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Microalgae and cyanobacteria as natural sources of antioxidant enzymes and enzyme inhibitors for Alzheimer's and diabetes

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ABSTRACT

Microalgae and cyanobacteria biomass can be cultivated in large amounts, producing a variety of bioactive compounds. As a result, various industries have begun to study the potential of this biomass in a wide range of applications such as biofuel production, environmental remediation for contaminated soil and water, food supplements, and as a source of feed for aquaculture. The cultivation conditions have a profound impact on microalgae biochemical composition. Therefore, the culture conditions must be tailored to the specific application of the biomass. This entails careful control of factors such as light exposure, nutrient concentration, and the application of stress conditions. To further enhance the value of microalgae biomass beyond its nutritional analysis, this review aims to explore the potential of the biomass as biofactories for producing antioxidant enzymes and inhibitors targeting Alzheimer's and diabetes diseases. Both chronic diseases are a growing concern due to an aging population and an increase in obesity rates. Microalgae when exposed to stressful conditions enhance the activity of antioxidant enzymes. However, further studies in the isolation and storage of these enzymes need to be performed. From the literature reviewed microalgae exhibited great potential in inhibiting key enzymes involved in Alzheimer's and Diabetes. The inhibitory potential was observed both in vitro and at a cellular level making them a promising natural alternative to current medication used to inhibit these enzymes.

1. Introduction

Microalgae and Cyanobacteria are microscopic, autotrophic organisms, they are easily cultivated with minimal resource demands, high adaptability to environmental conditions and independence from arable land for growth [1–5]. There has been a growing interest due to their emergence as a highly promising source of bioactive compounds and functional ingredients for pharmaceuticals, food, biofuel, cosmetic and healthcare industries [5–8]. This biomass is used as a dietary supplement and food in human nutrition, in agriculture and aquaculture [9]. The impact of diet on health has gained attention and awareness. No more is food considered only as a tool to satisfy hunger and meet our minimal metabolic requirements, with the potential of a good diet to prevent nutrition-related diseases and improve both physical and mental health, being intensively studied [10,11]. The World Health Organization (WHO) reported that dietary patterns and lifestyle habits constitute the main risk factors for the development of chronic diseases such as type 2 diabetes and hypertension [10]. Only a handful of microalgae and cyanobacteria species and their derivatives are approved for inclusion in the human diet, despite being highly recommended due to the high content of beneficial and bioactive compounds [12].

Some examples of the valuable compounds produced from these organisms are polyunsaturated fatty acids, carotenoids, and phenolic compounds [13]. Some species have shown antiviral, anti-inflammatory, and antitumor potentials [14]. There has been a growing demand for natural and bioactive compounds esteemed for their capacity to deliver health benefits. This biomass is a promising player for a biobased economy, as they serve as continuous and reliable

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source for safe bioactive natural products [15,16]. *Dunaliella salina* (Dunal) Teodoresco, a Chlorophyta, can accumulate high amounts of β-carotene when grown under specific conditions, with the ability to accumulate up to 80 % of β-carotene of total carotenoid content [16]. *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) (Flotow, 1844), a Chlorophyta, when stressed, becomes red due to the production of astaxanthin [3,17,18]. *Arthrospira platensis* Gomont, a Cyanobacteria (blue-green microalga), produces C-phycocyanin [19–22]. These three organisms exemplify the vast potential for natural and valuable bioactive compounds.

The market and production volumes of microalgae and Cyanobacteria has increased 5-fold since the start of this century and the diverse set of applications exhibits the potential to transition from the current fossil fuel-based economy to a circular bioeconomy [23]. The lipids from microalgae are a promising source of biofuel; however, the large-scale production of these lipids still faces a few bottlenecks [24]. Currently, a lot of focus is being placed on the accumulation of lipids and accumulation of biomass to increase the feasibility of microalgae as a biofuel source [24,25].

Microalgae are the base of the food chain in marine and coastal ecosystems and their key role in biogeochemical cycles of potential pollutants makes them very useful as bioindicators [26,27]. Microalgae and cyanobacteria produce specific enzymatic responses to stress caused by pollutants, meaning that these organisms can be used as early warning system to detect pollutants [27,28]. The fluctuation of environmental conditions including changes in salinity, temperature, light intensity, pH, and the presence of pollutants such as heavy metals like copper and pharmaceuticals or pesticides, causes oxidative stress in these organisms, which enhances antioxidant responses, increasing the activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) [25,29,30].

Microalgae possess a diverse set of bioactive molecules which has research targets for exploring the potential health benefits of this biomass [13]. In this review the microalgae potential to help in the treatment of Alzheimer's and Diabetes diseases is explored. Alzheimer's disease is a common form of dementia caused by a progressive loss of neurons. This causes a cognitive decline and mental deterioration, and it was recently proven that accumulation of reactive oxygen species (ROS) plays a role in the development of Alzheimer's disease [31,32]. Currently, no cure or effective therapy is available, and treatment consists of alleviating symptoms. There are two main factors which have the biggest impact on Alzheimer's patients: diet and the and inhibition of the cholinesterase enzymes, especially acetylcholinesterase (AChE) [32–34]. Microalgae and cyanobacteria possess bioactive compounds with the capacity to inhibit the cholinesterase enzymes [6–9]. Diabetes mellitus is a chronic metabolic disorder, characterized by hyperglycemia and it is often accompanied by many chronic vascular complications [35,36]. The control of blood glucose surges is critical for the treatment of Diabetes [35]. Hyperglycemia occurs when the pancreas fails to produce enough insulin or when the organism is unable to efficiently use the available insulin. Among the variations of Diabetes type 2, the last is the most common and represents 90 % of cases worldwide [36]. Generally, the disease develops in adulthood and is related to obesity, lack of exercise and a poor diet [36]. The reduction of carbohydrate uptake after a meal is one of the therapeutic approaches employed for the treatment of Diabetes. To achieve this, inhibitors of α-glucosidase and α-amylase are employed [35].

While numerous reviews explore the potential of microalgae and cyanobacteria in producing valuable products or for various biotechnological applications, few delve into their utilization for enzyme production. This review specifically examines the accumulation of antioxidant enzymes under diverse stress conditions. Additionally, it encompasses the extraction of enzymatic inhibitors, particularly inhibitors that target key enzymes associated with Alzheimer's and Diabetes diseases systematizing data for further research and inspire consumers to embrace the consumption of microalgae biomass.

2. Methodology

This systematic review was developed by searching in the Google Scholar database. Three major thematic groups were selected, antioxidant enzymes, antidiabetic, and anti-Alzheimer's compounds. The first group included stress factors either during growth or development stages and antioxidant enzymatic response. The second group included extraction of bioactive compounds, acetylcholinesterase inhibitors and butyrylcholinesterase inhibitors. The third group included extraction of bioactive compounds, α-amylase inhibitors, α-glucosidase inhibitors, and in vivo anti-diabetic effect. The software used for bibliographic management was Mendeley for Windows (v1.19.8). The primary keywords selected for database search were microalgae, activity, and in vitro. These were linked with several other words such as CAT, SOD, enzyme, AChE, BChE, α-amylase, α-glucosidase, protein extraction, protein purification and enzyme extraction. A total of research 193 works were considered. These were assessed considering the title, abstract and relevant data to remove irrelevant papers. Followed by the evaluation of the quality and data presented, 109 research works were considered eligible for this review. For this review the snowballing technique was employed of which 34 articles were added from the previous selected ones. Regarding the articles used for this critical review, 10 research papers are from 2000 to 2010, 83 are from 2011 to 2020 and 51 are from 2021 to 2024. Geographically they were from 37 countries and 11 were from the EU. Major research works used in this review were developed in China (n = 32), followed by India (n = 26), and Spain (n = 8). USA, South Africa, Egypt, Germany, Canada, Portugal, Brazil, Japan, Nigeria, UK, South Korea, Iran, Malaysia, Italy and Norway had between 2 and 7 reports each. Poland, Belgium, New Zealand, Colombia, Sweeden, Bangladesh, Indonesia, Algeria, Argentina, France, Mexico, Pakistan, Thailand, Estonia, Serbia and Chile were represented with one article each.

3. Microalgae and cyanobacteria as sources of antioxidant enzymes

The growing need for natural solutions to replace synthetic chemicals in food processing and the increasing consumer awareness to healthier food options is driving the enzyme market growth, which is anticipated to have a compound annual growth rate over 6.8 % from 2023 to 2032 [37]. The interest in antioxidant enzymes stands from their many industrial applications [38]. These biological catalysts protect the organism from oxidative stress and are currently studied to be harvested and added to food products or other items for preservation [38–40]. For instance, catalase (CAT) has been used in dairy and food enzymes markets in processes such as determining the quality of milk (catalase Mastitis test), packaging of food products (antioxidant enzyme system along with glucose oxidase to control the browning of fruit purees), cheese production (remove residues of hydrogen peroxide resultant from cold pasteurization, which in turn, is used to preserve the natural milk enzymes), and wine making (increasing its shelf-life and reducing alcohol content by oxygen removal) [41,42]. In these processes catalase is used for being safer, environmentally friendly, and cost-effective.

Besides food and beverage, antioxidant enzymes can be applied in several fields like personal care and cosmetics, textile, detergents, and bioremediation. For instance, engineered ascorbate peroxidase (APX) is used in electron microscopy for cellular imaging of a variety of mammalian organelles and specific proteins [43]. Superoxide dismutase (SOD) is applied in skin care as a safer cosmetic additive for its natural antioxidant ability with no toxic side effects. In this field SOD is used to prevent skin damaging, eliminate skin redness and swelling, assist in sun protection [44]. CAT has been used in bioremediation processes namely for the treatment of effluents resultant from the textile industries specifically to degrade the bleaching agent hydrogen peroxide into oxygen and water allowing to recycle the bleaching effluent in the dyeing

process [41].

Enzymes used for industrial purposes such as for food applications are mainly from microbial sources (fungi 50 % and bacteria 35 %) due to their easy, cost-effective, and consistent production, in contrast, to animal and plant sources [45,46]. CAT enzyme for industrial purposes can be produced from the following microbial sources: *Enterococcus faecalis* (Orla-Jensen 1919) Schleifer and Kilpper-Bälz 1984, *Aspergillus niger* van Tieghem, 1867, *Micrococcus luteus* (Schroeter 1872) Cohn 1872, *Bacillus maroccanus* n.sp., *Pyrobaculum calidifontisi* sp. nov., *Rhizobium radiobacter* (Smith & Townsend 1907) 2–1, *Ureibacillus thermosphaericus* (Andersson et al. 1996) Fortina et al. 2001 FZSF03, *Bacteroides fragilis* (Veillon and Zuber 1898) Castellani and Chalmers 1919 [42]. Many of these microbial sources (bacterial and fungal) are pathogenic or opportunistic pathogens. Therefore, a safety assessment of the production sources including the pathogenic and toxigenic potential is required by regulatory agencies [46]. To solve this problem enzymes industry are adopting the use of genetically modified microbes that overproduce enzymes with desired biochemical traits (as decreased production of unwanted secondary metabolites e.g., mycotoxins) and higher enzymatic activity [42,46]. However, the use of genetically modified organisms has controversial challenges that must be surpassed such as a lack of standardized regulation as well as safety and environmental concerns.

Microalgae and cyanobacteria are microorganisms that can growth faster in different modes of nutrition (autotrophic, heterotrophic and mixotrophic) with a consistent production of a desired biochemical trait [47]. For instance, they can produce enzymes which can be further increased by changing microalgal growth conditions. Microalgae and cyanobacteria can remove nutrients from water making it efficient for wastewater treatment reducing the cost of biomass production and making it suitable for circular bioeconomy biorefinery products, comprising an environmentally friendly and feasible alternative to microbial sources of enzymes production [47]. For instance, the diatom *Phaeodactylum tricornutum* (Bacillariophyceae) Bohlin, 1897 has a SOD production comparable to that of the yeast *Debaryomyces hansenii* Zopf (1889) which produces toxins as by-product, and cyanobacteria *Anabaena* strains were reported as potential organisms that produce hydrolytic enzymes [39]. Microalgae and cyanobacteria have similar advantages to the already used microbial sources in industry with similar enzyme contents that can be further enhanced making these organisms unexplored alternative sources to bacteria and fungi for enzyme production. The chlorophyta *Chlorella vulgaris* Beijerinck 1890 and the Cyanobacteria *Arthrospira platensis* displayed the presence of antioxidant enzymes and have the advantage of being recognized as safe for consumption [39]. In the following section, the role of antioxidant enzymes as well as the production of these enzymes by several microalgae and cyanobacteria and the strategies used for optimizing its production are presented.

3.1. Reactive oxygen species and oxidative stress

Reactive oxygen species (ROS) are naturally generated in the organism in response to changes in the environment [25]. The generated ROS are highly reactive species such as hydroxyl radical (HO^{-}), the superoxide radical (O_2^{-}) and hydrogen peroxide (H_2O_2) [48]. Normally these molecules are naturally controlled by the antioxidant system of the organism, which employs antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and non-enzymatic metabolites [25,29]. However, once a high concentration of ROS is accumulated in the cell, the antioxidant defence system fails to protect the cell components, leading to damage in proteins, lipids, carbohydrates, and desoxyribonucleic acid (DNA), ultimately resulting in cell death (Fig. 1) [25,29,48]. In higher organisms, the oxidative stress may lead to serious illnesses such as Alzheimer's disease, cancer, liver, and heart injury [23]. The antioxidant defence system of each organism is complex being composed of enzymatic and non-enzymatic defences,

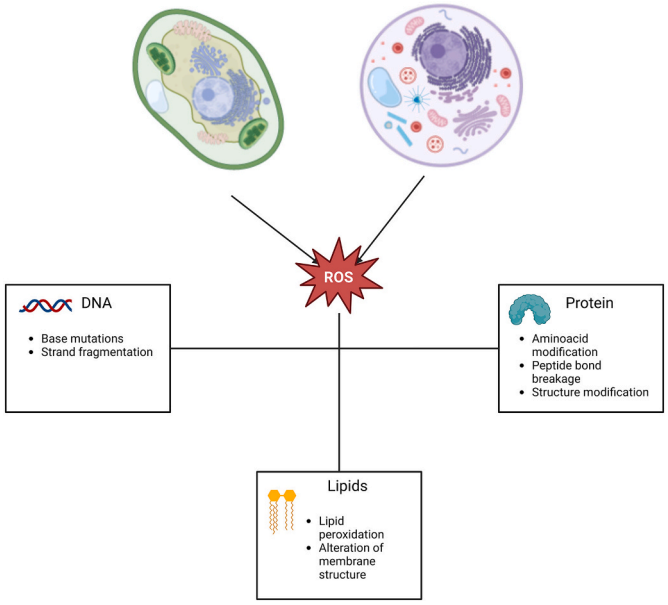

Fig. 1. Damage caused by ROS in different biomolecules leading to cell death Created with BioRender.com

that are intricately connected, as some antioxidant enzymes help to produce antioxidants, that in some cases act in the enzyme catalytic cycle [49].

3.2. Effect of stress factors on antioxidant enzymes production

Catalase (CAT) enzyme is responsible for converting H_2O_2 into water to prevent the formation of O_2^{-} ions [26]. Catalase activity increases under stress factors like nutrient limitation or temperature [24,29,48]. Superoxide dismutase (SOD) is responsible for converting O_2^{-} into H_2O_2 [26,50]. SOD together with CAT constitute the first line of defence against ROS generated in the organism (Fig. 2) [51,52]. SOD activity under nitrogen starvation significantly increases just as observed in the activity of catalase [8,24,53]. Ascorbate peroxidase (APX) is a H_2O_2 scavenger, ascorbate dependent and leads to the decomposition of H_2O_2 converting it into dehydroascorbate and H_2O [25]. This enzyme plays a major role in protecting higher plants from oxidative damage [54]. APX activation can be caused by the presence of metals such as Cr, Cu, Pb and Zn where the activity of APX from *Chlorella* sp. increased threefold when cultivated in 100 % tannery wastewater compared to the control group [54]. The effect of different stress conditions on the antioxidant enzymes from microalgae and Cyanobacteria are listed in Table 1. Nitrogen starvation induced in *Chlorella sorokiniana* (Chlorophyta) Shihira and R. W.Krauss 1965 greatly increases the activity of CAT and SOD, this increase was further enhanced when plant hormones were added to the culture medium, in which the activity reached 49.76 ± 4.72 and 81.41 ± 7.87 U/mg of protein of CAT and SOD, respectively [24]. When *Tetradesmus dimorphus* (formerly *Acutodesmus dimorphus*) (Chlorophyta) (Turpin) M.J.Wynne was starved of nitrogen, the activity of CAT and SOD increased. CAT activity reached 54.07 ± 2.79 U × 10^3/mg protein after 3 days and SOD activity reached 3857.92 ± 1052.67 U/mg of protein, decreasing to 1273.73 ± 155.25 U/mg of protein on the third day. APX activity reached 2.81 ± 0.11 U/mg of protein after two days of starvation and decreased to 2.06 ± 0.42 on the third day [8]. Cultivation of *Tetradesmus dimorphus* at higher temperatures also greatly increased the catalase activity. The maximum activity registered was 133.21 ± 22.74 U/mg of protein obtained after exposing the biomass to a cultivation temperature of 38 °C for three days, however the activity greatly decreased after longer periods where the activity dropped to 23.86 ± 0.81 U/mg of protein. The activity of APX when exposed to 38 °C

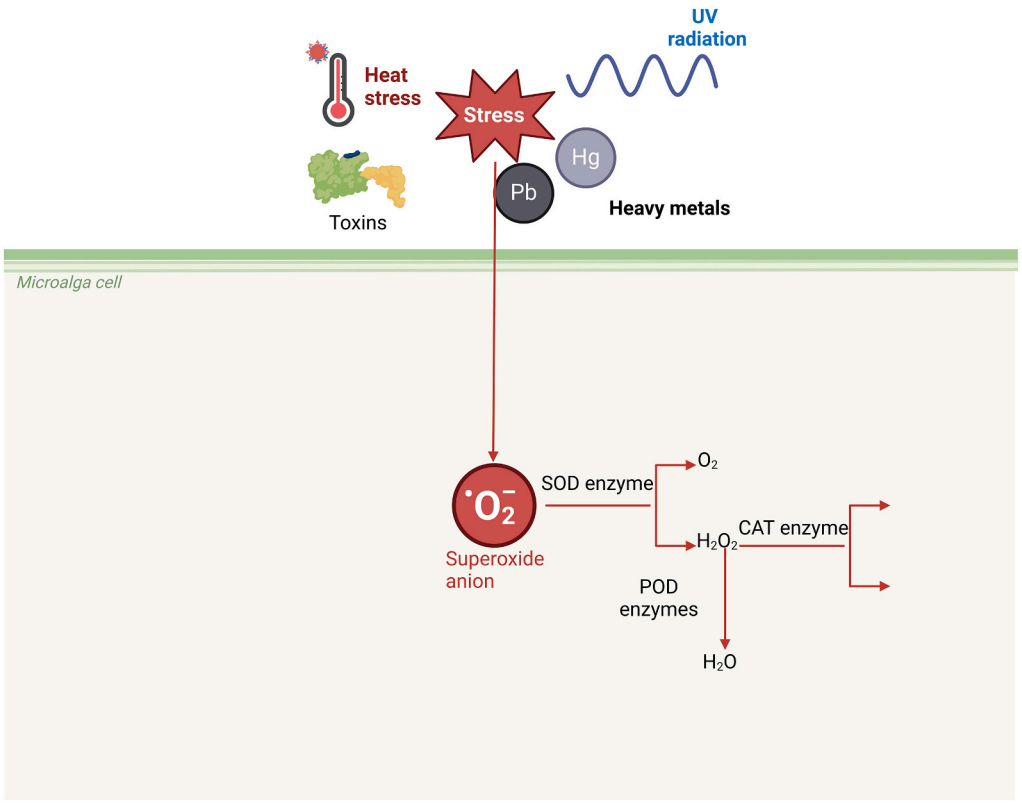

Fig. 2. Antioxidant mechanism in microalgal cells under different stress conditions. Superoxide dismutase (SOD) is the first enzyme in line against reactive oxygen species (ROS), scavenging the superoxide anion to produce oxygen (O_2) and hydrogen peroxide (H_2O_2). This later is further scavenged by peroxidase (POD) and catalase (CAT) enzymes to produce water. Scheme adapted from the template "Effects of Heat Stress in Crops" retrieved from https://app.biorender.com/biorender-templates/figures/all

Table 1
Effect of stress conditions on microalgae and cyanobacteria antioxidant enzymes production. Enzymes shown in this table: catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX).

Stress	Microalgae	Enzymes	Control activity	Activity under stress	References
Nitrogen limitation	*Chlorella sorokiniana*	CAT	18.80 ± 2.11 U/mg protein (100 % nitrate)	38.50 ± 4.23 U/mg protein (12.5 % nitrate)	[24]
		SOD	37.44 ± 4.54 U/mg protein (100 % nitrate)	70.70 ± 8.54 U/mg protein (12.5 % nitrate)	
Nitrogen limitation + diethyl aminoethyl hexonate (DA-6) hormone		CAT	38.50 ± 4.23 U/mg protein (12.5 % nitrate) without DA-6	49.76 ± 4.72 U/mg protein (12.5 % nitrate) with DA-6 10^{-9} M	
		SOD	70.70 ± 8.54 U/mg protein (12.5 % nitrate) without DA-6	81.41 ± 7.87 U/mg protein (12.5 % nitrate) with DA-6 10^{-9} M	
Naproxen (NPX) treatment	*Cymbella* sp.	CAT	20.62 U/mg without NPX	>35.65 U/mg for >10 mg/L NPX	[48]
		SOD	135.74 U/mg without NPX	195.15 U/mg at 1 mg/L NPX	
Nitrogen (N) starvation	*Tetradesmus dimorphus*	APX	6.60 ± 0.28 U/mg protein	2.28 ± 0.30 U/mg protein (1 day of N starvation)	[8]
		CAT	24.72 ± 1.17 U × 10^3/mg protein	54.07 ± 2.79 U × 10^3/mg protein (3 days of N starvation)	
		SOD	687.26 ± 222.87 U/mg protein	3857.92 ± 1052.67 U/mg protein (2 days of N starvation)	
Temperature	*Tetradesmus dimorphus*	APX	0.10 ± 0.004 U/mg protein (25 °C)	0.12 ± 0.044 U/mg protein (38 °C)	[25]
		CAT	20.54 ± 3.57 (x 10^3) (25 °C)	39.53 ± 1.97 (x 10^3) (38 °C)	
Tannery wastewater (TW) treatment	*Chlorella* sp.	APX	4.7 ± 0.2 U/mg protein (0 % TW)	11.2 ± 0.9 U/mg protein (100 % TW)	[54]
		CAT	0.09 ± 0.02 U/mg protein (0 % TW)	0.52 ± 0.06 U/mg protein (100 % TW)	
		SOD	3.8 ± 0.15 U/mg protein (0 % TW)	15.7 ± 0.9 U/mg protein (100 % TW)	

reached 4.21 ± 0.474 U/mg of protein after 9 days of cultivation whereas the control culture at 25 °C presented a activity of 0.10 ± 0.004 U/mg of protein [25]. When exposed to pollutants, the activity of antioxidant enzymes is generally increased as the cells take up increased amounts of the pollutant and this is linked to the accumulation of intracellular ROS [51]. The enzymes protect the organism until a critical concentration is reached, where the ROS accumulation is too great to handle, and the activity of the enzyme decreases due to cell damage and or cell death [29].

Pharmaceuticals are a class of pollutants that are rising in concentration as there are increasingly used [26,37,50]. Microalgae has been cultivated with carbamazepine in the medium to determine its toxicity and the capacity to biodegrade the pollutant [29,51]. The activity of antioxidant enzymes rose in a culture of *A. platensis* until a concentration

of 25 mg/L carbamazepine was reached where the activity of SOD reached over 6000 U/mg of protein while CAT reached over 250 U/mg of protein, further increase of carbamazepine concentration the activity of both SOD and CAT dropped drastically, with a final concentration of 100 mg/L of carmabazine the activity of SOD was under 2000 U/mg of protein and CAT was under 50 U/mg of protein [29]. Extracts of *Tetradesmus obliquus* (formerly *Scenedesmus obliquus*) (Chlorophyta) (Turpin) M.J.Wynne, 2016 where the activity of SOD decreased in all cultures and CAT activity increased drastically in the concentration of 200 mg/L [51]. Another class of pollutants rising in the environment are heavy metals, their levels have increased due to human activity [55,56]. Copper for example has an annual discharge of 9×10^6 tons [57]. Bioactivity assays revealed that under copper stress, *Tetradesmus lagerheimii* (formerly *Scenedesmus acuminatus*) (Chlorophyta) M.J.Wynne & Guiry showed a significant increase in POX, APX, and glutathione reductase (GR) activities, rising 1.7, 6, and 3-fold, respectively, compared to the control culture [57]. Conversely, *C. sorkiniana* demonstrated minor increases in POX and GR activity. However, it displayed a substantial increase in glutathione-s-transferase (GST) and SOD with an 8.7 and 2.4-fold increase, respectively, with notably higher in SOD levels than *T. lagerheimii* [57]. A rise in activity of SOD leads to a rise in activity of CAT as SOD converts the O_2^- into H_2O_2 which in turns leads to an increase of activity of CAT to convert the generated H_2O_2 it into H_2O [55].

Current antioxidant enzyme sources have a high activity, strain 2–1 identified as *Rhizobium radiobacter* (formerly known as *Agrobacterium tumefaciens*) produced 30,420 ± 1083 U/mg of protein of CAT in 20 h while *Micrococcus luteus* produced 10,938 ± 427 U/mg of protein in 34 h [58]. *Vibrio rumoiensis* sp. nov. was capable of producing 4092.46 ± 408.2 U/mg of protein in 48 h [59]. *Kluyveromyces marxianus* L3 strain after many optimizations and genetic modifications with a plasmid to overexpress the enzyme a yield of 1.4×10^6

U/L medium of superoxide dismutase [60,61]. A patented method by Suntory Ltd. (Japan) for SOD production using brewer's yeast yielded 8700 U/mg of protein [61,62]. The values observed in the bacterial and fungal species previously mentioned produce a high amount of both catalase and SOD and other organisms exist for the other antioxidant enzymes. In an industrial setting the organisms used are often genetically modified and culture conditions are highly optimized and thus produce far more antioxidant enzymes than microalgae [61]. Additionally, cultivation conditions, harvesting, cell lysis and purification processes are also highly optimized for enzyme production, and the overall process produces a high yield. Through genetic modification of microalgae, the production is expected to increase, as it has been demonstrated for several fungi species. Another challenge associated with microalgae is the cell lysis step, since they are known to possess a rigid cell wall that hampers enzyme and protein extraction. Regarding the enzyme/protein purification steps, some processes such as ammonium sulphate precipitation, ultrafiltration, affinity chromatography, and aqueous two-systems that are applied for bacteria protein isolation, are also applicable for microalgal biomass [63].

3.3. Extraction of antioxidant enzymes

To take advantage of enzymes for industrial purposes, they need to be first isolated. The main challenge in obtaining functional proteins from microalgae is the downstream processing required, thus there is a great need for an efficient and cost-effective protein extraction process [5,64]. Extraction conditions need to be carefully adjusted so that lysate of the tough cell wall of microalgae is achieved, especially when extracting enzymes, if conditions are too harsh, then the protein in the lysate will suffer denaturation making any target enzyme inactive [4,5,65,66]. Cell disruption pre-treatments are combined with buffer solutions, after cell disruption is achieved, and centrifugation is employed to remove the cell debris from the supernatant containing the crude protein extract [67]. The crude protein extract needs to be purified to remove other cell components such as lipids, nucleic acids, and pigments [5,66,68]. The purification techniques employed also need to be carefully adjusted to ensure that structure and activity of the target enzymes is maintained. The storage of antioxidant enzymes is another key factor to increase its applications. If properly stored, the enzymes last longer in their native form retaining their enzymatic activity, which allows them to be transported further and present more activity in the target products [69,70]. A comprehensive study on the stability of antioxidant enzymes extracted from *Dunaliella tertiolecta* (Chlorophyta) Teodoresco (Dunal) microalgae [69]. They concluded the storage temperature greatly affected the enzyme shelf life. Oxidative stress can be induced depending on the storage conditions leading to an increase of the activity of antioxidant enzymes to the induced stress. Freeze-dried extract loses activity over time, meaning that freeze-drying is recommended only for whole wet cells [69].

4. Enzyme inhibitors for Alzheimer's and diabetes

Enzymatic inhibitors, natural or artificial, help to regulate these catalysts trough inactivation of the target enzyme [71]. Enzymatic dysregulation has been linked to several medical disorders including, inflammatory, neurodegenerative, diabetes and cancer [72,73]. Thus, enzymatic inhibitors are of great interest to prevent or mitigate these conditions for e.g., inhibiting α-glucosidase decreases the speed in which starch is broken down and thus prevents a rapid increase in glucose levels which ultimately reduces postprandial hyperglycemia [74]. Currently one of the focus points in drug discovery, research and development are kinase and protease inhibitors. However, many of these inhibitors produce secondary undesired effects such as cardiotoxicity, hypertension, hypothyroidism, the mechanism in which these off target effects happen is largely unknown, but these effects are largely attributed to low specificity for the target enzyme [72,73,75]. Enzymatic inhibitors can be classified as competitive, noncompetitive, and uncompetitive. Competitive inhibitors compete with the substrate binding to the active site, the inhibition effect of these inhibitors depends on the number of active sites and relative concentration of the inhibitor compared to the substrate. Noncompetitive inhibitors bind to other parts of the enzyme and are typically irreversible. Uncompetitive inhibitors bind to the enzyme-substrate complex [72,76,77]. Currently several drugs serve as enzymatic inhibitors treating a diverse set of ailments, some examples include acarbose and miglitol for diabetes while galantamine and donepezil are used for Alzheimer's disease [74,78]. Due to the range of side effects of currently applied synthetic drugs new inhibitors are needed and natural products have diverse chemical structures, good efficiency and minimal adverse effects thus making them targets of high interest for new drug discovery [71,73,79,80]. The natural sources of inhibitors are often selected due to traditional knowledge or observation of interactions in nature [71]. Currently a lot of focus is being placed on marine microorganism for drug discovery, for e.g., brentuximab vedotin was extracted from a marine organism and is now approved by the FDA [81]. A lot of health ailments are caused by overactivity of enzymes and due to diverse set of side effects natural products are being examined as potential inhibitors with fewer side effects.

In the search of novel natural solutions to mitigate and prevent diseases that affect the quality of life of consumers several challenges must be overcome:

i) Evaluation of biological activities displayed by different microalgae and cyanobacteria species;
ii) Detailed biochemical characterization of the promising microalgae and cyanobacteria;
iii) Identify which biological molecules and possible synergistic effects are responsible for the desired biological activity;
iv) Purification process, upscaling processes and further clinical trials and application.

Microalgae are rich in natural bioactive substances like proteins, fatty acids, carotenoids polyphenols and polysaccharides [82]. The adaptability of microalgae to extreme conditions and environments underscores their potential as promising candidates for drug discovery, owing to the unique compounds synthesized along their evolutionary pathway [83]. Due to the high potential and diverse set of species of microalgae and cyanobacteria their biomass needs to be screened to find the species with highest biological activities. Currently few species have been screened and studies evaluating how culturing conditions affect bioactivity is almost nonexistent [82–84]. In the screening process testing different extraction conditions and solvents is crucial as these conditions will greatly affect the final product and thus the bioactivity of the extract [84]. When choosing the extraction solvent, careful consideration needs to be placed on the extraction capability as the higher the efficiency of the process the wider range of compounds are extracted from the biomass [85]. Choosing the extraction method is not straightforward due to the high diversity of the biomass and because conventional solid-liquid and liquid-liquid extraction consume high amounts of organic solvents, are labor intensive and are dependent on the proficiency of the operator, thus reproducibility of these methods is hard to achieve [85]. In this step upscaling needs to be considered, as the final downstream process needs to be as simple as possible and energy-efficient for a viable product [66]. After identifying promising biomass, a detailed characterization is in order, as the bioactive agents need to be identified and characterized for practical exploitation [86,87]. Some of the techniques employed to characterize the biomass are mass spectrometry (MS), chromatography including, gas chromatography (GC) and liquid chromatography (LC). Each technique has different applications and are thus appropriate to characterize different fractions of the biomass [86]. Completing the metabolomic characterization of the biomass, focus is placed on identifying the molecule responsible for the bioactive potential and evaluating possible synergetic effects between a group of molecules. Purifying bioactive compounds remains a significant challenge in the production of economically viable drugs from microalgae, exacerbated by the low yield of these compounds. Focus has been placed on biotechnological methods such as vectors to induce higher productivity, culturing conditions have also been explored to improve the yield of these compounds [88]. Bringing drug candidates from microalgae to the market is challenging due to extensive pre-clinical and clinical trials required to ensure that these compounds are safe so they can be approved by the FDA [88].

Regarding the investigation of microalgae and cyanobacteria potential as enzyme inhibitors sources for Alzheimer's and Diabetes several studies have been performed to overcome the first and second bottlenecks which are further discussed in the following sections.

4.1. Anti-Alzheimer potential of microalgae

Alzheimer's is a complex disease that affects mainly the brain, causing memory loss, loss of synapses, cell death and brain damage [13,89]. Many factors are associated with Alzheimer's disease such as low levels of acetylcholine, oxidative stress, and accumulation of metals [13,90]. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) play crucial roles in breaking down Acetylcholine (ACh) [13]. The decrease in concentration of acetylcholine affects the organism memory and body function, inhibition of these enzymes is currently considered one of the most widely accepted treatments for Alzheimer's disease, (Fig. 3) [14]. While there are a few medications available, like donepezil and galantamine, their prolonged usage can lead to various side effects, such as nausea, vomiting, dizziness, hepatoxicity, gastrointestinal disorders and renal failure [91]. Around 416 million individuals are reported to suffer from various forms of Alzheimer's [92]. Among this demographic, the likelihood of developing Alzheimer's escalates with age, and women constitute 54 % of the afflicted patients [92]. In Europe, it was predicted that 75 million people are affected by Alzheimer's disease, with an estimated range spanning between 59 and 93 million

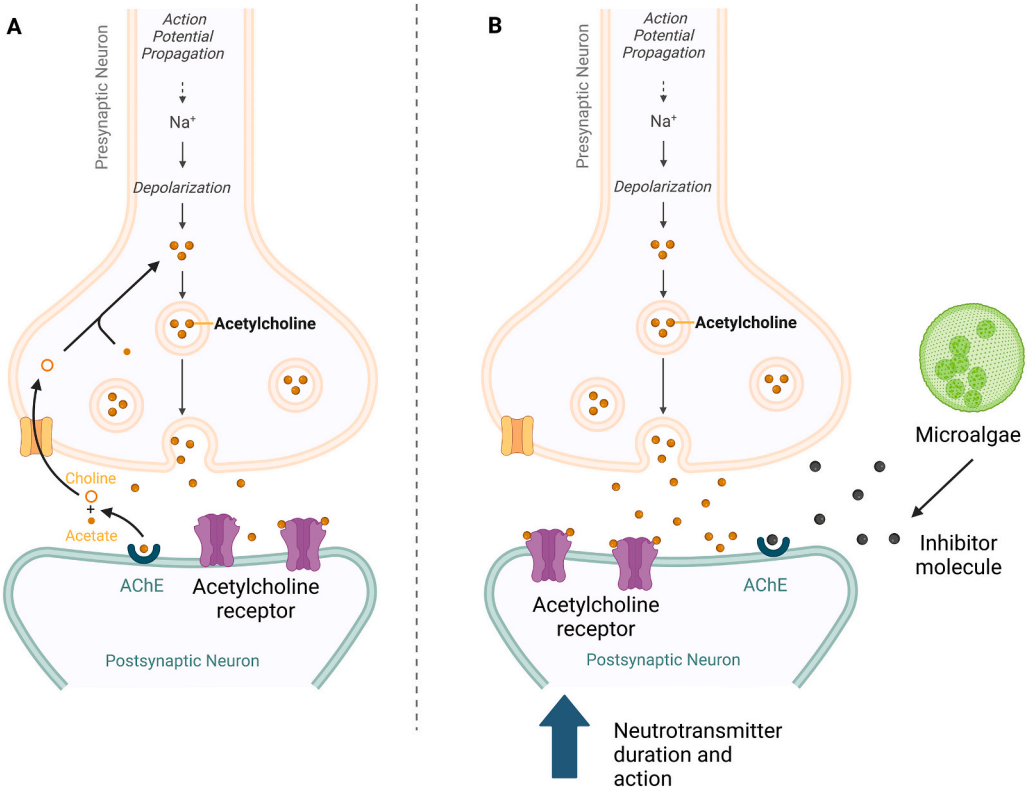

Fig. 3. Acetylcholinesterase and Butyrylcholinesterase inhibitors. Adapted from "*Alzheimer's Disease (AD) - Current Treatments*" by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates

patients [92]. These numbers highlight the growing concern of Alzheimer's disease due to an increasingly aging population, leading to more and more patients suffering from Alzheimer's disease [92]. Recent studies have suggested that Alzheimer's disease is linked to another chronic disease, Type II Diabetes (T2D) [93]. This is a concern since both diseases are age related, ongoing research suggests that patients with T2D are 50–60 % more likely to develop Alzheimer's disease. Around 80 % of patients with Alzheimer's disease have some form of Diabetes [93–95]. The link between Diabetes and Alzheimer's is so prevalent that some studies have started to refer to Alzheimer's as type III Diabetes (T3D) [94,96]. Given the range of side effects associated with currently used medications [13], there is a growing interest in natural sources of inhibitors [89], thus microalgae become increasingly appealing as in vitro inhibitory tests show promising results [9]. Not only are microalgae relatively easy to cultivate, but they also offer the advantage of manipulation to produce desired products. This versatility allows for different cultivation approaches to achieve specific goals [12].

Acetylcholinesterase is a serine hydrolase. This enzyme is mainly located in neuromuscular junctions and in cholinergic brain synapses. The enzyme is responsible for terminating the impulse of transmission by hydrolyzing acetylcholine into acetate and choline. This enzyme has a high specific catalytic activity degrading around 25,000 molecules of acetylcholine per second [97–99]. After association of the substrate the enzyme forms an unstable tetrahedral intermediate, this intermediate collapses forming an acyl enzyme and releasing a free alcohol. The acyl enzyme is then attacked by a water molecule and the free enzyme is regenerated trough a second tetrahedral intermediate [98,99].

The microalgae extracts are evaluated on their neuroprotective potential based on their IC50, concentration of the inhibitor required to inhibit 50 % of the enzyme. In the AChE inhibition assay the sample/extract is incubated with the substrate, acetylcholine iodide (AChI) and Ellman's reagent (DTNB). If the sample/extract has inhibitory activity, it bonds with the enzyme, stopping it from degrading acetylcholine. If the sample has no inhibitory activity or has low activity, the enzyme degrades the acetylcholine, exposing free thiol groups [16]. The reaction between DTNB and the free thiol groups results in the formation of a yellow product. Therefore, in this assay, the intensity of the yellow color at the end of the test directly correlates with the enzyme's activity. A more intense yellow color indicates higher enzyme activity, signifying lower activity of the sample as an inhibitor [9,31,89]. In recent years, a variety of microalgae extracts have been evaluated for their Anti-Alzheimer's potential by serving as Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) inhibitors.

Microalgae extracts which exhibit wide array of potencial to inhibit

Table 2
Summary table of different microalgae extracts and their Anti-Alzheimer's effect.

Biomass	Anti-Alzheimer's Effect	Extraction method	IC50		Sample	Reference
			AChE	BChE		
Dunaliella salina	AChE Inhibitor BChE Inhibitor	semi-pilot Speed Helix supercritical fluid extractor	73.14 ± 3.46 μg mL^{-1}	96.56 ± 6.58 μg mL^{-1}	Crude extract	[32]
Nitzschia amabilis *Arthrospira platensis* *Chlorella vulgaris*	AChE Inhibitor	Sonication using ddH_2O, soluble proteins fractioned with ammonium sulphate	1080 2040 2960 μg mL^{-1}	–	Protein Extract	[31]
Dunaliella salina	AChE Inhibitor	Compressed CO_2 extract Solid liquid extract	18.85 μg mL^{-1} 99.39 μg mL^{-1}	–	Carotenoids	[16]
Haematococcus lacustris *Tisochrysis lutea* *Porphyridium purpureum* *Nannochloropsis oceanica*	AChE Inhibitor BChE Inhibitor	Pressurized liquids extraction	87.14 ± 2.09 47.17 ± 3.40 89.21 ± 8.78 66.29 ± 3.29 μg mL^{-1}	113.11 ± 12.33 146.16 ± 8.40 101.24 ± 4.92 231.15 ± 13.62 μg mL^{-1}	Carotenoids	[6]
Chlorococcum sp. Hexane Ethanol Dichloromethane	AChE Inhibitor BChE Inhibitor	Sequential extraction on the same biomass shaking with the solvent for 2 days. Solvents used are hexane, dichloromethane, and ethanol.	13.83 ± 0.11 19.27 ± 0.27 μg mL^{-1}	14.09 ± 0.16 12.79 ± 0.10 μg mL^{-1}	Phenolic compounds	[9]
Dunaliella salina	AChE Inhibitor BChE Inhibitor	Freeze dried biomass mixed with 50:50 ethyl acetate/H2O and vortex applied.	18.85 ± 0.1 μg mL^{-1}	113.5 ± 11.5 μg mL^{-1}	Crude extract	[34]
Microcoleus autumnalis	AChE Inhibitor	*T1 Heptane extraction with thermomixer* *T2 Saponification Chloroform and* ethanolic KOH *T3* Co-solvent supercritical carbon dioxide 7 % ethanol	1399.0 ± 10.15 292.7 ± 9.39 65.8 ± 1.09 μg mL^{-1}	–	Phytosterols	[89]
Arthrospira platensis *Chlorella vulgaris* *Nannochloropsis occulta* *Porphyridium purpureum*	AChE Inhibitor BChE Inhibitor	Sonication using methanol/water/HCl (79/20/1)	40.89 ± 4.44 % 29.03 ± 3.33 % 8.66 ± 0.75 % 29.89 ± 2.26 % (Inhibition %)	31.68 ± 1.15 % 24.14 ± 3.00 % 6.85 ± 1.56 % 28.01 ± 1.39 % (Inhibition %)	Crude extract	[11]
Anabaena anomala *Anabaena oryzae* *Anabaena variabilis*	AChE Inhibitor	Soxhlet extraction using Methylene chloride/methanol (1/1)	25 ± 0.87 % 62 ± 1.3 % 49 ± 1.5 % (Inhibition %)	–	Crude extract	[33]

AChE and BChE are listed in Table 2. These are a diverse set of species including *Nitzschia amabilis* (formerly *Nitzschia laevis*) (Bacillariophyceae) H. Suzuki, *Arthrospira platensis*, *Chlorella vulgaris*, *Dunaliella salina*, *Haematococus lacustris*, *Tisochrysis lutea* (Haptophyta) Bendif et al. 2013, *Porphyridium purpureum* (formerly *Porphyridium cruentum*) (Rhodophyta) (S.F.Gray) Nägeli, *Nannochloropsis oceanica* D.J.Hibberd, 1981 and *Nannochloropsis oculata* (Eustigmatophyceae) (Droop 1955) Hibberd 1981. Among these, several stand out due to valuable biomolecules produced. Natural inhibitors are classified based on their potency, these classifications are low potency ($200 < IC50 < 1000$ μg mL^{-1}), moderate potency ($20 < IC50 < 200$ μg mL^{-1}) and high potency ($IC50 < 20$ μg mL^{-1}) [100].

According to the articles reviewed the highest neuroprotective effect is observed in microalgae from the Chlorophyceae class. Two species belonging to this class exhibited high inhibitory potential, *Dunaliella salina* and *Haematococcus lacustris.* The lowest IC50 recorded could be classified as a high potency inhibitor, with a concentration of 18 μg mL^{-1}, this result was obtained by two different studies using extracts of *D. salina* [16,34]. The bioactive compounds responsible for the inhibition of cholinesterase enzymes from microalgae have not yet been identified. However, studies that obtain extracts with high contents of carotenoids and polyphenols report higher inhibitory potential for these enzymes and carotenoids have exhibited neuroprotective effects in other studies, both *D. salina* and *H. lacustris* are rich in carotenoids [6,9,16,32]. The high content of carotenoids present in these microalgae could be responsible for their neuroprotective activity. Extraction of carotenoids from *D. salina* produce biomolecules with high bioactive potential. Almost all the extracts from this study exhibited moderate potency, with the solid-liquid extraction recording an IC50 of 99.39 μg mL^{-1} and the most potent extract, achieved under conditions of 325 bar and 45 °C, exhibited an IC50 of 18.85 μg mL^{-1}, reaching a level of an inhibitor with high potency [16]. Another carotenoid rich extract from *D. salina* biomass exhibited moderate inhibitory potency for both cholinesterase enzymes with IC50 values of 73.14 and 96.56 μg mL^{-1} for AChE and BChE, respectively [32]. Carotenoidsrichextractsfrom four microalgaespecies obtained promising results. The extracts of *Haematococcus lacutris, Tisochrysis lutea* Bendif et al. 2013*, Porphyridium purpureum* (S.F. Gray) Nägeli and *Nannochloropsis oceanica* exhibited positive results. Both *H. lacutris* and *P. cruentum* had an IC50 close to 90.0 μg mL^{-1} for AChE and an IC50 of approximately 100.0 μg mL^{-1} for BChE [6]. *T. lutea* and *N. oceanica* had the lowest IC50 for AChE, reaching 47.17 and 66.29 μg mL^{-1}, respectively [6]. However, the IC50 of these extracts for BChE was much higher, with values of 146.16 and 231.15 μg mL^{-1} [6]. These results further reinforce the notion that microalgae carotenoids exhibit neuroprotective activity. The high concentration of carotenoids has additional advantages, since the carotenoids from these microalgae are associated with antioxidant activity making them tackle two factors of a multifactorial disease [16]. Other fractions explored include the phenolic fraction of microalgae, where phenolic rich extract from *Chlorococcum* sp., using ethanol and dichloromethane as solvents, and identified the resulting extracts as potent inhibitors for both AChE and BChE. The IC50 values recorded were 13.83 μg mL^{-1} and 19.27 μg mL^{-1} for AChE, when using ethanol or dichloromethane, respectively. Additionally, for BChE, the IC50 values stood at 14.09 μg mL^{-1} when using ethanol as a solvent and 12.79 μg mL^{-1} when using dichloromethane [9]. Protein extract have also been evaluated, however these results exhibited weak inhibition capacity, the best result was 1008 μg mL^{-1} obtained by the *Nitzschia amabilis* extract [31]. Phytosterol-rich extracts exhibited moderate inhibitory potency. The study used three extraction methods that were applied on *Microcoleus autumnalis* (formerly *Phormidium autumnale*) (Cyanobacteria) Gomont 1892 biomass: T1 heptane extraction, T2 saponification and T3 pretreatment with bead-beating and then extracting in a supercritical fluid extractor using CO_2 and ethanol [89]. The T1 extract had low potency, with an IC50 value of 1399.0 μg mL^{-1}. Additionally, this study highlighted another extract with low potency, closer to a moderate inhibitor, the T3 extract, which exhibited an IC50 of 292.7 μg mL^{-1}. The T2 extract exhibited a moderate potency inhibitor profile, with a IC50 of 65.8 μg mL^{-1} [89].

Currently limited studies exist where the neuroprotective activity is measured true the inhibition of cholinesterase enzymes and the number of studies that reach an IC50 value is even more limited. Obtaining the IC50 value is crucial as it allows the direct comparison between studies, this comparison allows researchers to identify the bioactive fraction of the biomass with highest neuroprotective activity and thus direct further research in the right direction. Other plant sources and vegetables also exhibit anti-Alzheimer's potential. An ethanolic extract of *Convulvulus betonicifolia* Miller subsp. exhibited an IC50 1.946 μg mL^{-1} against AChE [101]. A phenolic extrac from *Limonium spathulatum* exhibited an IC50 of 3.28 ± 0.16 and 26.64 ± 0.96 μg/mL against AChE and BChE, respectively [102]. A range of different vegetables used in Thai food exhibited inhibition against AChE and BChE, although most only exhibited a low inhibition percentage at a 1 mg mL^{-1} concentration. A notable mention is *Eryngium foetidum* with an inhibition percentage of 58.57 ± 0.51 % and 32.85 ± 0.47 % for AChE and BChE, respectively [103]. A methanolic extract of *Satureja cuneifolia* obtained an IC50 of 63.69 and 23.17 μg/mL against AChE and BChE, respectively [104]. Essential oils of *Elaeagnus umbellata* exhibited an IC50 of 48 and 90 μg mL^{-1} against AChE and BChE, respectively [105]. These values are comparable to those obtained by microalgae, as most results reported by other plant sources either present a high inhibitory capacity or moderate capacity. Although most extracts of microalgae did not achieve a high inhibitory capacity, the studies presented still illustrate the neuroprotective activity of a diverse set of species and bioactive fractions. No specific bioactive component responsible for the inhibition of the enzymes has been identified in these studies. The carotenoid fraction and polyphenols have exhibited either moderate or high inhibitory capacity depending on the species. Thus, further research is needed to further enhance the neuroprotective activity of these extract and to identify the specific components responsible for the inhibition.

4.2. Anti-diabetic potential of microalgae

Diabetes mellitus (DM) is a chronic metabolic disease characterized by abnormal high levels of glucose in the bloodstream (hyperglycaemia) [106]. Chronic hyperglycaemia is associated with excessive oxidative stress and inflammation [107], which leads to the damage of essential macromolecules, such as lipids and DNA [36]. Promotes the development of several long-term complications, including neuropathy, nephropathy, retinopathy, cataract, and cardiovascular diseases, among others [106,108–110]. Considering its high prevalence worldwide, it is considered a public health problem according to the International Diabetes Federation (IDF), which reports that approximately 540 million adults, aged between 20 and 79 years, are currently affected, reaching 10.5 % of the adult population [111]. The IDF estimates that this number will increase to 643 million by 2030 and 783 million by 2045 [111]. According to the World Health Organization (WHO), the Diabetes mortality rate between 2000 and 2019 rose by 3 % and predicts that the disease will be the seventh cause of death worldwide by 2030 [112,113].

Diabetes mainly consists of type 1 (T1DM) and type 2 Diabetes (T2DM), with the latter being the most prevalent with 90 % of all DM cases [114]. T1DM, also known as insulin-dependent Diabetes, is characterized by a deficiency in insulin production, which results from the destruction of pancreatic β-cells by the immune system, thus preventing blood glucose from entering body cells [115]. T2DM or non-insulin-dependent Diabetes is characterized by insulin resistance and β-cells failure [116,117]. In this case, skeletal muscle and liver cells do not respond properly to any concentration of insulin, causing glucose to accumulate in the bloodstream, thus driving to hyperglycaemia [113,118]. T2DM is mostly diagnosed in adults from 40 years of age, and it is associated with genetic risk factors and inadequate lifestyle habits, such as lack of physical exercise and unhealthy diet [36,119]. As mentioned previously T2DM is linked to Alzheimer's disease (AD) and

thus, by treating Diabetes it helps to prevent the formation of AD.

The main objective of treating Diabetes is to control the glucose levels in the bloodstream [90,116]. For the treatment of T1DM, the administration of insulin via intravenous injection is commonly employed [113]. For individuals with Type 2 Diabetes (T2DM), maintaining blood glucose at a favourable level can be achieved through the adoption of a healthy diet, regular physical exercise, and the use of prescribed oral medications [120]. Various treatments with different mechanisms of action against hyperglycaemia have been documented [114]. One such approach involves inhibiting α-amylase and α-glucosidase enzymes to regulate postprandial hyperglycaemia [121]. The α-amylase enzyme is found in both saliva and the pancreas, and its function is to break down polysaccharides into smaller oligosaccharides and disaccharides. Subsequently, the α-glucosidase enzyme in the small intestine further hydrolyses these, leading to the formation of glucose, which is then released into the bloodstream, after absorption, through the intestinal epithelium [35,122]. A schematic representation of α-glucosidase action is displayed in Fig. 4. Another approach to manage T2DM is to inhibit the dipeptidyl peptidase-4 (DPP-IV) enzyme, which is involved in the deactivation of incretins hormones such as glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) [123,124]. These hormones are responsible for up to 70 % of the total postprandial insulin secretion by the pancreatic β-cells [125], and both contribute positively to the maintenance of glucose homeostasis and normal glucose tolerance [124,126]. Oral drugs such as acarbose, biguanide and voglibose have been utilised as α-amylase and α-glucosidase inhibitors, to control postprandial hyperglycaemia, but they are associated with side effects [121,127]. Synthetic DPP-IV inhibitors have also been developed, including sitagliptin and vildagliptin, both exhibiting significant antidiabetic effects, but also accompanied with adverse effects [126]. The high costs of current T2DM treatments may also pose an inconvenient for individuals with Diabetes [117].

Given the disadvantages of current therapies, attention has shifted towards natural resources due to their non-toxicity and proven effectiveness in managing T2DM disease [128]. Among these, microalgae are characterized by a high content in bioactive compounds with application in the food and pharmaceutical industries [129], and while these microorganisms can be utilised for their antioxidant, anticancer, and anti-inflammatory properties, among others [11], this review focuses on their applicability in T2DM management. The antidiabetic properties of microalgae and cyanobacteria have been documented [119,130,131]. Several studies have demonstrated ameliorating effects in diabetic mice when treated with microalgae supplementations, including the reduction of blood glucose, glycated haemoglobin (HbA1c), triglycerides, total cholesterol, and LDL-C levels. Accompanied with these effects, the increase in HDL-c levels and the attenuation of body weight gain is also a usual observation in diabetic mice [17,132–135]. The attenuation of the oxidative stress in diabetic rats, fed with a diet enriched with the alga *Nannochloropsis gaditana* (Droop 1955) Hibberd 1981, avoiding further complications associated with the disease [133]. Phycocyanin from *Arthrospira platensis* activated the ATK and AMPK-α signalling pathway in diabetic rats, increasing the glucose uptake into cells [22,136]. Activation of such signalling pathway in diabetic mice upon administration of Auxenochlorella pyrenoidosa (formerly *Chlorella pyrenoidosa*) (Chlorophyta) H. Chick, 1903 functional formulations [136]. AKT and AMPK-α proteins lead to the activation/expression of GLUT4. This later protein is then translocated glucose through the cell membrane enhancing the glucose uptake into body cells [136,137].

The focus of the present review is placed on in vitro studies on inhibiting α-amylase, α-glucosidase, and DPP-IV inhibition by microalgal extracts, (Tables 3–5). Among the diverse set of microalgae species reported to possess antidiabetic activities, *Chlorella vulgaris* from the class Trebouxiophyceae exhibited the highest activity against α-amylase.

A methanolic extract from *Chlorella vulgaris* obtained an IC50 value of 2.66 μg mL^{-1} while the standard, Acarbose, obtained an IC50 of 2.85 μg mL^{-1}. 14 bioactive compounds where later identified from the methanolic extract trough gas chromatography–mass spectrometry analysis. The compound responsible for the inhibition of the enzyme was not identified; however, it was noted that the most abundant bioactive compound present is Neophytadiene. In silico approach revealed that the compound C3, [1-Acetyl-7-bromo-5-(2-chlorophenyl)-5-ethoxy-2-oxo-3,4-dihydro-1,4-benzodiazepin-3-yl] acetate, had a higher binding affinity for the enzyme compared to acarbose, with free Gibbs energy values for the C3 compound and acarbose of −8.3 and − 7.7 $kcalmol^{-1}$, respectively [138]. The higher binding afinity of the C3 compound may be indicative of the inhibition capacity of the extract, and the isolation and purification of this compound is needed to confirm the source of the inhibitory activity from the methanolic extract. A Novel microalga from the class Chlorophyceae, *Scenedesmus bajacalifornicus* L.A.Lewis & Flechtner BBKLP-07 [Tetradesmus bajacalifornicus L.A.Lewis & Flechtner], reported IC50 values on α-amylase inhibition between 98.74 and

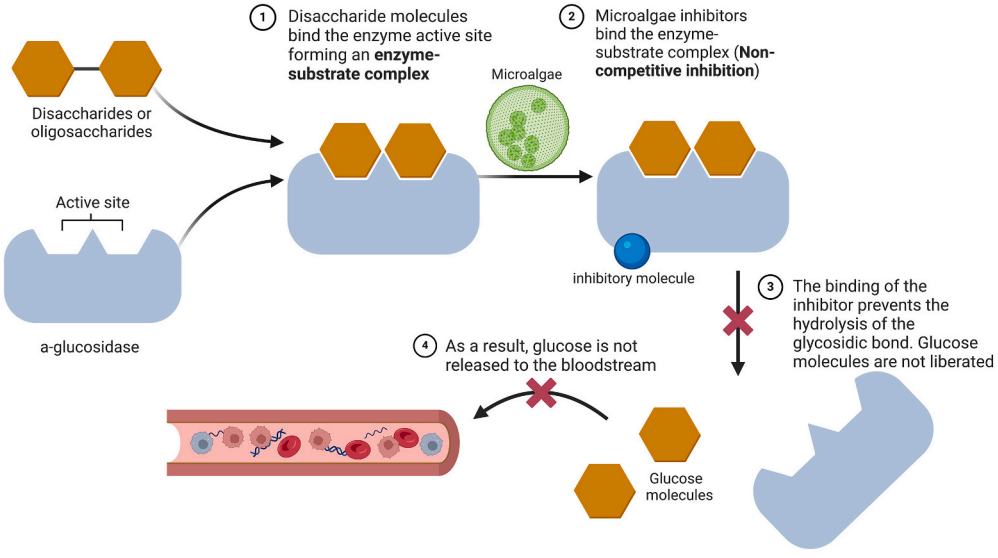

Fig. 4. Inhibition of α-glucosidase enzyme by microalgae extracts. In this scheme, we opted for the non-competitive inhibition to simplify the representation. Upon inhibitory molecule binding, the enzyme-substrate complex is deactivated, thus glucose units are not released, lowering post-prandial hyperglycaemia. Adapted from "Enzymatic Mechanism (Layout)" by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates.

Table 3
IC50 values or percentage of inhibition on α-amylase activity.

Biomass	Sample	IC50	Reference
Arthrospira platensis	Purified phycocyanin	231.45 ± 0.47 μg mL^{-1}	[19]
Arthrospira platensis (peptides)	USW-extracted protein at 120 °C	Inhibition percentage of 62 % at 1 mg mL^{-1}	[123]
	Synthetized peptides GVPMPNK, RNPFVFAPTLLTVAAR, and LRSELAAWSR with 95 % of purity, after In Silico studies.	236.2, 1077, and 313.3 μg mL-1, respectively	
Arthrospira platensis	Phycocyanin with 2.911 of purity	Inhibition of 51.129 ± 2.658 % at 1000 μg mL^{-1}	[121]
Chlorella vulgaris	Methanolic extract (14 bioactive compounds identified)	2.66 μg mL-1	[138]
Porphyridium purpureum *Chlorella vulgaris* *Arthrospira platensis* *Nannochloropsis oculata*	Crude extracts	7.50 ± 2.68 mg mL^{-1} 28.72 ± 8.30 mg mL^{-1} 31.04 ± 5.29 mg mL^{-1} 12.69 ± 5.53 mg mL^{-1}	[11]
Chlamydomonas reinhardtii *Chlorella vulgaris* *Haematococcus lacustris* *Monoraphidium dybowskii* *Parachlorella Kessleri* *Tetradesmus obliquus*	Amino acid fractions (after hydrolyzation)	230 ± 9.5 μg mL^{-1} 258 ± 6.7 μg mL^{-1} 261 ± 8.7 μg mL^{-1} 287 ± 10 μg mL^{-1} 294 ± 7.5 μg mL^{-1} 298 ± 9.0 μg mL^{-1}	[140]
Chlamydomonas sp. *EL5* *Chloroidium saccharophilum.* Mon*1* *Nostoc* sp	Ethanol extracts	Inhibition of 51.0 ± 1.6 % Inhibition of 59.4 ± 1.5 % Inhibition of 64.9 ± 0.8 % (At 5 mg mL^{-1})	[149]
Rhodosorus sp. SCSIO-45730	Three polysaccharide fractions: RSP-1, RSP-2, and RSP-3	- 0.56 mg mL^{-1} 8.78 mg mL^{-1}	[142]
S. bajacalifornicus BBKLP-07	Chloroform extract Acetone extract Ethanol extract Methanol extract Aqueous solvents extract	220.18 μg mL^{-1} 249.71 μg mL^{-1} 193.33 μg mL^{-1} 98.74 μg mL^{-1} 150.32 μg mL^{-1}	[85]

Table 4
IC50 values or percentage of inhibition on α-glucosidase activity.

Biomass	Sample	IC50	Reference
Arthrospira platensis	Purified phycocyanin	198.11 ± 0.25 μg mL^{-1} (β-glucosidase inhibition)	[19]
Arthrospira platensis (peptides)	USW-extracted protein at 120 °C	Inhibition percentage of 90 % at 1 mg mL^{-1}	[123]
	Synthetized peptides GVPMPNK, RNPFVFAPTLLTVAAR, and LRSELAAWSR with 95 % of purity.	151.5, 164.5, and 134.2 μg mL^{-1}, respectively	
Arthrospira platensis	Polysaccharide isolates PSP1, PSP2, PSP3 with decreasing MW.	7.38 mg mL-1, 1.56 mg mL-1, 0.85 mg mL^{-1}, respectively.	[128]
Arthrospira platensis	Supercritical carbon dioxide (SCCO2) extract	307 ± 2.0 μg mL^{-1}	[150]
Mychonastes homosphaera	Acetone extract dominated by terpenoids and alkaloids	From 3.45 ± 0.58 to 1.61 ± 0.68 mg mL^{-1}	[85]
Mychonastes homosphaera	Acetone and hexane extracts.	3.28 mg mL-1 for acetone extract and 4.07 mg mL^{-1} for hexane extract	[82]
Chlorella vulgaris	Bioactive scaled-up hydrolysate with 45 % of protein.	31 % of inhibition at a concentration of 30 mg mL^{-1} of hydrolysates	[151]
Chlamydomonas reinhardtii *Chlorella vulgaris* *Haematococcus lacustris* *Monoraphidium dybowskii* *Parachlorella Kessleri* *Tetradesmus obliquus*	Amino acid fractions	259 ± 8.4 μg mL^{-1} 267 ± 8.5 μg mL^{-1} 242 ± 7.7 μg mL^{-1} 255 ± 9.4 μg mL^{-1} 313 ± 11 μg mL^{-1} 278 ± 10 μg mL^{-1}	[140]
Euglena gracilis	Purified polysaccharide fraction	0.58 mg mL^{-1}	[143]
Haematococcus lacustris	Synthesized astaxanthin-s-allyl cysteine biconjugate.	3.98 μM for *Saccharomyces cerevisiae* α-glucosidase, and 1.6 and 6.4 μM for mammalian α-glucosidase sucrase and maltase, respectively.	[18]
Haematococcus lacustris	All-trans astaxanthin with a purity over 97 %	67.95 ± 0.03 μM	[125]
Leptolyngbya sp. KC45	Ethanol extract	5.47 mg mL^{-1}	[144]
Rhodosorus sp. SCSIO-45730	Three polysaccharide fractions with different compositions: RSP-1, RSP-2, and RSP-3	2.31, 0.03, and 0.63 mg mL^{-1}, respectively	[145]

249.71 μg mL^{-1} using solvents with different polarities [139]. These results are quite promising for a relatively unexplored species, therefore the importance of conducting further studies in this microalga arises.

Some proteins and their derivatives namely, peptides and amino-acids have demonstrated antidiabetic activities. C-phycocyanin is a phycobiliprotein which has been reported to posses antidiabetic properties. Purified phycocyanin obtained from *Arthrospira platensis* yielded IC50 values of 231.45 ± 0.47 and 198.11 ± 0.25 μg mL^{-1} on *α*-amylase and *β*-glucosidase activities, respectively [19]. C-phycocyanin inhibited salivary *α*-amylase with an average inhibition percentage of 51.129 ± 2.658 %, this result was obtained with a C-phycocyanin purity of 2.911 at 1000 ppm [121]. Three bioactive peptides were identified from C-phycocyanin extracts from *A. platensis*, GVPMPNK (GK), RNPFVFAPTLLTVAAR (RR) and LRSELAAWSR (LR). Among these peptides, LR presented the highest inhibitory activity against α-amylase, α-glucosidase and DPP-IV, presenting IC50 values of 313.6, 134.2 and 167.3 μg mL^{-1}, respectively [123]. Amino acid fractions from other microalgae sources such as *Chlorella vulgaris* (IBRC-50026), *Tetradesmus obliqus* (IBRC-50130) [[Turpin) Kützing 1833], *Chlamydomonas reinhardtii* P.A.Dang. (IBRC-50113), *Haematococcus lacustris* (IBRC-5175), *Monoraphidium dybowskii* (Wołoszyńska) Hindák & Komárkova Legnerová 1969 (IBRC-5005), and *Parachlorella kessleri* (Fott et Nováková) Krienitz et al. (IBRC-5028) exhibited IC50 values between

Table 5
IC50 values or percentage of inhibition on DPP-IV activity.

Biomass	Sample	IC50	Reference
Arthrospira platensis (peptides)	USW-extracted protein at 120 °C	Inhibition percentage 49 % at 2 mg mL^{-1}	[123]
	Synthetized peptides GVPMPNK, RNPFVFAPTLLTVAAR, and LRSELAAWSR with 95 % of purity.	192.3, 181.2, and 167.3 μg mL^{-1}, respectively.	
Arthrospira platensis	Butanol extract	Inhibition of 7–70 % at concentrations of 200–5000 μg mL^{-1}	[117]
Arthrospira platensis	Phycobiliprotein hydrolysates (PBPH)	4.059 mg mL^{-1} PBPH-pepsin 5.603 mg mL^{-1} PBPH-trypsin 5.257 mg mL^{-1} PBPH-alcalase 3.819 mg mL^{-1} PBPH-papain 4.195 PBPH-bromelain	[20]
Arthrospira platensis	Hydrolysate peptides from alcalase protease	Inhibition of 47 % at 1000 μg mL^{-1}	[146]
Arthrospira platensis	Tryptic hydrolysates from purified phycobiliproteins	IC50 value falls in the range between 0.5 and 1.0 mg mL^{-1}	[152]
Arthrospira platensis	Peptic (SP) and tryptic (TP) protein hydrolysates from *Spirulina* biomass	3.4 mg mL-1 for SP and 3.0 mg mL^{-1} for ST	[125]
Auxenochlorella pyrenoidosa	Peptic (CP) and tryptic (CT) protein hydrolysates from *Chlorella pyrenoidosa* biomass	Inhibition of 63.7 % for CP and 69.6 % for CT, both at a concentration of 5.0 mg mL^{-1}	[144]
Porphyridium purpureum *Phaeodactylum tricornutum*	Protein hydrolysates from proteolytic preparations of Alcalase and Flavourzyme	2.28 ± 0.36 mg mL^{-1} 2.68 ± 0.19 mg mL^{-1}	[145]

230 ± 8.7 μg mL^{-1} and 298 ± 9.0 μg mL^{-1} for α-amylase inhibition and between 242 ± 7.7 μg mL^{-1} and 313 ± 11 μg mL^{-1} for α-glucosidase. These results highlight that amino acid fractions from microalgae may be an important source for antidiabetic products in the pharmaceutical and food industries [140].

The antidiabetic properties of four microalga biomasses, *Nannochloropsis oculata, Porphyridium purpureum* (Bory de Saint-Vincent) K.M. Drew & R. Ross, 1965*, Arthrospira platensis, and Chlorella vulgaris* was evaluated, where the lowest IC50 values were obtained by *N. oculata and P. purpureum.* The lower IC50 values in these two microalgae species were associated with the higher polyphenolic and carotenoid content present in these species [11]. The carotenoids and polyphenols are strong antioxidants, and this may be the source of their inhibitory activity against enzymes related to diabetes and Alzheimer's. A synthetic form of astaxanthin, all-trans astaxanthin, with a purity of 97 % yielded an IC50 of 67.95 ± 0.03 μmol L^{-1}, approximately equivalent to 41.8 μg mL^{-1}. Even though this result is obtained with synthetic astaxanthin, *it still* highlights the antidiabetic potential of microalgae since the carotenoid is highly produced by *Haematococcus lacustris* [141]. Another class of bioactive compound with demonstrated antidiabetic activity are polysaccharides. The polysaccharide fraction RSP-2 extracted from *Rhodosorus* sp. SCSIO-45730 yielded IC50 for *α*-amylase and *α*-glucosidase values of 0.56 and 0.05 mg mL^{-1} against the IC50 values of acarbose 0.25 and 0.03 mg mL^{-1} for *α*-amylase and *α*-glucosidase, respectively [142]. A purified polysaccharide fraction from microalga *Euglena gracilis* (Euglenophyta) Ehrenberg, 1830, presented an IC50 of 0.58 mg mL^{-1} on α-glucosidase inhibition, while the value for acarbose was 2.28 mg mL^{-1} [143]. Secondary metabolites extracted from *Mychonastes homosphaera* (formerly *Chlorella minutissima*) (Chlorophyta) M. Beijerinck, 1890 yielded an IC50 of 3.13 ± 0.63 mg mL^{-1} while acarbose obtained an IC50 of 1.99 ± 0.49 mg mL^{-1}. Through various purification steps the IC50 values of the SIV-P2 obtained an IC50 of 1.61 ± 0.68 mg.mL^{-1}. The main classes of bioactive compounds identified in this fraction were terpenoids, alkaloids and fatty acids [85].

From the studies evaluated the polarity of the solvent has had an impact on the inhibitory strength of the extract. Extracts from the microalga *Tetradesmus bajacalifornicus* (formerly *Scenedesmus bajacalifornicus*) (Chlorophyta) BBKLP-07 using different solvents of increasing polarity found greater inhibition capacity on α-amylase activity as the polarity of the solvent increased, obtaining the highest value for the aqueous extracts [139]. A similar study using the microalgae *Mychonastes homosphaera*, and extractions with solvents of increasing polarity, revealed that the highest inhibitory capacity came from the acetone extract rather than the aqueous extract on α-glucosidase inhibition activity [85]. Another important aspect is the molecular weight (MW) of the biomolecules responsible for the *α*-amylase, *α*-glucosidase and DPP-IV inhibitions, and some studies have reported that lower MW compounds are more efficient at inhibiting those enzymes [128,142,144]. On the evaluation of antidiabetic properties of three different polysaccharide fractions against α-amylase and *α*-glucosidase activities, [128,142] state that the extracts of lower MW polysaccharides exert greater inhibition on these enzymes. However, other factors apart from MW also contribute to the suppression of enzyme activity. In contrast to these outcomes, four polysaccharide fractions from microalga *Euglena gracilis* through ultrafiltration found that the fraction with highest MW (EGP-2) had the best inhibition properties on α-glucosidase [143]. The DPP-IV inhibition from non-hydrolysed (the control) and hydrolysed protein extracts of microalgae *P. purpureum* and *Phaeodactylum tricornutum* found lower IC50 values for the hydrolysed samples, which were characterized with a higher quantity of small MW peptides (most of these being of <0.5 kDa) when compared to the controls [145]. *A. platensis* extracts revealed a higher inhibitory activity against DPP-IV with higher hydrolysis times of proteins [146].

The results reported clearly demonstrate the inhibitory potential of microalgae and Cyanobacteria extracts against the enzymes related to diabetes. This is especially true for extracts rich in carotenoids, polyphenols, and polysaccharides. Other plant and vegetable sources presented antidiabetic activity comparable to the articles explored in this review. Essential oils from *Elaeagnus umbellata* exhibited an IC50 of 120 and 110 μg mL^{-1} against α-glucosidase and α-amylase, respectively [105]. Decoctions and infusions of *Limonium algarviense* Erben and *Camellia sinensis* (L.) Kuntze exhibited inhibitory potentials against diabetic enzymes. *Limonium algarviense* exhibited higher capacity to inhibit yeast α-glucosidase while *Camellia sinensis* had higher potential against rat α-glucosidase. Neither species was able to inhibit α-amylase as the IC50 was over 10 mg mL^{-1}. An infusion and decoction of *Limonium algarviense* exhibited an IC50 of 0.05 ± 0.00 and 0.04 ± 0.00 mg mL^{-1}, respectively, against yeast α-glucosidase. An infusion and decoction of *Camellia sinensis* exhibited an IC50 of 0.12 ± 0.00 and 0.12 ± 0.00 mg mL^{-1}, respectively, against rat α-glucosidase [147]. The shell of *Pistacia terebinthus* fruits exhibited IC50 values of 0.19 and 35.99 mg mL^{-1} against α-glucosidase and α-amylase, respectively [148]. From the articles reviewed, the antidiabetic activity of C-phycocyanin has been confirmed both in vitro and in silico, and the presence of bioactive peptides has also been confirmed, with the highest inhibitory activity being obtained by the LR peptide with the sequence LRSELAAWSR. *Chlorella vulgaris* exhibited the lowest IC50 against *α*-amylase, and from its extracts a bioactive component with a higher binding affinity for the enzyme compared to acarbose has been identified to be [1-Acetyl-7-bromo-5-(2-chlorophenyl)-5-ethoxy-2-oxo-3,4-dihydro-1,4-benzodiazepin-3-yl] acetate. Future studies are needed to both continue to screen microalgae species to identify those with inhibitory activity but also to further identify more bioactive compounds responsible for

the inhibition of these enzymes. Additionally future studies are needed to evaluate the antidiabetic effect in vivo.

5. Conclusion

Microalgae biomass has a diverse set of applications, and each day more studies are being made to explore the full potential of this biomass. It has exhibited the potential for extraction of antioxidant enzymes and inhibitors for enzymes linked to both Alzheimer's and Diabetes. Under stress factors, the antioxidant response of microalgae can be modulated, and its production enhanced, resulting in a higher activity of antioxidant enzymes. In conclusion, the utilization of microalgae biomass as biofactories for antioxidant enzymes presents a promising avenue for further research and development especially in the cultivation and isolation processes. While existing literature highlights the significant potential of microalgae biomass in providing inhibitors for enzymes associated with Alzheimer's and Diabetes, additional efforts are needed to optimize extraction and isolation conditions. Isolating and purifying the active compounds responsible for inhibitory activity is paramount, alongside exploring the synergistic effects of different compounds. Despite the necessity for more extensive research, the encouraging results thus far underscore the viability of microalgae biomass as a rich source of inhibitors targeting key enzymes implicated in Alzheimer's and Diabetes. This underscores the importance of continued exploration in this field for potential therapeutic applications.

CRediT authorship contribution statement

Kilian Odenthal: Writing – original draft, Investigation. **Emmanuel Nunes:** Writing – original draft, Investigation. **Nuno Nunes:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Tomásia Fernandes:** Writing – review & editing, Supervision, Funding acquisition. **Igor A. Fernandes:** Writing – review & editing, Project administration, Funding acquisition. **Miguel A.A. Pinheiro de Carvalho:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] M. Nanda, V. Kumar, N. Fatima, V. Pruthi, M. Verma, P.K. Chauhan, M.S. Vlaskin, A.V. Grigorenko, Detoxification mechanism of organophosphorus pesticide via carboxylestrase pathway that triggers de novo TAG biosynthesis in oleaginous microalgae, Aquat. Toxicol. 209 (2019) 49–55, https://doi.org/10.1016/j.aquatox.2019.01.019.

[2] Y. Qiao, J. Rong, H. Chen, C. He, Q. Wang, Non-invasive rapid harvest time determination of oil-producing microalgae cultivations for biodiesel production by using chlorophyll fluorescence, Front. Energy Res. 3 (2015) 163215, https://doi.org/10.3389/fenrg.2015.00044.

[3] A. Paula, M. Cristiana, P. Fradinho, L. Gouveia, I. Sousa, A. Raymundo, J. M. Franco, Novel foods with microalgal ingredients – effect of gel setting conditions on the linear viscoelasticity of *Spirulina* and *Haematococcus* gels, J. Food Eng. 110 (2012) 182–189, https://doi.org/10.1016/j.jfoodeng.2011.05.044.

[4] N. Unterlander, P. Champagne, W.C. Plaxton, Lyophilization pretreatment facilitates extraction of soluble proteins and active enzymes from the oil-accumulating microalga *Chlorella vulgaris*, Algal Res. 25 (2017) 439–444, https://doi.org/10.1016/j.algal.2017.06.010.

[5] L. Grossmann, S. Ebert, J. Hinrichs, J. Weiss, Effect of precipitation, lyophilization, and organic solvent extraction on preparation of protein-rich powders from the microalgae *Chlorella protothecoides*, Algal Res. 29 (2018) 266–276, https://doi.org/10.1016/j.algal.2017.11.019.

[6] R. Gallego, A. Valdés, Z.J. Suárez-Montenegro, J.D. Sánchez-Martínez, A. Cifuentes, E. Ibáñez, M. Herrero, Anti-inflammatory and neuroprotective evaluation of diverse microalgae extracts enriched in carotenoids, Algal Res. 67 (2022), https://doi.org/10.1016/j.algal.2022.102830.

[7] H.E. Touliabah, D.A. Refaay, Enhancement of anticancer, antibacterial, and acetylcholinesterase inhibition activities from *Oscillatoria sancta* under starvation conditions, Water (Switzerland) 15 (2023) 1–15, https://doi.org/10.3390/w15040664.

[8] K. Chokshi, I. Pancha, A. Ghosh, S. Mishra, Nitrogen starvation-induced cellular crosstalk of ROS-scavenging antioxidants and phytohormone enhanced the biofuel potential of green microalga *Acutodesmus dimorphus*, Biotechnol. Biofuels 10 (2017) 1–12, https://doi.org/10.1186/s13068-017-0747-7.

[9] T.A. Olasehinde, A.O. Olaniran, A.I. Okoh, Cholinesterase inhibitory activity, antioxidant properties, and phytochemical composition of *Chlorococcum* sp. extracts, J. Food Biochem. 45 (2021) 1–9, https://doi.org/10.1111/jfbc.13395.

[10] WHO, Diet, Nutrition and the Prevention of Chronic Diseases; WHO Technical Report Series No. 916; WHO: Geneva, Switzerland, 2003.

[11] M.V. Vieira, I.P. Turkiewicz, K. Tkacz, C. Fuentes-Grünewald, L.M. Pastrana, P. Fuciños, A. Wojdyło, P. Nowicka, Microalgae as a potential functional ingredient: evaluation of the phytochemical profile, antioxidant activity and in-vitro enzymatic inhibitory effect of different species, Molecules 26 (2021), https://doi.org/10.3390/molecules26247593.

[12] R.A. Bekheit, W.M. Aboulthana, W.M. Serag, Bio- and Phyto-chemical study on *Nannochloropsis oculata* algal extract incorporated with gold nanoparticles, *in vitro* study, Front. Sci. Res. Technol. 5 (2023) 1–17, https://doi.org/10.21608/fsrt.2022.170128.1073.

[13] T.A. Olasehinde, A.O. Olaniran, A.I. Okoh, P. Koulen, Therapeutic potentials of microalgae in the treatment of Alzheimer’s disease, Molecules 22 (2017), https://doi.org/10.3390/molecules22030480.

[14] G.T. Grossberg, Cholinesterase inhibitors for the treatment of Alzheimer’s disease: getting on and staying on, Curr. Ther. Res. Clin. Exp. 64 (2003) 216–235, https://doi.org/10.1016/S0011-393X(03)00059-6.

[15] T. Chiong, C. Acquah, S.Y. Lau, E.H. Khor, M.K. Danquah, Microalgal-Based Protein By-products: Extraction, Purification, and Applications, Elsevier Inc., 2016, https://doi.org/10.1016/B978-0-12-802391-4.00012-4.

[16] M. Bueno, C. Vitali, J.D. Sánchez-Martínez, J.A. Mendiola, A. Cifuentes, E. Ibáñez, M. Herrero, Compressed CO2 technologies for the recovery of carotenoid-enriched extracts from *Dunaliella salina* with potential neuroprotective activity, ACS Sustain. Chem. Eng. 8 (2020) 11413–11423, https://doi.org/10.1021/acssuschemeng.0c03991.

[17] M. Wang, H. Ma, S. Guan, T. Luo, C. Zhao, G. Cai, Y. Zheng, X. Jia, J. Di, R. Li, H. Cui, Astaxanthin from: *Haematococcus pluvialis* alleviates obesity by modulating lipid metabolism and gut microbiota in mice fed a high-fat diet, Food Funct. 12 (2021) 9719–9738, https://doi.org/10.1039/d1fo01495a.

[18] P. Sakayanathan, C. Loganathan, A. Iruthayaraj, P. Periyasamy, K. Poomani, V. Periasamy, P. Thayumanavan, Biological interaction of newly synthesized astaxanthin-s-allyl cysteine biconjugate with *Saccharomyces cerevisiae* and mammalian α-glucosidase: *in vitro* kinetics and *in silico* docking analysis, Int. J. Biol. Macromol. 118 (2018) 252–262, https://doi.org/10.1016/j.ijbiomac.2018.06.027.

[19] G. Prabakaran, P. Sampathkumar, M. Kavisri, M. Moovendhan, Extraction and characterization of phycocyanin from *Spirulina platensis* and evaluation of its anticancer, antidiabetic and antiinflammatory effect, Int. J. Biol. Macromol. 153 (2020) 256–263, https://doi.org/10.1016/j.ijbiomac.2020.03.009.

[20] J. Liu, X. Bai, P. Fu, *In silico* and *in vitro* assessment of bioactive peptides from *Arthrospira platensis* phycobiliproteins for DPP-IV inhibitory activity, ACE inhibitory activity, and antioxidant activity, J. Appl. Phycol. 34 (2022) 1497–1511, https://doi.org/10.1007/s10811-022-02732-z.

[21] P. Sakayanathan, C. Loganathan, S. Kandasamy, R.V. Ramanna, K. Poomani, P. Thayumanavan, *In vitro* and *in silico* analysis of novel astaxanthin-s-allyl cysteine as an inhibitor of butyrylcholinesterase and various globular forms of acetylcholinesterases, Int. J. Biol. Macromol. 140 (2019) 1147–1157, https://doi.org/10.1016/j.ijbiomac.2019.08.168.

[22] S. Hao, F. Li, Q. Li, Q. Yang, W. Zhang, Phycocyanin protects against high glucose high fat diet induced diabetes in mice and participates in AKT and AMPK signaling, Foods 11 (2022), https://doi.org/10.3390/foods11203183.

[23] W. Gongi, N. Cordeiro, J.L.G. Pinchetti, S. Sadok, H. Ben Ouada, Extracellular polymeric substances with high radical scavenging ability produced in outdoor

cultivation of the thermotolerant chlorophyte *Graesiella* sp, J. Appl. Phycol. 33 (2021) 357–369, https://doi.org/10.1007/s10811-020-02303-0.
[24] A. Giridhar Babu, X. Wu, A.N. Kabra, D.P. Kim, Cultivation of an indigenous *Chlorella sorokiniana* with phytohormones for biomass and lipid production under N-limitation, Algal Res. 23 (2017) 178–185, https://doi.org/10.1016/j.algal.2017.02.004.
[25] K. Chokshi, I. Pancha, K. Trivedi, B. George, R. Maurya, A. Ghosh, S. Mishra, Biofuel potential of the newly isolated microalgae *Acutodesmus dimorphus* under temperature induced oxidative stress conditions, Bioresour. Technol. 180 (2015) 162–171, https://doi.org/10.1016/J.BIORTECH.2014.12.102.
[26] S. Wang, Z. Wang, M. Chen, H. Fang, D. Wang, Co-exposure of freshwater microalgae to tetrabromobisphenol A and sulfadiazine: oxidative stress biomarker responses and joint toxicity prediction, Bull. Environ. Contam. Toxicol. 99 (2017) 438–444, https://doi.org/10.1007/s00128-017-2153-z.
[27] P. Lozano, C. Trombini, E. Crespo, J. Blasco, I. Moreno-Garrido, ROI-scavenging enzyme activities as toxicity biomarkers in three species of marine microalgae exposed to model contaminants (copper, Irgarol and atrazine), Ecotoxicol. Environ. Saf. 104 (2014) 294–301, https://doi.org/10.1016/j.ecoenv.2014.03.021.
[28] X. Wang, J. Miao, L. Pan, Y. Li, Y. Lin, J. Wu, Toxicity effects of p-choroaniline on the growth, photosynthesis, respiration capacity and antioxidant enzyme activities of a diatom, *Phaeodactylum tricornutu*, Ecotoxicol. Environ. Saf. 169 (2019) 654–661, https://doi.org/10.1016/J.ECOENV.2018.11.015.
[29] Q. Wang, W. Liu, X. Li, R. Wang, J. Zhai, Carbamazepine toxicity and its co-metabolic removal by the cyanobacteria *Spirulina platensis*, Sci. Total Environ. 706 (2020) 135686, https://doi.org/10.1016/j.scitotenv.2019.135686.
[30] S. Du, F. Meng, W. Duan, Q. Liu, H. Li, X. Peng, Oxidative stress responses in two marine diatoms during acute n-butyl acrylate exposure and the toxicological evaluation with the IBRv2 index, Ecotoxicol. Environ. Saf. 240 (2022) 113686, https://doi.org/10.1016/j.ecoenv.2022.113686.
[31] M.A.J. Alzahrani, C.O. Perera, R. Sabaragamuwa, Y. Hemar, Assessment of bioactive potential of aqueous protein extracts from diatoms *Nitzschia laevis*, *Spirulina platensis*, and *Chlorella vulgaris*, J. Aquat. Food Prod. Technol. 28 (2019) 177–193, https://doi.org/10.1080/10498850.2019.1571551.
[32] R. Gallego, A. Valdés, J. David, S. Martínez, Z.J. Suárez, Study of the potential neuroprotective effect of *Dunaliella salina* extract in SH - SY5Y cell model, Anal. Bioanal. Chem. 414 (2022) 5357–5371, https://doi.org/10.1007/s00216-021-03819-1.
[33] D.A. Refaay, M.I. Abdel-hamid, A.A. Alyamani, M.A. Mougib, D.M. Ahmed, A. Negm, A.M. Mowafy, A.A. Ibrahim, R.M. Mahmoud, Growth optimization and secondary metabolites evaluation of *Anabaena variabilis* for acetylcholinesterase inhibition activity, Plants 11 (2022) 1–16, https://doi.org/10.3390/plants11060735.
[34] J.D. Sánchez-martin, A.R. Garcia, G. Alvarez-rivera, A. Vald, M.A. Brito, A. Cifuentes, *In vitro* study of the blood–brain barrier transport of natural compounds recovered from agrifood by-products and microalgae, Int. J. Mol. Sci. 24 (2022), https://doi.org/10.3390/ijms24010533.
[35] J. Feng, X.W. Yang, R.F. Wang, Bio-assay guided isolation and identification of α-glucosidase inhibitors from the leaves of *Aquilaria sinensis*, Phytochemistry 72 (2011) 242–247, https://doi.org/10.1016/j.phytochem.2010.11.025.
[36] M.F. de A. Alves, F.K. de A. Barreto, M.A. de Vasconcelos, L.G. do Nascimento Neto, R.F. Carneiro, L.T. da Silva, C.S. Nagano, A.H. Sampaio, E.H. Teixeira, Antihyperglycemic and antioxidant activities of a lectin from the marine red algae, *Bryothamnion seaforthii*, in rats with streptozotocin-induced diabetes, Int. J. Biol. Macromol. 158 (2020) 773–780, https://doi.org/10.1016/j.ijbiomac.2020.04.238.
[37] Global Market Insights Inc, Global market insights: insights to innovation, in: Enzym. Mark. Size – By Prod. (Proteases, Lipases, Carbohydrases, Polym. Nucleases, Phytases, Catal. Appl. Food Beverage, Process. Food, Dairy, Bak. Confect. Forecast). 2023-2032, 2023.
[38] U.K. Roy, B.V. Nielsen, J.J. Milledge, Antioxidant production in *Dunaliella*, Appl. Sci. 11 (2021), https://doi.org/10.3390/app11093959.
[39] B. dos S.A.F. Brasil, F.G. de Siqueira, T.F.C. Salum, C.M. Zanette, M.R. Spier, Microalgae and cyanobacteria as enzyme biofactories, Algal Res. 25 (2017) 76–89, https://doi.org/10.1016/j.algal.2017.04.035.
[40] A. Bafana, S. Dutt, S. Kumar, P.S. Ahuja, Superoxide dismutase: an industrial perspective, Crit. Rev. Biotechnol. 31 (2011) 65–76, https://doi.org/10.3109/07388551.2010.490937.
[41] J. Kaushal, S. Mehandia, G. Singh, A. Raina, S.K. Arya, Catalase enzyme: application in bioremediation and food industry, Biocatal. Agric. Biotechnol. 16 (2018) 192–199, https://doi.org/10.1016/j.bcab.2018.07.035.
[42] M.O. Okpara, Microbial enzymes and their applications in food industry: a mini-review, Adv. Enzym. Res. 10 (2022) 23–47, https://doi.org/10.4236/aer.2022.101002.
[43] J.D. Martell, T.J. Deerinck, Y. Sancak, T.L. Poulos, K. Vamsi, G.E. Sosinsky, M. H. Ellisman, A.Y. Ting, L. Jolla, G. Hospital, L. Jolla, Engineered ascorbate peroxidase as a genetically-encoded reporter for electron microscopy, Nat. Biotechnol. 30 (2012) 1143–1148, https://doi.org/10.1038/nbt.2375.
[44] M. Zheng, Y. Liu, G. Zhang, Z. Yang, W. Xu, Q. Chen, The applications and mechanisms of superoxide dismutase in medicine, food, and cosmetics, Antioxidants 12 (2023), https://doi.org/10.3390/antiox12091675.
[45] S. Raveendran, B. Parameswaran, S.B. Ummalyma, A. Abraham, A.K. Mathew, A. Madhavan, S. Rebello, A. Pandey, Applications of microbial enzymes in food industry, Food Technol. Biotechnol. 56 (2018) 16–30, https://doi.org/10.17113/ftb.56.01.18.5491.
[46] M. Deckers, D. Deforce, M.A. Fraiture, N.H.C. Roosens, Genetically modified micro-organisms for industrial food enzyme production: an overview, Foods 9 (2020), https://doi.org/10.3390/foods9030326.
[47] S.B. Ummalyma, R. Sirohi, A. Udayan, P. Yadav, A. Raj, S.J. Sim, A. Pandey, Sustainable microalgal biomass production in food industry wastewater for low-cost biorefinery products: a review, Phytochem. Rev. 22 (2023) 969–991, https://doi.org/10.1007/s11101-022-09814-3.
[48] T. Ding, K. Lin, B. Yang, M. Yang, J. Li, W. Li, J. Gan, Biodegradation of naproxen by freshwater algae *Cymbella* sp. and *Scenedesmus quadricauda* and the comparative toxicity, Bioresour. Technol. 238 (2017) 164–173, https://doi.org/10.1016/j.biortech.2017.04.018.
[49] C. Yang, J. Zhou, S. Liu, P. Fan, W. Wang, C. Xia, Allelochemical induces growth and photosynthesis inhibition, oxidative damage in marine diatom *Phaeodactylum tricornutum*, J. Exp. Mar. Biol. Ecol. 444 (2013) 16–23, https://doi.org/10.1016/j.jembe.2013.03.005.
[50] C. Song, Y. Wei, J. Sun, Y. Song, S. Li, Y. Kitamura, Biodegradation and metabolic fate of thiamphenicol via *Chlorella* sp. UTEX1602 and L38, Bioresour. Technol. 296 (2020) 122320, https://doi.org/10.1016/j.biortech.2019.122320.
[51] J.Q. Xiong, M.B. Kurade, R.A.I. Abou-Shanab, M.K. Ji, J. Choi, J.O. Kim, B. H. Jeon, Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana* and *Scenedesmus obliquus* and the determination of its metabolic fate, Bioresour. Technol. 205 (2016) 183–190, https://doi.org/10.1016/j.biortech.2016.01.038.
[52] W. Zhang, M. Zhang, K. Lin, W. Sun, B. Xiong, M. Guo, X. Cui, R. Fu, Eco-toxicological effect of Carbamazepine on *Scenedesmus obliquus* and *Chlorella pyrenoidosa*, Environ. Toxicol. Pharmacol. 33 (2012) 344–352, https://doi.org/10.1016/j.etap.2011.12.024.
[53] M.M.S. Ismaiel, Effect of nitrogen regime on antioxidant parameters of selected prokaryotic and eukaryotic microalgal species, Acta Physiol. Plant. 38 (2016) 1–12, https://doi.org/10.1007/s11738-016-2170-2.
[54] K.V. Ajayan, C.C. Harilal, M. Selvaraju, Phycoremediation resultant lipid production and antioxidant changes in green microalgae *Chlorella* Sp, Int. J. Phytoremed. 20 (2018) 1144–1151, https://doi.org/10.1080/15226514.2017.1413333.
[55] S.E. Sabatini, Á.B. Juárez, M.R. Eppis, L. Bianchi, C.M. Luquet, M. del C. Ríos de Molina, Oxidative stress and antioxidant defenses in two green microalgae exposed to copper, Ecotoxicol. Environ. Saf. 72 (2009) 1200–1206, https://doi.org/10.1016/j.ecoenv.2009.01.003.
[56] B. Xia, Q. Sui, X. Sun, Q. Han, B. Chen, L. Zhu, K. Qu, Ocean acidification increases the toxic effects of TiO2 nanoparticles on the marine microalga *Chlorella vulgaris*, J. Hazard. Mater. 346 (2018) 1–9, https://doi.org/10.1016/j.jhazmat.2017.12.017.
[57] S.M. Hamed, S. Selim, G. Klöck, H. AbdElgawad, Sensitivity of two green microalgae to copper stress: growth, oxidative and antioxidants analyses, Ecotoxicol. Environ. Saf. 144 (2017) 19–25, https://doi.org/10.1016/j.ecoenv.2017.05.048.
[58] M. Nakayama, T. Nakajima-Kambe, H. Katayama, K. Higuchi, Y. Kawasaki, R. Fuji, High catalase production by *Rhizobium radiobacter* strain 2-1, J. Biosci. Bioeng. 106 (2008) 554–558, https://doi.org/10.1263/JBB.106.554.
[59] I. Yumoto, H. Iwata, T. Sawabe, K. Ueno, N. Ichise, H. Matsuyama, H. Okuyama, K. Kawasaki, Characterization of a facultatively psychrophilic bacterium, *Vibrio rumoiensis* sp. nov., that exhibits high catalase activity, Appl. Environ. Microbiol. 65 (1999) 67–72, https://doi.org/10.1128/AEM.65.1.67-72.1999.
[60] S. Raimondi, D. Uccelletti, D. Matteuzzi, U.M. Pagnoni, M. Rossi, C. Palleschi, Characterization of the superoxide dismutase SOD1 gene of *Kluyveromyces marxianus* L3 and improved production of SOD activity, Appl. Microbiol. Biotechnol. 77 (2008) 1269–1277, https://doi.org/10.1007/S00253-007-1270-8.
[61] A. Bafana, S. Dutt, S. Kumar, P.S. Ahuja, Superoxide dismutase: an industrial perspective, Crit. Rev. Biotechnol. 31 (2011) 65–76, https://doi.org/10.3109/07388551.2010.490937.
[62] S. Asami, T. Kusumi, T. Amachi, H. Yoshizumi, Process for production of superoxide dismutase, in: European Patent Application EP 19870218472, 1987.
[63] R. Gupta, N. Gupta, P. Rathi, Bacterial lipases: an overview of production, purification and biochemical properties, Appl. Microbiol. Biotechnol. 64 (2004) 763–781, https://doi.org/10.1007/s00253-004-1568-8.
[64] K.W. Chew, S.R. Chia, S.Y. Lee, L. Zhu, P.L. Show, Enhanced microalgal protein extraction and purification using sustainable microwave-assisted multiphase partitioning technique, Chem. Eng. J. 367 (2019) 1–8, https://doi.org/10.1016/j.cej.2019.02.131.
[65] J. Wang, S. Qin, J. Lin, Q. Wang, W. Li, Y. Gao, Phycobiliproteins from microalgae: research progress in sustainable production and extraction processes, Biotechnol. Biofuels Bioprod. 16 (2023), https://doi.org/10.1186/s13068-023-02387-z.
[66] L. Grossmann, J. Hinrichs, J. Weiss, Cultivation and downstream processing of microalgae and cyanobacteria to generate protein-based technofunctional food ingredients, Crit. Rev. Food Sci. Nutr. 60 (2020) 2961–2989, https://doi.org/10.1080/10408398.2019.1672137.
[67] S.P. Cuellar-Bermudez, I. Aguilar-Hernandez, D.L. Cardenas-Chavez, N. Ornelas-Soto, M.A. Romero-Ogawa, R. Parra-Saldivar, Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins, Microb. Biotechnol. 8 (2015) 190–209, https://doi.org/10.1111/1751-7915.12167.
[68] S. Khanra, M. Mondal, G. Halder, O.N. Tiwari, K. Gayen, T.K. Bhowmick, Downstream processing of microalgae for pigments, protein and carbohydrate in industrial application: a review, Food Bioprod. Process. 110 (2018) 60–84, https://doi.org/10.1016/j.fbp.2018.02.002.

[69] U.K. Roy, B.V. Nielsen, J.J. Milledge, Effect of post-harvest conditions on antioxidant enzyme activity in *Dunaliella tertiolecta* biomass, Biocatal. Agric. Biotechnol. 27 (2020), https://doi.org/10.1016/j.bcab.2020.101661.
[70] B.B. Moura, N.M. Silveira, E.C. Machado, R.V. Ribeiro, Effects of storage time and freeze-drying on the activity of antioxidant enzymes in sugarcane leaves, Rev. Bras. Bot. 39 (2016) 373–376, https://doi.org/10.1007/s40415-015-0229-8.
[71] D. Chettri, A.K. Verma, Biological significance of carbohydrate active enzymes and searching their inhibitors for therapeutic applications, Carbohydr. Res. 529 (2023) 108853, https://doi.org/10.1016/j.carres.2023.108853.
[72] A. Pesaresi, Nonallosteric activation of posttranslational modification enzymes by active site-directed inhibitors, Comput. Struct. Biotechnol. J. 23 (2024) 34–42, https://doi.org/10.1016/j.csbj.2023.11.019.
[73] J. Zhang, W. Li, B. Zhang, G. Zhang, C. Liu, Screening of angiotensin converting enzyme inhibitors from natural products via origami microfluidic paper-based analytical devices with colorimetric detection, J. Pharm. Biomed. Anal. 238 (2024) 115833, https://doi.org/10.1016/j.jpba.2023.115833.
[74] P. Sharma, V. Sharma, S. Mathpal, D. Tewari, S. Chandra, S. Tamta, Identification of alpha-glucosidase enzyme inhibitors from phytochemicals via integrated deep learning, molecular docking, molecular dynamics simulation, and MMPBSA analysis, S. Afr. J. Bot. 167 (2024) 48–61, https://doi.org/10.1016/j.sajb.2024.01.061.
[75] M. Khan, N. Ambreen, F. Saleem, M.A. Lodhi, F. Jan, M. Kamal, U. Salar, M. Taha, K.M. Khan, Nicotinonitriles as potential inhibitors of α-glucosidase, tyrosinase, and urease enzymes: synthesis, characterization, *in vitro*, and *in silico* studies, J. Mol. Struct. 1305 (2024) 137742, https://doi.org/10.1016/j.molstruc.2024.137742.
[76] M. Kuddus, Introduction to food enzymes, in: Enzym. Food Biotechnol. Prod. Appl. Futur. Prospect., Academic Press, 2018, pp. 1–18, https://doi.org/10.1016/B978-0-12-813280-7.00001-3.
[77] J.A. Berges, M.R. Mulholland, Enzymes and Nitrogen Cycling, Academic Press, 2008, https://doi.org/10.1016/B978-0-12-372522-6.00032-3.
[78] G. Marucci, M. Buccioni, D.D. Ben, C. Lambertucci, R. Volpini, F. Amenta, Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease, Neuropharmacology 190 (2021), https://doi.org/10.1016/j.neuropharm.2020.108352.
[79] T. Noori, A.R. Dehpour, A. Sureda, E. Sobarzo-Sanchez, S. Shirooie, Role of natural products for the treatment of Alzheimer's disease, Eur. J. Pharmacol. 898 (2021) 173974, https://doi.org/10.1016/j.ejphar.2021.173974.
[80] H. Luo, Y. Qian, J. Qi, X. Liu, A novel strategy for screening angiotensin-converting enzyme inhibitors from natural products based on enzyme-immobilized ligand fishing combined with active-site blocking and directional enrichment, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1195 (2022) 123203, https://doi.org/10.1016/j.jchromb.2022.123203.
[81] J.S. Hwang, G.J. Kim, H.G. Choi, M.C. Kim, D. Hahn, J.W. Nam, S.J. Nam, H. C. Kwon, J. Chin, S.J. Cho, H. Hwang, H. Choi, Identification of antiangiogenic potential and cellular mechanisms of Napyradiomycin A1 isolated from the marine-derived *Streptomyces* sp. YP127, J. Nat. Prod. 80 (2017) 2269–2275, https://doi.org/10.1021/acs.jnatprod.7b00211.
[82] K. S, H. P, A. Malik, S. Koushalya, P. Hariprasad, M. Anushree, *Chlorella minutissima* as a functional food: evaluation on nutritional profile and antioxidant potential of the metabolites, Biomass Convers. Biorefin. (2022) 13, https://doi.org/10.1007/s13399-022-02401-8.
[83] A. Trincone, C.A. Llewellyn, G.V. Subhash, C. Lauritano, J.H. Andersen, E. Hansen, M. Albrigtsen, L. Escalera, F. Esposito, K. Helland, K.Ø. Hanssen, G. Romano, A. Ianora, Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities, Front. Mar. Sci. 1 (2016) 68, https://doi.org/10.3389/fmars.2016.00068.
[84] K. Phinyo, K. Ruangrit, J. Pekkoh, Y. Tragoolpua, T. Kaewkod, K. Duangjan, C. Pumas, N. Suwannarach, J. Kumla, W. Pathom-aree, W. Gu, G. Wang, S. Srinuanpan, Naturally occurring functional ingredient from filamentous thermophilic Cyanobacterium *Leptolyngbya* sp. KC45: phytochemical characterizations and their multiple bioactivities, Antioxidants 11 (2022), https://doi.org/10.3390/antiox11122437.
[85] K. Selvaraju, V. Raguraman, H.N. Yadav, P. Hariprasad, A. Malik, Spectral characterization and binding dynamics of bioactive compounds from *Chlorella minutissima* against α-glucosidase: an *in vitro* and *in silico* approach, Algal Res. 75 (2023) 103281, https://doi.org/10.1016/j.algal.2023.103281.
[86] J.P. Pérez, A.A. Muñoz, C.P. Figueroa, C. Agurto-Muñoz, Current analytical techniques for the characterization of lipophilic bioactive compounds from microalgae extracts, Biomass Bioenergy 149 (2021) 106078, https://doi.org/10.1016/J.BIOMBIOE.2021.106078.
[87] O.M. Skulberg, Microalgae as a source of bioactive molecules-experience from cyanophyte research, J. Appl. Phycol. 12 (2000) 341–348, https://doi.org/10.1023/A:1008140403621.
[88] V. Balasubramaniam, R.D.N. Gunasegavan, S. Mustar, J.C. Lee, M.F.M. Noh, Isolation of Industrial Important Bioactive Compounds from Microalgae, Mol. 2021, Vol. 26, Page 943 26, 2021, p. 943, https://doi.org/10.3390/MOLECULES26040943.
[89] M.B. Fagundes, G. Alvarez-Rivera, J.A. Mendiola, M. Bueno, J.D. Sánchez-Martínez, R. Wagner, E. Jacob-Lopes, L.Q. Zepka, E. Ibañez, A. Cifuentes, Phytosterol-rich compressed fluids extracts from *Phormidium autumnale* cyanobacteria with neuroprotective potential, Algal Res. 55 (2021), https://doi.org/10.1016/j.algal.2021.102264.
[90] N. Cortes, R.A. Posada-Duque, R. Alvarez, F. Alzate, S. Berkov, G.P. Cardona-Gómez, E. Osorio, Neuroprotective activity and acetylcholinesterase inhibition of five Amaryllidaceae species: a comparative study, Life Sci. 122 (2015) 42–50, https://doi.org/10.1016/j.lfs.2014.12.011.
[91] T.B. Ali, T.R. Schleret, B.M. Reilly, W.Y. Chen, R. Abagyan, Adverse effects of cholinesterase inhibitors in dementia, according to the pharmacovigilance databases of the United-States and Canada, PLoS One 10 (2015), https://doi.org/10.1371/journal.pone.0144337.
[92] A. Gustavsson, N. Norton, T. Fast, L. Frölich, J. Georges, D. Holzapfel, T. Kirabali, P. Krolak-Salmon, P.M. Rossini, M.T. Ferretti, L. Lanman, A.S. Chadha, W.M. van der Flier, Global estimates on the number of persons across the Alzheimer's disease continuum, Alzheimers Dement. 19 (2023) 658–670, https://doi.org/10.1002/alz.12694.
[93] M. Barbagallo, Type 2 diabetes mellitus and Alzheimer's disease, World J. Diabetes 5 (2014) 889, https://doi.org/10.4239/wjd.v5.i6.889.
[94] X. Yuan, H. Wang, F. Zhang, M. Zhang, Q. Wang, J. Wang, The common genes involved in the pathogenesis of Alzheimer's disease and type 2 diabetes and their implication for drug repositioning, Neuropharmacology 223 (2023) 109327, https://doi.org/10.1016/j.neuropharm.2022.109327.
[95] S. Paul, J. Bhardwaj, B.K. Binukumar, Cdk5-mediated oligodendrocyte myelin breakdown and neuroinflammation: implications for the link between type 2 diabetes and Alzheimer's disease, Biochim. Biophys. Acta Mol. basis Dis. 1870 (2024) 166986, https://doi.org/10.1016/j.bbadis.2023.166986.
[96] K. Moayedi, S. Orandi, R. Ebrahimi, M. Tanhapour, M. Moradi, M. Abbastabar, A. Golestani, A novel approach to type 3 diabetes mechanism: the interplay between noncoding RNAs and insulin signaling pathway in Alzheimer's disease, J. Cell. Physiol. 237 (2022) 2838–2861, https://doi.org/10.1002/jcp.30779.
[97] M.B. Colovic, D.Z. Krstic, T.D. Lazarevic-Pasti, A.M. Bondzic, V.M. Vasic, Acetylcholinesterase inhibitors: pharmacology and toxicology, Curr. Neuropharmacol. 11 (2013) 315–335, https://doi.org/10.2174/1570159x11311030006.
[98] V. Tougu, Acetylcholinesterase: mechanism of catalysis and inhibition, Curr. Med. Chem. Nerv. Syst. Agents 1 (2005) 155–170, https://doi.org/10.2174/1568015013358536.
[99] T.L. Rosenberry, Strategies to resolve the catalytic mechanism of acetylcholinesterase, J. Mol. Neurosci. 40 (2010) 32–39, https://doi.org/10.1007/s12031-009-9250-3.
[100] T.C. Dos Santos, T.M. Gomes, B.A.S. Pinto, A.L. Camara, A.M. De Andrade Paes, Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer's disease therapy, Front. Pharmacol. 9 (2018) 1–14, https://doi.org/10.3389/fphar.2018.01192.
[101] Z. Bingol, H. Kızıltaş, A.C. Gören, L.P. Kose, M. Topal, L. Durmaz, S.H. Alwasel, İ. Gulcin, Antidiabetic, anticholinergic and antioxidant activities of aerial parts of shaggy bindweed (*Convulvulus betonicifolia* Miller subsp.) – profiling of phenolic compounds by LC-HRMS, Heliyon 7 (2021), https://doi.org/10.1016/j.heliyon.2021.e06986.
[102] W. Mazouz, N.E.H. Haouli, L. Gali, T. Vezza, C. Bensouici, S. Mebrek, T. Hamel, J. Galvez, S. Djeddi, Antioxidant, anti-alzheimer, anti-diabetic, and anti-inflammatory activities of the endemic halophyte *Limonium spathulatum* (Desf.) kuntze on LPS-stimulated RAW264 macrophages, S. Afr. J. Bot. 135 (2020) 101–108, https://doi.org/10.1016/j.sajb.2020.08.021.
[103] U. Suttisansanee, P. Thiyajai, P. Chalermchaiwat, K. Wongwathanarat, K. Pruesapan, S. Charoenkiatkul, P. Temviriyanukul, Phytochemicals and *in vitro* bioactivities of aqueous ethanolic extracts from common vegetables in thai food, Plants 10 (2021) 1–18, https://doi.org/10.3390/plants10081563.
[104] P. Taslimi, E. Köksal, A.C. Gören, E. Bursal, A. Aras, Ö. Kılıç, S. Alwasel, İ. Gülçin, Anti-Alzheimer, antidiabetic and antioxidant potential of *Satureja cuneifolia* and analysis of its phenolic contents by LC-MS/MS, Arab. J. Chem. 13 (2020) 4528–4537, https://doi.org/10.1016/j.arabjc.2019.10.002.
[105] N. Nazir, M. Zahoor, F. Uddin, M. Nisar, Chemical composition, *in vitro* antioxidant, anticholinesterase, and antidiabetic potential of essential oil of *Elaeagnus umbellata* Thunb, BMC Complement. Med. Ther. 21 (2021) 1–13, https://doi.org/10.1186/s12906-021-03228-y.
[106] N. Cardullo, V. Muccilli, L. Pulvirenti, A. Cornu, L. Pouységu, D. Deffieux, S. Quideau, C. Tringali, C-glucosidic ellagitannins and galloylated glucoses as potential functional food ingredients with anti-diabetic properties: a study of α-glucosidase and α-amylase inhibition, Food Chem. 313 (2020), https://doi.org/10.1016/j.foodchem.2019.126099.
[107] B.O. Yusuf, M.T. Yakubu, M.A. Akanji, Chromatographic fractions from *Chrysophyllum albidum* stem bark boost antioxidant enzyme activity and ameliorate some markers of diabetes complications, J. Tradit. Complement. Med. 11 (2021) 336–342, https://doi.org/10.1016/j.jtcme.2021.01.005.
[108] H. Yaribeygi, S.L. Atkin, A. Sahebkar, A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress, J. Cell. Physiol. 234 (2019) 1300–1312, https://doi.org/10.1002/jcp.27164.
[109] S. Ovais, H. Pushpalatha, G.B. Reddy, P. Rathore, R. Bashir, S. Yaseen, A. Dheyaa, R. Yaseen, O. Tanwar, M. Akthar, M. Samim, K. Javed, Synthesis and biological evaluation of some new pyrazoline substituted benzenesulfonylurea/thiourea derivatives as anti-hyperglycaemic agents and aldose reductase inhibitors, Eur. J. Med. Chem. 80 (2014) 209–217, https://doi.org/10.1016/j.ejmech.2014.04.046.
[110] R. Ottanà, R. MacCari, M. Giglio, A. Del Corso, M. Cappiello, U. Mura, S. Cosconati, L. Marinelli, E. Novellino, S. Sartini, C. La Motta, F. Da Settimo, Identification of 5-arylidene-4-thiazolidinone derivatives endowed with dual activity as aldose reductase inhibitors and antioxidant agents for the treatment of diabetic complications, Eur. J. Med. Chem. 46 (2011) 2797–2806, https://doi.org/10.1016/j.ejmech.2011.03.068.
[111] Diabetes Facets and Figures | International Diabetes Federation, (n.d.). https://idf.org/about-diabetes/diabetes-facts-figures/ (accessed February 20, 2024).

[112] World Health Organization, Diabetes (2023). https://www.who.int/news-room/fact-sheets/detail/diabetes (accessed February 20, 2024).
[113] N. Kerru, A. Singh-Pillay, P. Awolade, P. Singh, Current anti-diabetic agents and their molecular targets: a review, Eur. J. Med. Chem. 152 (2018) 436–488, https://doi.org/10.1016/j.ejmech.2018.04.061.
[114] Y. Wu, Y. Ding, Y. Tanaka, W. Zhang, Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention, Int. J. Med. Sci. 11 (2014) 1185–1200, https://doi.org/10.7150/ijms.10001.
[115] O. Vahidi, K.E. Kwok, R.B. Gopaluni, F.K. Knop, A comprehensive compartmental model of blood glucose regulation for healthy and type 2 diabetic subjects, Med. Biol. Eng. Comput. 54 (2016) 1383–1398, https://doi.org/10.1007/s11517-015-1406-4.
[116] Y. Ou, L. Lin, X. Yang, Q. Pan, X. Cheng, Antidiabetic potential of phycocyanin: effects on KKAy mice, Pharm. Biol. 51 (2013) 539–544, https://doi.org/10.3109/13880209.2012.747545.
[117] J.M.A. Hannan, P. Ansari, S. Azam, P.R. Flatt, Y.H.A. Abdel Wahab, Effects of *Spirulina platensis* on insulin secretion, dipeptidyl peptidase IV activity and both carbohydrate digestion and absorption indicate potential as an adjunctive therapy for diabetes, Br. J. Nutr. 124 (2020) 1021–1034, https://doi.org/10.1017/S0007114520002111.
[118] M. Safavi, A. Foroumadi, M. Abdollahi, The importance of synthetic drugs for type 2 diabetes drug discovery, Expert Opin. Drug Discovery 8 (2013) 1339–1363, https://doi.org/10.1517/17460441.2013.837883.
[119] K. Tamel Selvan, J.A. Goon, S. Makpol, J.K. Tan, Therapeutic potentials of microalgae and their bioactive compounds on diabetes mellitus, Mar. Drugs 21 (2023), https://doi.org/10.3390/md21090462.
[120] H. Kakinuma, T. Oi, Y. Hashimoto-Tsuchiya, M. Arai, Y. Kawakita, Y. Fukasawa, I. Iida, N. Hagima, H. Takeuchi, Y. Chino, J. Asami, L. Okumura-Kitajima, F. Io, D. Yamamoto, N. Miyata, T. Takahashi, S. Uchida, K. Yamamoto, (1 S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio- d -glucitol (TS-071) is a potent, selective sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for type 2 diabetes treatment, J. Med. Chem. 53 (2010) 3247–3261, https://doi.org/10.1021/jm901893x.
[121] H. Siti Halimatul Munawaroh, G.G. Gumilar, F. Nurjanah, G. Yuliani, S. Aisyah, D. Kurnia, A.P. Wulandari, I. Kurniawan, A. Ningrum, A.K. Koyande, P.L. Show, *In-vitro* molecular docking analysis of microalgae extracted phycocyanin as an anti-diabetic candidate, Biochem. Eng. J. 161 (2020) 107666, https://doi.org/10.1016/j.bej.2020.107666.
[122] Y. Deng, L. Huang, C. Zhang, P. Xie, J. Cheng, X. Wang, L. Liu, Novel polysaccharide from *Chaenomeles* speciosa seeds: structural characterization, α-amylase and α-glucosidase inhibitory activity evaluation, Int. J. Biol. Macromol. 153 (2020) 755–766, https://doi.org/10.1016/j.ijbiomac.2020.03.057.
[123] S. Hu, X. Fan, P. Qi, X. Zhang, Identification of anti-diabetes peptides from *Spirulina platensis*, J. Funct. Foods 56 (2019) 333–341, https://doi.org/10.1016/j.jff.2019.03.024.
[124] A.K. Singh, Dipeptidyl peptidase-4 inhibitors: novel mechanism of actions, Indian, J. Endocrinol. Metab. 18 (2014) 753–759, https://doi.org/10.4103/2230-8210.141319.
[125] G. Aiello, Y. Li, G. Boschin, C. Bollati, A. Arnoldi, C. Lammi, Chemical and biological characterization of *spirulina* protein hydrolysates: focus on ACE and DPP-IV activities modulation, J. Funct. Foods 63 (2019) 103592, https://doi.org/10.1016/j.jff.2019.103592.
[126] R. Godinho, C. Mega, E. Teixeira-De-Lemos, E. Carvalho, F. Teixeira, R. Fernandes, F. Reis, The place of dipeptidyl Peptidase-4 inhibitors in type 2 diabetes therapeutics: a "me too" or "the special one" antidiabetic class? J. Diabetes Res. 2015 (2015) https://doi.org/10.1155/2015/806979.
[127] Y. Jia, Z. Xue, Y. Wang, Y. Lu, R. Li, N. Li, Q. Wang, M. Zhang, H. Chen, Chemical structure and inhibition on α-glucosidase of polysaccharides from corn silk by fractional precipitation, Carbohydr. Polym. 252 (2021) 117185, https://doi.org/10.1016/j.carbpol.2020.117185.
[128] J. Liu, X. Zhu, L. Sun, Y. Gao, Characterization and anti-diabetic evaluation of sulfated polysaccharide from *Spirulina platensis*, J. Funct. Foods 95 (2022) 105155, https://doi.org/10.1016/j.jff.2022.105155.
[129] E. Ibañez, A. Cifuentes, Benefits of using algae as natural sources of functional ingredients, J. Sci. Food Agric. 93 (2013) 703–709, https://doi.org/10.1002/jsfa.6023.
[130] T. Bito, E. Okumura, M. Fujishima, F. Watanabe, Potential of *Chlorella* as a dietary supplement to promote human health, Nutrients 12 (2020) 1–21, https://doi.org/10.3390/nu12092524.
[131] J. Zheng, T. Inoguchi, S. Sasaki, Y. Maeda, M.F. Mccarty, M. Fujii, N. Ikeda, K. Kobayashi, N. Sonoda, R. Takayanagi, Phycocyanin and phycocyanobilin from *Spirulina platensis* protect against diabetic nephropathy by inhibiting oxidative stress, Am. J. Phys. Regul. Integr. Comp. Phys. 304 (2013) 110–120, https://doi.org/10.1152/ajpregu.00648.2011.
[132] W.M. Aboulthana, A.M. El-Feky, N.E. Ibrahim, R.K. Sahu, A.E.K.B. El-Sayed, Evaluation of the pancreatoprotective effect of *Nannochloropsis oculata* extract against streptozotocin-induced diabetes in rats, J. Appl. Pharm. Sci. 8 (2018) 46–58, https://doi.org/10.7324/JAPS.2018.8607.
[133] W. Nacer, F.Z. Baba Ahmed, H. Merzouk, O. Benyagoub, S. Bouanane, Evaluation of the anti-inflammatory and antioxidant effects of the microalgae *Nannochloropsis gaditana* in streptozotocin-induced diabetic rats, J. Diabetes Metab. Disord. 19 (2020) 1483–1490, https://doi.org/10.1007/s40200-020-00681-3.
[134] F. Navarro, A. Toimil, S. Ramírez, Y. Montero, J.L. Fuentes, J.S. Perona, M.Á. Castaño, R. Pásaro, J.M. Vega, C. Vílchez, The acidophilic microalga *Coccomyxa onubensis* and atorvastatin equally improve antihyperglycemic and antihyperlipidemic protective effects on rats fed on high-fat diets, J. Appl. Phycol. 32 (2020) 3923–3931, https://doi.org/10.1007/s10811-020-02280-4.
[135] T.T. Li, Z.R. Huang, R.B. Jia, X.C. Lv, C. Zhao, B. Liu, *Spirulina platensis* polysaccharides attenuate lipid and carbohydrate metabolism disorder in high-sucrose and high-fat diet-fed rats in association with intestinal microbiota, Food Res. Int. 147 (2021) 110530, https://doi.org/10.1016/j.foodres.2021.110530.
[136] Z. Huang, J. Chen, C. Wang, M. Xiao, Y. Zhu, N. Li, Z. Huang, B. Liu, Y. Huang, Antidiabetic potential of *Chlorella pyrenoidosa* functional formulations in streptozocin-induced type 2 diabetic mice, J. Funct. Foods 103 (2023), https://doi.org/10.1016/j.jff.2023.105489.
[137] K.C. Nandipati, S. Subramanian, D.K. Agrawal, Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance, Mol. Cell. Biochem. 426 (2017) 27–45, https://doi.org/10.1007/s11010-016-2878-8.
[138] S. Sahoo, M. Samantaray, M. Jena, V. Gosu, P.P. Bhuyan, D. Shin, B. Pradhan, *In vitro* and *in silico* studies to explore potent antidiabetic inhibitor against human pancreatic alpha-amylase from the methanolic extract of the green microalga *Chlorella vulgaris*, J. Biomol. Struct. Dyn. 0 (2023) 1–11, https://doi.org/10.1080/07391102.2023.2244592.
[139] L. Patil, B.B. Kaliwal, Microalga Scenedesmus bajacalifornicus BBKLP-07, a new source of bioactive compounds with in vitro pharmacological applications, Bioprocess Biosyst. Eng. 42 (2019) 979–994, https://doi.org/10.1007/s00449-019-02099-5.
[140] R. Siahbalaei, G. Kavoosi, M. Noroozi, Protein nutritional quality, amino acid profile, anti-amylase and anti-glucosidase properties of microalgae: inhibition and mechanisms of action through *in vitro* and *in silico* studies, Lwt 150 (2021) 112023, https://doi.org/10.1016/j.lwt.2021.112023.
[141] X. Du, X. Wang, X. Yan, Y. Yang, Z. Li, Z. Jiang, H. Ni, Hypoglycaemic effect of all-trans astaxanthin through inhibiting α-glucosidase, J. Funct. Foods 74 (2020) 104168, https://doi.org/10.1016/j.jff.2020.104168.
[142] N. Wang, Z. Chen, J. Lv, T. Li, H. Wu, J. Wu, H. Wu, W. Xiang, Characterization, hypoglycemia and antioxidant activities of polysaccharides from *Rhodosorus* sp. SCSIO-45730, Ind. Crop. Prod. 191 (2023) 115936, https://doi.org/10.1016/j.indcrop.2022.115936.
[143] X. Huang, Y. Wen, Y. Chen, Y. Liu, C. Zhao, Structural characterization of *Euglena gracilis* polysaccharide and its *in vitro* hypoglycemic effects by alleviating insulin resistance, Int. J. Biol. Macromol. 236 (2023) 123984, https://doi.org/10.1016/j.ijbiomac.2023.123984.
[144] Y. Li, G. Aiello, E.M.A. Fassi, G. Boschin, M. Bartolomei, C. Bollati, G. Roda, A. Arnoldi, G. Grazioso, C. Lammi, Investigation of *Chlorella pyrenoidosa* protein as a source of novel angiotensin i-converting enzyme (Ace) and dipeptidyl peptidase-iv (dpp-iv) inhibitory peptides, Nutrients 13 (2021), https://doi.org/10.3390/nu13051624.
[145] J. Stack, A.V. Le Gouic, P.R. Tobin, F. Guihéneuf, D.B. Stengel, R.J. FitzGerald, Protein extraction and bioactive hydrolysate generation from two microalgae, *Porphyridium purpureum* and *Phaeodactylum tricornutum*, J. Food Bioact. 1 (2018) 153–165, https://doi.org/10.31665/jfb.2018.1134.
[146] S. Villaró, S. Jiménez-Márquez, E. Musari, R. Bermejo, T. Lafarga, Production of enzymatic hydrolysates with *in vitro* antioxidant, antihypertensive, and antidiabetic properties from proteins derived from *Arthrospira platensis*, Food Res. Int. 163 (2023) 1–9, https://doi.org/10.1016/j.foodres.2022.112270.
[147] M.J. Rodrigues, M. Oliveira, V. Neves, A. Ovelheiro, C.A. Pereira, N.R. Neng, J.M.F. Nogueira, J. Varela, L. Barreira, L. Custódio, Coupling sea lavender (*Limonium algarvense* Erben) and green tea (*Camellia sinensis* (L.) Kuntze) to produce an innovative herbal beverage with enhanced enzymatic inhibitory properties, S. Afr. J. Bot. 120 (2019) 87–94, https://doi.org/10.1016/j.sajb.2017.12.003.
[148] M. Akyuz, L. Yabo-Dambagi, T. Kilic, A. Cakir, Antidiabetic, neuroprotective and antioxidant potentials of different parts of *Pistacia terebinthus* fruits, S. Afr. J. Bot. 147 (2022) 443–456, https://doi.org/10.1016/j.sajb.2022.01.040.
[149] S. Khemiri, N. Khelifi, C. Messaoud, I. Smaali, Bioprospecting of microalgae for a potential use as enzyme inhibitors, anti-ageing and prebiotic agents, Biocatal. Agric. Biotechnol. 51 (2023) 102759, https://doi.org/10.1016/j.bcab.2023.102759.
[150] M.G. Mallikarjun, U.S. K, S. R, G.A. Ravishankar, Supercritical CO2 extraction of functional compounds from *Spirulina* and their biological activity, J. Food Sci. Technol. 52 (2015) 3627–3633, https://doi.org/10.1007/s13197-014-1426-3.
[151] S.A. Cunha, E.R. Coscueta, P. Nova, J.L. Silva, M.M. Pintado, Bioactive hydrolysates from *Chlorella vulgaris*: optimal process and bioactive properties, Molecules 27 (2022), https://doi.org/10.3390/molecules27082505.
[152] Y. Li, G. Aiello, C. Bollati, M. Bartolomei, A. Arnoldi, C. Lammi, Phycobiliproteins from *Arthrospira Platensis* (*Spirulina*): a new source of peptides with dipeptidyl peptidase-IV inhibitory activity, Nutrients 12 (2020) 1–11 (doi:10.3390%2Fnu12030794).