyric acid (Wei et al., 2013).

The fermentation of microalgae with Clostridium has applications in manufacturing biofuels. Using fermentation with C. butyricum, the microalgae Spirogyra sp. (Pinto et al., 2016) and Chlorella vulgaris are employed to produce H2. By limiting nitrogen accessibility throughout the culture period, Pinto et al. (2016) found that the number of carbohydrates increased by 36% (w/w), resulting in the formation of 146.3 mL H2/g of microalga (d.b.) after mild acid hydrolysis with sulfuric acid. While Liu et al. (2012) discovered that 1.5% HCl increased the conversion of microalgal biomass to reducing sugars by up to 57% (d.b.), they also found that sodium hydroxide (NaOH) in combination with the enzyme endo-glucanase produced comparable results.

Acetic acid fermentation

Through the bacterial cellular oxidation transformation of diluted, purified alcohol or alcoholic blends from different fruits and grains, acetic acid bacteria are used to create vinegar (Ribereau-Gayon et al., 2021). The species used in vinegar manufacturing belong to the genera Acetobacter, Gluconacetobacter, Gluconobacter, and Komagataeibacter, all members of the Acetobacteraceae family. These bacteria can prevent the release of acetic acid into the fermentative medium after they oxidize ethanol to acetic acid. (Gomes et al.,2018).

Mohd et al.,2017, explored the practice of sugar hydrolysate gained from enzymatic saccharification of microalgae (Chlorella sp. and Tetraselmis suecica) as a fermentation feedstock for acetic acid production, a significant biochemical in the polymer, paint and food manufacturing industries. When microalgal hydrolysate fermentated with minimum carbohydrate (<2 g/L) combined with bacterium C. saccharoperbutylacetonicum, produced stream of organic acids (acetic acid and butyric acid) and it was discovered to follow acidogenesis pathway. As evidenced by the researchers acetic acid developed as the prime produce in the hydrolysate fermentation of both species (up to 92 wt% of Chlorella's fermentation products and 80 wt% of T. suecica's fermentation products). The investigation attained good yeild of fermentation harvests: 0.1g fermentation products/g sugar for T. suecica and 0.13 g fermentation products/g sugar for Chlorella.

Alcoholic fermentation

Although certain moulds and genetically modified *Escherichia coli* may generate alcohol, yeasts are the primary organisms in ethanolic fermentation. (Terefe et al.,2019). The yeast species frequently utilised is S. cerevisiae. To produce ATP, NADH, or further biological precursors such as 3-phosphoglycerate/pyruvate utilizing glucose, yeast first produces pyruvate through the glycolytic route (Aranda et al., 2011). The pyruvate is then decarboxylated to produce acetaldehyde, which is then reduced to alcohol (Ciani et al.,2013). The production of biofuels from algae is the alcoholic fermentation, Biofuels are produced from living organisms that comprise energy from geographically current carbon fixation (Hossain et al.,2015).

Irrespective of the nature of algae, the pre-requisite for ethanol making is an energetic delivery of carbohydrates. Product yield can be enhanced by employing diverse enzymes such as cellulase , xylanase , glucoa mylase , α-amylase, β-glucosidase , laminarinase with dissimilar compositions and concentrations of these enzymes (Uchida et al.,2014). Preprocessing by acid hydrolysis with H_2SO_4 combined with pressure, distinct concentrations of HCl, phosphoric acid (H_3PO_4), alkaline hydrolysis by NaOH, pH alterations, and ultrasonication are used as pretreatments (Lee et al.,2015; Adams et al.,2008). The most used yeast is S. cerevisiae (Lee et al.,2015). Additionally, Pseudomonas sp. and E. coli bacteria have been employed (Adams et al.,2008). To produce enhanced amounts of bioethanol, genetically modified microorganisms have been developed to be more capable of hydrolyzing and fermenting microalgae. (Trivedi et al.,2015). Biofuel manufacture with distinct algae utilizing yeasts to harvest alginate lyase and alcohol dehydrogenase at the same time augmented the production of bioethanol (Zhang et al.,2018).

Enzyme production through mould fermentation

Moulds can be utilized to harvest enzymes for applications like detergents and technical applications in industries such as textiles, leather, pulp, paper, fuel, food and feed (Demain and Martens,2017). Different substrates for the mold *Botrytis cinerea* that encourage protease creation were tested for the production of proteases, and detergent enzymes. The substrates employed were Spirulina, Cheatomorpha, gelatin powder, sheet gelatin, casein, soy powder and fish flour. The finest outcomes were attained with Spirulina (Abidi et al.,2008). Fucoidan hydrolytic enzymes were produced by fermentation of microalgae with A. niger and Mucor sp., which had improved consequences specially when microalgae was microwaved as preprocessing For the production of enzymes, fermentation of microalgae has been done through diverse bacteria, and fungi or a blend of together (Pervez et al.,2017).

Bioactive compounds gained in the fermentation of microalgae

Bioactive compounds deliver health gains besides the basic nutritional value. They possess valuable properties, for instance, antioxidant effects, regulation of enzymes, diminish receptor activities, and control of gene expression. Microalgae can produce metabolites with outstanding healthiness aids and preserving assets (Pessione and Cirrincione,2016). Some bioactive composites formed via fermentation comprise bacteriocins, metabolic enzymes, amino acids and peptides, short-chain fatty acids, vitamins, antioxidants, anti-inflammatory agents, and exopolysaccharides (Sevgili and Erkmen, 2019) .

Table 1: Bioactive Products obtained through microalgae fermentation.

Bioactive effect	Microalgae	Compound	Microorganism (Species)	Reference
Antioxidant capacity	*A. platensis*	phenolic compounds (salicylic, synaptic, chlorogenic, quimic and caffeic acids)	*Bacteria (Bifidobacterium bifidum, L. casei, Bifidobacterium infantis, Bifidobacterium longum and L. lactis)*	Liu et al., 2011
	Spirulina maxima	β-carotene	*L. plantarum*	Castro et al.,2019
	Pavlova lutheri	bioactive peptides and amino acids (arginine, glycine and proline)	*Hansenula polymorpha, L. brevis and Candida rugopelliculosa*	Qian et al.,2012 Ryu et al.,2012
Antimicrobial properties	Microalgal extracts	oligosaccharides	*Flavobacterium sp., LAB and yeast C. utilis*	Eom et al.,2013
	A. platensis	Total phenolic content.	LAB	Martelli et al., 2020
Anticoagulant properties	*Chlorella sorokiniana*	sulfated polysaccharides	---	Misurcova et al. 2015
	Picochlorum sp	sulfated polysaccharides	---	Zahra et al.,2022
Anti-inflammatory potential	*A. platensis*	Lipopolysaccharide (LPS)	yeasts *(S. cerevisiae, C. utilis)* *bacteria* (*Lactobacillus sp. L. brevis, L. acidophilus, L. casei, L. lactis, B. bifidum, B. infantis, B. subtilis*)	Lee et al.,2011; Wijesinghe et al.,2013; Mun et al.,2019
Neuroprotective and UV protective effect	*A. platensis*	---	lactic acid bacteria (*L. acidophilus, B. bifidum, L. casei, B. infantis, B. longum and L. lactis*)	Choi et al.,2018

Bioactive compounds with antioxidant capacity

The antioxidant capability of fermented microalgae differs progressively and standards can fluctuate based on the antioxidant capacity test being used.

The highest total phenolic content was found in *A. platensis* after 36 hours of fermentation (19 mg Gallic acid equivalent (GAE)/g), compared to levels at 24, 48, and 60 hours (16 mg GAE/g at all fermentation periods), and unfermented models (7 mg GAE/g). The 36-hour fermented samples (65 mol Trolox/g) and 48-hour fermented samples (60 mol Trolox/g) showed the highest rates of oxygen radical antioxidant capacity (ORAC). While antioxidant activity peaked after 24 hours of fermentation (21 mg Ascorbic acid equivalent (AEE)/g) as measured by DPPH radical scavenging capacity. Castro et al., 2019, attributed these variations to the formation of C-phycocyanin and phenolic compounds because these composites react differently to various antioxidant capacity experiments.

When fermented with *L. acidophilus, B. bifidum, L. casei, B. infantis, B. longum, and L. lactis, A. platensis* demonstrated an enhanced DPPH radical scavenging capacity (28% of inhibition) compared to the unfermented control. At concentrations between 9 and 19 mg/mL of microalgae, the concentration-dependent effect in DPPH scavenging capacity of fermented and unfermented was more pronounced. The level of phenol in the fermented microalgae was also purposefully higher (34 mg/g GAE) than in the control samples (20 mg/g GAE). According to Liu et al. (2011), these results were related to increased phycocyanobilin and the formation of phenolic chemicals such gallic acid.

P. lutheri microalgae fermented with *C. rugopelliculosa* has a dose-dependent ability to scavenge free radicals (0.01-1000 g/mL of fermented microalgae), especially hydroxyl radicals. According to Ryu et al. (2012), this antioxidant ability was mostly ascribed to the production of bioactive peptides and amino acids (arginine, glycine, and proline) that can act as electron donors and react with hydroxyl radicals to transform them into more stable molecules, stopping radical chain reactions. Similar to this, after 12 days of fermentation, P. lutheri which had been combined with *H. polymorpha* displayed higher levels of lipid peroxidation inhibition activity. In terms of hydroxyl, superoxide, alkyl-radical scavenging activity, and DPPH, fermented microalgae had IC50 values of 0.024 mg/mL for hydroxyl, 0.064 mg/mL for superoxide, 0.187 mg/mL for alkyl-radical scavenging activity, and 0.257 mg/mL for DPPH. The scavenging percentages for DPPH, alkyl, hydroxyl, and superoxide were 72.4, 84.0, 93.5, and 94%, respectively, when the same concentration (1 mg/mL) of fermented algae was utilised (Qian et al., 2012). According to these scientists, the release of hydrophobic amino acids such alanine, leucine, and valine is thought to be the cause of the increase in antioxidant capacity (Qian et al., 2012). The free radical scavenging activity of microalgae *P. lutheri* fermented with *C. rugopelliculosa* was found to be dose-dependent (0.01-1000 g/mL of fermented microalgae), primarily for hydroxyl radicals. According to Ryu et al. (2012), the bioactive peptides and amino acids (arginine, glycine, and proline) were mostly responsible for this antioxidant activity since they could act as electron donors and interact with hydroxyl radicals.

Correspondingly, *P. lutheri* showed lipid peroxidation inhibition activity when fermented with *H. polymorpha* for 12 days with advanced ranges. The IC50 value for fermented microalgae was 0.024 mg/mL for hydroxyl, 0.064 mg/mL for superoxide, 0.187 mg/mL for alkyl-radical scavenging activity, and 0.257 mg/mL for DPPH, which represents the concentration at which the radicals created by the chemical systems are scavenged by 50%. The scavenging percentages for DPPH, alkyl, hydroxyl, and superoxide were 72.4, 84.0, 93.5, and 94%, respectively, when 1 mg/mL of fermented algae was utilised (Qian et al., 2012). These researchers hypothesised that hydrophobic amino acids like alanine, leucine, and valine

were the cause of the increase in antioxidant capacity. For *P. lutheri*, an enzymatic pretreatment using cellulase was occasionally requested to collapse the cell wall components, whereas yeast was introduced before fermentation for *H. polymorpha* cultured with *L. brevis* (Ryu et al., 2012).

When fermented with *L. plantarum* and ultrasonic extraction (2 mg/g), S. maxima produces higher antioxidants (attributable to its -carotene), in comparison to conservative water extraction (1mg/g) techniques. The amount of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger activity in the fermented S. maxima was advanced (50%), in comparison to water or ultrasound was castoff without fermentation (32% and 36.%, respectively) (Choi et al.,2018).

Bioactive substances having antimicrobial qualities

Phlorotannins, polysaccharides, polyunsaturated fatty acids, and carotenoids are examples of bioactive substances that may have antimicrobial effects on a variety of pathogens. Fermentation can enhance the creation of these composites (Eom et al., 2013). Martelli et al., 2020 investigated fermenting microalgae with Flavobacterium sp., LAB and yeast C. utilis to harvest composites with antimicrobial properties. *Pseudomonas aeruginosa* was effectively inhibited by microalgal oligosaccharides, but not *E. coli, Erwinia carotovora, Salmonella typhi, Streptococcus mutans, Staphylococcus aureus,* or Xanthomonas campestris (An et al.,2009). *Listeria monocytogenes, Salmonella spp. enterica, Bacillus cereus, E. coli,* and S. aureus growth was observed to be inhibited in both fermented and unfermented microalgae samples (Eom et al., 2013). Significantly higher antimicrobial activity was obtained when algae extracts were found with *L. casei* and *L. paracasei*, towards *Salmonella* spp. and *S. aureus.* Inappropriately, all the extracts missed their antimicrobial activity over prolonged periods (Eom et al.,2013).

Phlorotannins were isolated and identified by Eom et al. in 2013 whose content decreased after 5 days of fermentation. Phlorotannins significantly improved during the first day of fermentation and showed the strongest antibacterial action against methicillin-resistant S. aureus (MRSA) and methicillin-susceptible S. aureus (MSSA). According to Martelli et al., A. platensis samples that had undergone fermentation had lower total phenolic content than ones that hadn't. At concentrations between 0.625 and 20 mg/mL, both unfermented and LAB-fermented A. platensis did not inhibit the development of various E. coli strains or two S. aureus strains. The findings demonstrated that the microalgae supported the growth of the pathogenic bacteria, which was attributed to an abundance of protein (Liu et al., 2011).

Bioactive substances having anticoagulant qualities

Conventionally, heparin is employed as a profitable anticoagulant, yet it is tough to harvest and it is associated with side effects like thrombocytopenia (Pushpamali et al.,2008). So, there has been a curiosity in the development of novel compounds with anticoagulant capabilities. Some research observed that microalgae possess anticoagulant properties, that can be improved with fermentation, mostly credited to the production of diverse polysaccharides (Nikapitiya et al.,2007).

Utilising the activated partial thromboplastin time (aPTT), prothrombin time (PT), DPPH, and ABTS assays, isolated sulfated polysaccharides' anticoagulant activity was evaluated. *Chlorella sorokiniana* and *Chlorella* sp. (N4) sulfated polysaccharides had anticoagulant properties. The existence of several factors, including the sulphate content and

their binding site, the monosaccharide residue, and the glycoside bond that are involved in the polysaccharide's effectiveness, may be the cause of Chlorella sorokiniana's anticoagulant capabilities. As a highly sulfated mucopolysaccharide, microalgal sulfated polysaccharides possess anticoagulant properties similar to heparin (Misurcova et al. 2015). Due to its sulphate group-containing anionic nature, sPS is suitable for bio applications including anticoagulant medicines (Marques et al. 2016).

Prothrombin time (PT) and activated partial thromboplastin time (APPT) assays were used to measure the anticoagulant effects of exopolysaccharides concentrations in microalgal strains such *Chlorella sorokiniana, Chlorella* sp. (L2), Chlorella sp. (D1), Chlorella sp. (N4), and Picochlorum sp. Various EPS concentrations (0.05–2 mg/mL) were combined with control plasma samples, and the mixture was then incubated at 37 °C for 60 s. For two minutes, the mixture and preheated aPTT assay reagent were incubated at 37 °C. Finally, 0.25 mol/L of pre-warmed calcium chloride was added, and the clotting time was measured. Control plasma and EPS samples were combined at varied quantities (0.025–0.2 mg/mL) for the prothrombin time (PT) assay. Clotting time was then measured after the addition of the pre-warmed PT test reagent (Li et al. 2016). Heparin was utilised as a reference drug (0–0.05 mg/mL).

Using the PT and aPTT assay, the anticoagulant activities of sPS and heparin as a control were assessed. In the aPTT test, C. sorokiniana sPS increased the clotting time by more than 38 s at 10 g/mL. Clotting time was lengthened (up to 14 s) by the PT activity of Chlorella sp. (N4) sPS and C. sorokiniana at a concentration of 200 g/mL. It was discovered that aPTT and PT activity of Chlorella sp. (N4) sPS and C. sorokiniana stood lower than heparin. The aPTT and PT times were quickly accelerated by heparin; the clotting time was more than 60 s at 5 g/mL for aPTT and 13.69 s at 25 g/mL for PT. So, a higher concentration of sPS was required to achieve a result similar to that of heparin. Majdoub et al. 2009 revealed that sPS isolated from Arthrospira platensis has five times less anticoagulant effect than heparin in the aPTT experiment.

Some researchers found that, in addition to sulphate concentration, structural variations of sPS, including the sulfate's dispersal form, location, and chemical conformation, primarily the monosaccharide parts, may affect the substance's anticoagulant activity (Li et al. 2015). Chlorella sp. (N4) and C. sorokiniana displayed greater activity than other chlorella strains for the anticoagulant effect. This may be due to the same carbohydrate content as that of heparin sugar (mg)/g dry biomass in sPS isolated from Chlorella sp. and C. sorokiniana (N4). The C. sorokiniana sPS, which has anticoagulant qualities, may be a promising anticoagulant candidate with reduced bleeding risk in heart ischemic disease and stroke. Clotting time was lengthened (up to 14 s) by the PT activity of Chlorella sp. (N4) sPS and C. sorokiniana at a concentration of 200 g/mL. The maximum PT activity was seen in sPS extracted from Chlorella sp. (N4), but no anticoagulant action was seen in sPS extracted from Chlorella sp. (D1) despite the high sulfate/sugar ratio.

Bioactive compounds with anti-inflammatory potential

The anti-inflammatory activity of microalgae can be enhanced by the fermentation of microalgae due to its polysaccharide content (Mun et al.,2017). Thus fermentation united with enzyme-aided extraction, has been examined for polysaccharides production. To improve the anti-inflammatory property of different microalgae, the microbes like the lactic acid bacteria Lactobacillus sp. (Mun et al.,2017), L. brevis (Lee et al.,2011), *L. acidophilus, L. casei, L.*

lactis, B. bifidum, B. infantis, and *B. longum* and the spore-forming, gram-positive bacteria *B. subtilis* (Lin, et al.,2016) and the yeasts *S. cerevisiae* (Lee et al.,2011) and *C. utilis* (Lee et al.,2011; Wijesinghe et al.,2013) have been used.

A. platensis fermented in lactic acid showed dose-dependent antiinflammatory activity (Choi et al., 2018). Nitric oxide (NO), which is produced by immune and inflammatory cell types, has a variety of beneficial biological features, including stronger antibacterial and antiviral actions. However, NO can also cause damage to the target tissues during the inflammation process (Lin et al., 2016). Using a lipopolysaccharide (LPS)-stimulated RAW 265 cell culture, the inhibitive impact of the fermented microalgae on NO generation was confirmed. The formation of NO was suppressed when fermented microalgae at a concentration of 4.8 mg/mL were removed. According to Choi et al. (2018), concentrations between 0.6 and 3.0 mg/mL reduced NO generation by 40–50%.

Bioactive substances with additional health effects

A. platensis and *Spirulina maxima,* two fermented microalgae, have been studied for their potential neuroprotective (Choi et al., 2018) and UV-protecting (Liu et al., 2011) properties. In comparison to the unfermented control, *A. platensis* fermented with a mixture of lactic acid bacteria (*L. acidophilus, B. bifidum, L. casei, B. infantis, B. longum, and L. lactis*) displayed improved UV protective abilities. UVB-induced HaCa T cells were used to determine this extent (Liu et al., 2011). The antioxidant capacity of S. maxima has been associated with valuable health-related properties, like neuroprotective abilities, which are enhanced through fermentation (Choi et al.,2018). These properties, which were linked to the -carotene content, were enhanced by fermentation with *L. plantarum* and ultrasonic extraction in conjunction with cautious extraction techniques (water extraction).

Fermented Microalgae as Novel Functional Foods

To produce novel functional foods, certain observations were made to evaluate the potential of using microalgal biomass or extract as the only substrate for LAB or yeast fermentation. These studies sought to determine how microbial fermentation altered the nutritional conformation, sensory traits, and functional features of microalgae. LAB cultures produce chemicals with nutraceutical properties by hydrolyzing the microalgae's cell wall polymers into simpler compounds (Perez-Alva et al., 2022).

In comparison to the results found for the unfermented Spirulina biomass, Spirulina wet biomass fermented with *Lactiplantibacillus (Lpb.) plantarum* produces more total phenolic compounds, C-phycocyanin, and free methionine as well as improved antioxidant power, protein disintegration, and the release of bioactive peptides after 72 hours of fermentation (De et al., 2019). *A.platensis* was found to be a suitable substrate for the development of the probiotic bacterium Lpb. plantarum ATCC 8014, according to Niccolai et al., 2022, and the fermentation product showed a noticeably increased level of antioxidant properties (80%) and total phenolic content (318%). More cellular antioxidant capacity and total phenolic content were found in the biomass of *A. platensis* fermented by L. plantarum, according to Jamnik et al.,2022. Additionally, during the first 24 hours of fermentation, there was an extreme LAB count, an increase in lactic acid concentration, and a decrease in pH. The fermented *A. platensis* may have new food preparations that promote microbial solidity because of the lower pH and

lack of pathogenic microorganisms. Furthermore, compared to the unfermented sample, the enhanced non-protein nitrogen level indicates higher protein degradation and bioavailability as well as a decrease in fat content. Interestingly, the microalga *P. lutheri* has been suggested as a potential source of natural antioxidants since when it was fermented with the yeast *Hansenula polymorpha*, it showed strong antioxidant activity (Qian et al., 2012). A. maxima biomass fermented by an Lpb. plantarum strain was found to have neuroprotective effects and to improve memory, according to Choi et al., 2018. combination of the fermentation process and a 4-hour, 40 kHz, ultrasonic extraction of -carotene. The high levels of beta-carotene and other biologically active components produced by LAB fermentation, which enhance the brain-derived neurotrophic factor (BDNF)/p-CREB signalling pathways and prevent dementia in mice, were attributed to this sample's higher antioxidant capacity.

Numerous observations were made to determine how various microbial species and treatments affected the sensory qualities (taste, flavour, aroma, and colour attributes) of fermented spirulina. Sahin et al.'s study from 2022, which looked at the fermentation process of three yeast cultures, *D. hansenii*, *K. marxianus*, and *S. cerevisiae*, successfully showed a decrease in the usual odours of spirulina (seaweed, cardboard, earthy/muddy, and cereal). To improve the volatile component form, reduce off flavours through deodorization, and hydrolyze the protein, Bao et al. (2018) utilised a variety of Lactic Acid Bacilli strains and a Bacillus subtilis to Spirulina biomass. Due to the removal of undesirable aromatic components associated with spirulina, the combined fermentation of spirulina by *Lpb. plantarum* and *B. subtilis* successfully produced an appealing aroma in the final product, while additional volatile compounds, such as acetoin (responsible for a creamy flavour), ethyl L(-)-lactate, lactic acid, and (R, R)-2,3-butanediol were produced by fermentation.

Microalgal fermentation for potential use in innovative food production

Microalgae	Activity	Microorganism	Reference
Spirulina	higher content of total phenolic compounds, C-phycocyanin, release of bioactive peptides,	Lactiplantibacillus (***Lpb***.) ***plantarum***)	Perez-Alva et al.,2022 De et al.,2019.
A. platensis	antioxidant activity	*Lpb. plantarum* ATCC 8014,	Niccolai et al. 2019
A. platensis	Total phenolic content	Lactiplantibacillus (***Lpb***.) ***plantarum***)	Jamnik et al. 2022
P. lutheri	high antioxidant activity	yeasts *H. polymorpha*	Qian et al.,2012
A. maxima	neuroprotective effect and the memory-enhancing activity	Lactiplantibacillus (***Lpb***.) ***plantarum***)	Choi et al.,2018
C. vulgaris	enhancing enzyme activity	LAB	Scieszka and Klewicka, 2020
Spirulina	sensory properties	yeasts: *D. hansenii*, *K. marxianus*, and *S. cerevisiae*	Sahin et al.,2022

Spirulina	volatile component profile	LAB strains and a *B. subtilis*	Bao et al.2018

Conclusion

Microalgae are regarded as a cutting-edge source of highly valuable, organically active composites, such as polysaccharides and polypeptides, which have beneficial effects on health (including antioxidant, anti-inflammatory, antibacterial, and anticoagulant qualities). The fermentation of microalgae could progress the issue of bioactive composites, thus improving the advantageous properties. The target composite and microalgae features will govern the kind of microorganism, the kind of fermentation (submerged or solid-state), and the parameters influencing fermentation. Application of pre-treatment or a mixture of pre-treatments will encourage an advanced liberation of the bioactive composites, but then an extreme procedure will cause the deprivation of the composite united with the loss of the bioactive characteristics.

The fermented microalgae area is in its early stages and has been only moderately discovered; the outcomes gained for spirulina are inspiring and lead to additional research on additional microalgal species. For enhancing the fermentation method, management of the conformation of microalgae may also be vital. We will need further research to pinpoint and isolate the precise bioactive chemicals produced by microalgal fermentation essential to numerous biological processes.

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Available data is provided in the publication.

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Declarations

Conflicts of interest The authors declare no confict of interest

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