Review

Antioxidant Production in *Dunaliella*

Uttam Kumer Roy [1,2,*], Birthe Vejby Nielsen [2] and John James Milledge [2]

[1] School of Architecture, Building and Civil Engineering, Loughborough University, Epinal Way, Loughborough, Leicestershire LE11 3TU, UK

[2] Algae Biotechnology Research Group, Faculty of Engineering and Science, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK; b.v.nielsen@greenwich.ac.uk (B.V.N.); J.J.Milledge@greenwich.ac.uk (J.J.M.)

[*] Correspondence: Uttam.roy@recipharm.com

Abstract: Microalgae have become an attractive natural source of a diverse range of biomolecules, including enzymatic and non-enzymatic antioxidants; nevertheless, economically sustainable production of such compounds from microalgae biomass is still challenging. The main hurdles are: (a) increasing microalgae yield; (b) achieving optimal cultivation conditions; (c) energy-efficient and cost-effective downstream processing (extraction and purification); (d) optimal storage of post-processed antioxidant molecules. This review provides a detailed overview of enzymatic and non-enzymatic antioxidants in the cellular metabolism of the commercially important microalgae *Dunaliella*, industrial applications of antioxidant enzymes, strategies to enhanced antioxidant accumulation in cells, and the opportunities and limitations of current technologies for antioxidant enzymes production from microalgae biomass as an alternative to common microbial sources.

Keywords: *Dunaliella*; antioxidant enzymes; cultivation conditions; post-harvest processing

1. Introduction

Microalgae are singled celled micro-bio-factories capable of producing a wide variety of high-value compounds (carbohydrates, proteins, lipids, carotenoids, phycobiliproteins, phenolic, polyunsaturated fatty acids and antioxidants) used in the pharmaceutical, nutraceutical, cosmetic, and food processing industries [1–10]. Microalgal biomass can be added directly as a nutrient enhancer in animal feeds; as an enhancer for improving the quality of food; and as a stabiliser for maintaining the colour and flavour of food products [3,11–14]. The global microalgae market has gradually expanded, and worldwide sales are expected to exceed US $3.2 billion by the end of 2030 [15]. Recent studies have demonstrated the potential of microalgae to produce antioxidant molecules [16–19]. The antioxidant capacity and antioxidant activity of small molecules (β-carotene, astaxanthin, and phenolic compounds) have also been explored extensively [10,20,21]. Nevertheless, antioxidant enzymes have received little attention.

The green halotolerant microalgae *Dunaliella* can grow in a wide range of salt concentrations, from 0.05 M to 5.5 M NaCl [22,23]. Currently, 28 species of *Dunaliella* have been identified, of which 23 species live in saline environments, and 5 are rare species that inhabit freshwater [22]. Commercially, *Dunaliella* is cultivated in several countries, such as Australia, China, Israel, and India, with pilot-scale projects in Chile, Spain, Iran and Portugal [24–26] and considered one of the best sources of β carotene. *Dunaliella* has also been suggested as a sustainable source for industrial bioprocessing for the production of protein [27], biodiesel [4,28,29], colouring agent [30], and antioxidants [31].

Currently, antioxidant enzymes are sourced from microbial and animal sources [32,33]. Though there is no commercial production of antioxidant enzymes from *Dunaliella*, several reports have shown that *Dunaliella* can increase its levels of enzymatic (mainly catalase, peroxidase and superoxide dismutase) and non-enzymatic (carotenoids, phenolic, ascorbate and glutathione) antioxidants to mitigate stress induced by exogenous abiotic fac-

tors [31,34–39]. Its high adaptability to exogenous stressors (oxidative and osmosis) and the lack of a rigid cell wall [40] could make *Dunaliella* an ideal natural source for antioxidant production. *Dunaliella* can efficiently and sustainably produce large volumes of biomass without competing for cultivable land and fresh water [25].

2. Antioxidants

Extensive research on the effect of ROS in humans has demonstrated a substantial link between free radicals and more than sixty different health conditions, including ageing, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis [41,42]. Consumption of higher levels of dietary antioxidant enzymes, as well as antioxidant molecules-enriched food or antioxidant supplements, has been found to reduce the risk of free radical-related health issues [41].

2.1. Classification of Antioxidants

Antioxidants (enzymatic or non-enzymatic) are classified depending on their mode of activity as primary antioxidants (hydrogen or electrons donors) or secondary antioxidants (oxygen scavengers or chelating agents) [43,44]. Antioxidants can also be grouped according to size, solubility, mode of action or structure, Figure 1. Abundant enzymatic antioxidants in microalgae are superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione transferase (GST). Non-enzymatic antioxidants consist of compounds such as Vitamin C (ascorbate), glutathione, carotenoid, phenolic compounds, proline, glycine, polyamine, PUFA, and some metals (Cu, Zu) [45–47]. Most enzymatic antioxidants and some non-enzymatic antioxidants (glutathione, ascorbate) are hydrophilic and mainly present in the cellular fluids (cytosol or cytoplasmic matrix), whereas the hydrophobic antioxidants (carotenoid, tocopherol) are primary located in the cell membranes [48].
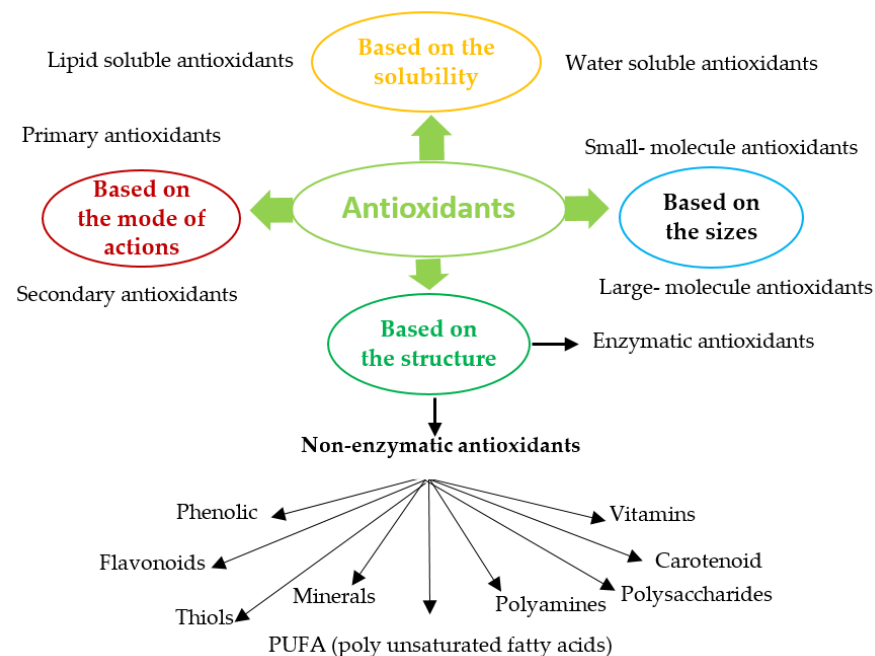

Figure 1. Classification of antioxidants [41,44,48–50].

2.2. Antioxidant Enzymes

Antioxidant enzymes prevent or delay the oxidation of other molecules by neutralising reactive oxygen species (ROS) [48]. These enzymes eliminate ROS by reducing the energy of free radicals or by donating electrons to free radicals [42], and as such, constitute the first level of defence in the cells antioxidant network. Some molecules are not involved directly in the scavenging of free radicals but rather enhance other antioxidant molecules' activity

and may also be classified as antioxidants [41]. The majority of antioxidant enzymes are metalloenzymes and contain a metal ion in their catalytic site [45].

2.3. Commercial Applications of Antioxidant Enzymes

Antioxidants, or antioxidant-enriched extracts, are commercially used to prevent oxidative processes and to maintain the flavor, texture, and colour of food during storage. They also find uses as refining, bleaching, deodorising agents in the food processing industries [51–55], in extending the shelf life of lubricating oil and reducing vehicular emissions [56] and in stabilisation of synthetic fibre, rubber, thermoplastic, and adhesives by stopping autocatalytic reactions [57]. In cosmeceutical products, antioxidant compounds are used to prevent skin ageing and UV-induced skin damage, and treat the appearance of wrinkles and erythema due to inflammation [58–61]. Industrial applications of antioxidants are listed in Table 1. Global demand for antioxidants was valued at ~USD 2.25 billion in 2014 and grew at a CAGR (compounds annual growth rate) of ~5.5% between 2015 and 2020 [62]. This increasing global demand is driving the search for synthetic and natural-derived antioxidants.

Table 1. Commercial uses of enzymatic and non-enzymatic antioxidant.

Antioxidants		Applications	Ref
Natural antioxidants	SOD	Added to cosmetic products to protect against skin damage	[49,52,60,63,64]
		Protect against lipid peroxidation, heat, and cold stress in poultry production	
		As a therapeutic agent for treatment of inflammatory disorders	
		Normal cells protector during radiotherapy for cancer patients	
	CAT	Eliminate excessive H_2O_2 in the textile industry, pulp, and paper industry used for bleaching fibres and pulp, and as a bactericidal disinfectant in food processing and in the pharmaceutical industry	[51,57,65]
		In aesthetics (mask treatment) to increase cellular oxygenation in the upper layers of facial epidermis	
		Reducing the risk of diabetes mellitus	
	GPX	Immune system booster	[42,66]
	GST	Protective role against neurogenerative diseases	[66,67]
		Decreases the risk of tumours of the head and neck, oral, cavity and colon	
	Glutathione	Anti-wrinkle formation, and as a modifier of skin smoothness	[68]
	Vitamins	As a food preservative and bread improver, protective activity against heart diseases, reduced the risk of colorectal adenomas and prostate cancer, reduction of thyroid hormone levels	[69–73]
	Flavonoids	As cancer preventive agents, protection against type 2 diabetes Functional food additive	[74–77]
	Carotenoid	Anticancer agents, additive to cosmetics and multivitamin preparation Food colouring agent, pro-vitamin A in food and animal feed	[29,38,78]
	PUFA	Prevention of heart and inflammatory diseases	[79,80]
Synthetic Antioxidants	BHA	Extending the shelf life of vegetable oil, frying oil, animal feed, cereals, chewing gum, potato flakes and cosmetic products	[81–83]
	BHT	Increasing the shelf life of animal fats, chewing gum, animal feed, vegetable oils	[82,83]
	TBHQ	Used as preservative for enhancing storage stability of vegetable oils, margarine, fish oil, fried foods, essential oils, nuts, edible animal fats, butterfat, and packed fried foods	[82]
	Propyl gallate	As an antioxidant agent in foods and vegetable oil	[82]
BHA = butylated hydroxyl anisole; TBHQ = tert-butylhydroquinone; BHT = butylated hydroxyl toluene			

Commercially, synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), α-tocopherol and propyl gallate are used in foods, food packaging, cosmetics and pharmaceutical products [82,84]. However, the physical properties of BHT and BHA (high volatility and instability at elevated temperature), strict

legislation on the use of synthetic food additives, and the carcinogenic nature of some synthetic antioxidants [85–88] have shifted the attention to finding antioxidants from natural sources that are pharmacologically potent and have low or no toxicity.

2.4. Generation and Detoxification of ROS

ROS is a collective term for oxygen-derived products (free radicals and non-radicals reactive derivatives of oxygen). They are produced in cellular compartments either exogenously (in response to environmental stress such as UV radiation or xenobiotics) or endogenously (from the intracellular metabolic pathway, enzymatic activities, mitochondrial respiration, or photosynthesis) [66]. Accumulation of ROS leads to oxidative stress in cells and causes damage to cellular macromolecules, including proteins, lipids, carbohydrates and DNA [89]. Various sources of ROS and corresponding modes of biochemical metabolism are summarised in Table 2.

Table 2. Production of ROS in cells during biological metabolism and their corresponding neutralising antioxidants.

ROS	Reaction	Life Span	Function	Sources of ROS	Scavenging Antioxidants
Singlet ($^1O_2$) First excited electronic state of O_2	Chlorophyll triplet state (Chl) is generated sue to insufficient energy dissipation during photosynthesis $Chl + {}^3O_2 \rightarrow {}^1O_2$ Reduction of transition metal (Fe^{3+}) $O_2^{\bullet-} + Fe^{3+} \rightarrow {}^1O_2 + Fe^{2+}$	3 μs (appx.) 4 μs in H_2O 100 μs in polar solvents	Gene up-regulation, molecular defense against photo-oxidative stress	Chloroplast	β-carotene, lycopene, tocopherol, ASc, plastoquinone, and proline
Superoxide radical ($O_2^{\bullet-}$) One electron reduction of $^3O_2$	Reduction of oxygen ($^3O_2$) during electron transport during the photosynthesis process in chloroplasts or during oxidative phosphorylation in the mitochondria $^3O_2 + e^- \rightarrow O_2^{\bullet-}$ $^3O_2 + Xanthine$ $(^{Xanthine\ oxidase}) \rightarrow O_2^{\bullet-} + uric\ acid$ $^3O_2 + NADPH$ $(^{NADPH\ oxidase}) \rightarrow O_2^{\bullet-} + NADP^+ + H^+$	2–4 μs	Triggering the formation of more ROS which further participates in membrane lipid peroxidation	PSI in Chloroplast oxidative phosphorylation in mitochondria Peroxisomes Plasma membrane	SOD, ASc, glutathione (GSH), flavonoids, Cu
H_2O_2 Two electron reduction of $^3O_2$ or univalent reduction of $O_2^{\bullet-}$	Protonation reaction (acidic conditions) $O_2^{\bullet-} + H^+ + HO_2^{\bullet} \rightarrow H_2O_2 + 2O_2$ Glycolate + O_2 $(^{Glycolate\ oxidase}) \rightarrow H_2O_2 + Glyoxylate$ Fatty acids $(^{\beta-oxidation}) \rightarrow Acetyl\ coenzyme + H_2O_2$	1 ms	Act as a signaling molecule (low concentration of H_2O_2), at high concentration of H_2O_2 triggers tolerance to various stress, as a regulator of physiological processes (photorespiration and photosynthesis)	Chloroplast, Mitochondria, and Peroxisome	CAT, POD (GPX and APX), peroxiredoxin, ASc, tocopherol, GSH, β-carotene, Se flavonoids, lipoic acid,

Table 2. *Cont.*

ROS	Reaction	Life Span	Function	Sources of ROS	Scavenging Antioxidants
$HO_2\bullet$	Protonation of superoxide ions $O_2{}^{\bullet-} + H^+ \rightarrow HO_2{}^{\bullet}$		Attacks PUFA in the negatively charged membrane surface	Mitochondria, microsomes and peroxisomes	
HO• (Three electron reduction of $^3O_2$)	Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + HO^- + Fe^{3+}$ Haber-Weiss reactions: $O_2{}^{\bullet-} + H_2O_2 \rightarrow HO^{\bullet} + HO^- + O_2$	1 ps	Attack unsaturated fatty acids in membranes	Mitochondria	ASc, GSH, flavonoids, lipoic acid, proline
Nitric oxide (NO•)	L-arginine + O_2 Nitric oxide synthase $\rightarrow NO^{\bullet} +$ Citrulline		Intercellular messenger, the quencher of $O_2{}^{\bullet-}$, defense against various protozoa, fungi, and mycobacteria	Peroxisome, cytosol	GSH
Peroxynitrite (ONOO$^-$)	$NO^{\bullet} + O_2{}^{\bullet-} \rightarrow ONOO^-$		React with amino acids residues in enzymes causing inactivation		Peroxiredoxin, Uric acid
Lipid hydroperoxide	Oxidation of PUFA		Tissue injuries and diseases	Mitochondrial membrane PUFA	

The majority of ROS are generated when electrons leak from the chloroplastic electron transport systems during photosynthesis, from the mitochondrial electron transport chain during photorespiration, and from the peroxisomal membrane electron transport chain (Figure 2) [90]. In plant cells, 1–2% of O_2 consumption leads to the formation of superoxide ($O_2{}^{\bullet-}$), and 1–5% of mitochondrial O_2 consumption leads to the generation of H_2O_2 [45]. $O_2{}^{\bullet-}$ is generated during oxidisation of unsaturated fatty acids [91], from the activity of cytochrome P450 [92] and the cytochrome *b*5 family members [93,94]. Also, $O_2{}^{\bullet-}$ is produced in the peroxisome where xanthine oxidases catalyse the oxidation of xanthine and hypoxanthine to uric acid [45], and in the plasma membrane due to the reduction of O_2 by NADPH oxidases [49].
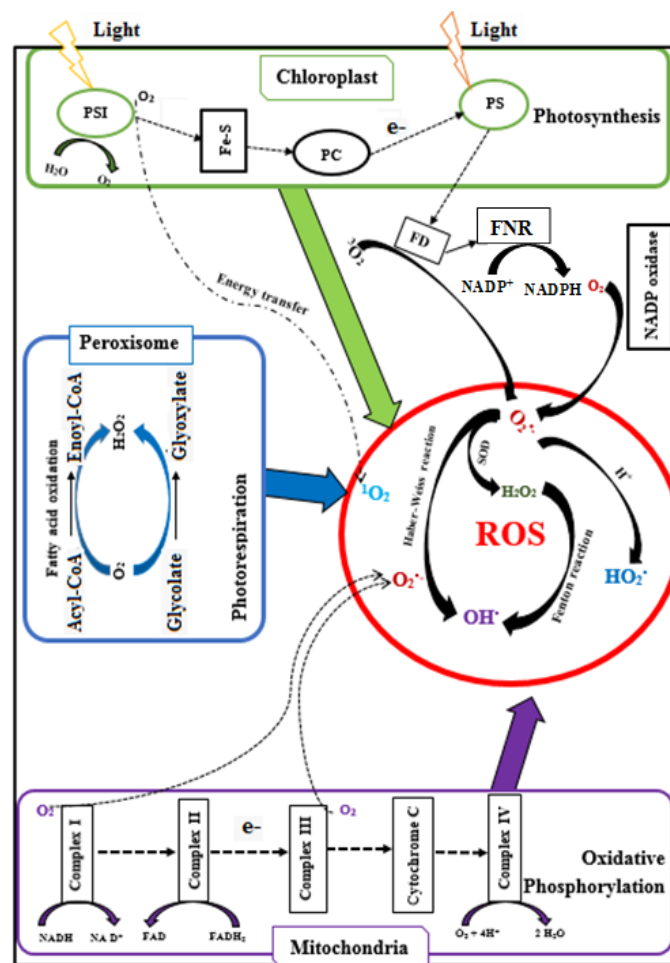
Figure 2. Generation of ROS by energy transfer and during intracellular metabolic pathways in microalgae (FD; ferredoxin, FNR; ferredoxin-NADP$^+$ oxidoreductase; PSI-Photosystem I; PSII-Photosystem II, PC-plastocyanin [90,95]).

Superoxide ions ($O_2^{\bullet-}$) are converted into hydrogen peroxide (H_2O_2) by the catalytic activity of SOD. H_2O_2 is also produced in peroxisomes when glycolate from the photorespiration is recycled. In addition, H_2O_2 can be formed by D-amino acid oxidase, urate oxidase, flavin oxidase, L-α-hydroxy acid oxidase, and fatty acyl-CoA oxidase, and by cell wall peroxidases. Reactive $OH^{\bullet}$ is produced from the reaction of $O_2^{\bullet-}$ and H_2O_2 at neutral pH and ambient temperature (Haber–Weiss reaction) or from H_2O_2 during the Fenton reaction [96]. In contrast, the addition of a proton (H^+) to $O_2^{\bullet-}$ generates perhyroxyl radicals ($HO_2^{\bullet}$). In some cases, intracellular ROS could also form during auto-oxidation of small molecules (epinephrine, flavins, and hydroquinones) [97,98]. The singlet oxygen ($^1O_2$) is generated from the reaction of oxygen ($^3O_2$) with the triplet state of chlorophyll produced by the dissipation of insufficient energy during photosynthesis [45].

Under normal physiological conditions, ROS is neutralised by the cells' antioxidant systems where antioxidant enzymes and antioxidant molecules maintain the delicate intracellular redox balance and mitigate undesirable cellular damage caused by ROS, Table 2 [48,50].

Different isozymes of SOD exist (Mn-SOD in mitochondria and peroxisome, Fe-SOD in the chloroplast, and Cu/Zn-SOD isozyme in cytosol) but they all participate in scavenging of $O_2^{\bullet-}$. In addition to SOD, some antioxidants molecules (Vitamin C, glutathione, etc.) also eliminate $O_2^{\bullet-}$ [45]. Further, the individual or cumulative catalytic activity of catalase or peroxidases decompose H_2O_2 into H_2O and O_2. CAT, peroxidases (GPX, APX) and SOD show a synergistic effect in the scavenging of $O_2^{\bullet-}$. In addition to eliminating H_2O_2, GPX

can protect cells by preventing intracellular lipid peroxidation [48]. APX may be more efficient compared to CAT or GPX in detoxification of H_2O_2 due to its higher affinity for H_2O_2. APX reduces H_2O_2 into H_2O in chloroplasts, cytosol, mitochondria and peroxisomes, and in the apoplastic space using Ascorbic acid (ASc) as an electron donor [99].

Algal cells accumulate ASc with 30–40% remaining in the chloroplast. Ascorbic acid is water-soluble and acts as a potent antioxidant because of its ability to donate electrons in enzymatic and non-enzymatic reactions [100]. It protects cells by directly scavenging $O_2^{\bullet-}$, $HO_2^{\bullet-}$ and regenerating the tocopherol from tocopheroxyl radicals [101]. All intracellular compartments generate the reduced form of glutathione, which plays a role as an excellent scavenger of many ROS such as $O_2^{\bullet-}$, $HO^{\bullet}$, O_3, NO_2, lipid hydroperoxides [50] due to the redox-active thiol group that becomes oxidised when GSH reduces ROS [102]. Carotenoid also protects cells from light-induced oxidative stress by quenching $^1O_2$ or dissipating excess heat (excitation energy) or scavenging peroxy radicals [45,48].

As the accumulation of enzymatic and non-enzymatic antioxidants in the cell depends on the external environment, manipulating cultivation conditions could enhance the intracellular antioxidant levels.

3. Cultivation Conditions

In addition to carbon, light energy, and water, *Dunaliella* requires certain mineral nutrients for growth. A suitable medium can be prepared from natural or artificial seawater enriched with nutrients (carbon, nitrogen, phosphorous, sulphur, iron, and magnesium), trace metals, and vitamins [40]. Carbon dioxide (CO_2), inorganic carbon (e.g., $NaHCO_3$, Na_2CO_3), and organic carbon (e.g., sodium acetate, glucose, glycerol) can be used as carbon sources. Nitrate, ammonia, and urea are commonly used as nitrogen sources for the synthesis of amino acids, nucleotides, chlorophylls and phycobilins [103]. Phosphorous is needed for several metabolic processes (ATP, DNA, RNA, and phospholipids). Iron acts as a cofactor for many enzymes (e.g., ferredoxins, catalases, nitrogenases, nitrates), and sulphur is needed for the biosynthesis of specific amino acids (cysteine, methionine). Magnesium is required for t chlorophyll biosynthesis, and other trace minerals serve as cofactors for various enzymes. *Dunaliella* needs light and temperature to assimilate carbon during photosynthesis and enhance biomass productivity and growth [104]. Growth conditions can be classified into three types based on energy and carbon sources (Table 3).

Table 3. Characteristics of microalgae growth modes [105,106].

Characteristics	Photo-Autotrophic	Heterotrophic	Mixotrophic
Carbon assimilation process	Photosynthesis	Aerobic respiration	Photosynthesis and aerobic respiration
Energy sources	Light (solar or artificial)	Organic carbon (e.g., glucose, acetic acid glycerol, wastewaters)	Light and organic carbon
Carbon sources	Inorganic carbon (CO_2, $NaHCO_3$)	Organic carbon	Inorganic and organic
Light energy	Mandatory	Not required	Not essential
Photo-inhibition effect	High	No effect	Low
Growth-limiting factor	Light	Oxygen	Light and oxygen
Photo-oxidative damage risk	High	Very low	Protected by accumulating oxygen
Types of bioreactors	Photo-bioreactors and open ponds	Fermenters	Photo-bioreactors, open ponds
Availability of bioreactor's vessels	Photo-bioreactor	Commercially available	Bioreactor

Table 3. *Cont.*

Characteristics	Photo-Autotrophic	Heterotrophic	Mixotrophic
Bioreactor's surface tovolume ratio (m^2/m^3)	High	Low	High
Sterility	Usually sanitised	Sterility required	Not required
Contamination risk	Low	High	Medium
Harvesting cost	High due to the low density of biomass	Low due to high biomass concentration	Low due to high biomass concentration
Bioreactor set up cost (per unit of volume)	High	Low	High
Bioreactor operation cost (per kg of biomass)	Low	Medium	High
Scale-up	Design optimisation required	Easy	Design optimisation required
Efficiency in wastewater treatment	Low	High	High
Growth rate	Low	Medium	High
Biomass density (g L^{-1})	Low	Medium	High
Lipids (%)	Low	High	High
Proteins (%)	High	Low	Medium
Carbohydrates (%)	Low	High	Low
Chlorophyll and β-carotene (%)	High	Low	Medium
Lutein (%)	Medium	High	High

Photoautotrophic culturing is the most common strategy for growing *Dunaliella* biomass. However, in large cultures, cell-shading becomes an issue, limiting light penetration into the culture, leading to lower amounts of biomass [107]. In mixotrophic cultivation, the microalgae use CO_2 and organic carbon (acetate, glucose) simultaneously along with light energy; respiratory and photosynthetic metabolism operates concurrently [105]. This increases biomass sproduction and results in a higher lipid production compared to that observed in photoautotrophic cultures [107–128], though at increased cultivation costs.

Although heterotrophic cultivation of algae [106] eliminates the requirements for light and facilitates biomass production in the dark, attempts to grow *D. para* heterotrophically were unsuccessful [110]. However, some *Dunaliella* strains can grow heterotrophically using organic carbon (such as tryptone, yeast extract, and urea, etc.) as an energy source, together with nutrients [128–133]. No studies have been published supporting the heterotrophic growth of *Dunaliella* strains at a commercial scale.

Dunaliella can grow phototrophically at temperatures ranging between 10 °C and 30 °C and in a wide variety of media, where the maximum cells density range between 0.3×10^6 cells mL^{-1} and 24×10^6 cells mL^{-1} [25]. In addition, these species are halotolerant and can grow in a high saline medium (0.5–4.0 M NaCl) [111]. *Dunaliella* strains can adapt to grow under quite different nutrients conditions, which demonstrates the diversity of these strains [112–115]. The essential photoautotrophic growth parameters of microalgae (*Dunaliella*) are shown in Figure 3.
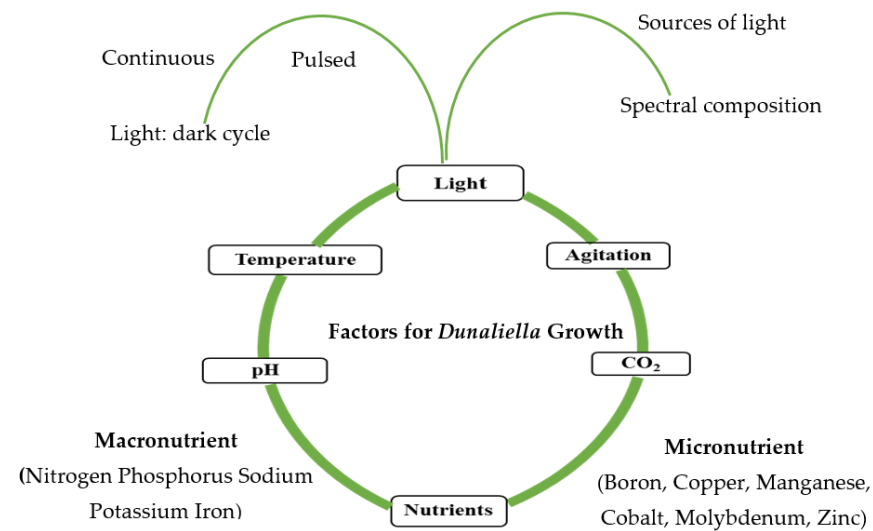
Figure 3. Phototrophic growth parameters essential for *Dunaliella*.

Using available technologies, *Dunaliella* biomass production costs are approximately US $4.92 kg^{-1}, which, for biodiesel production, is exceptionally high. Production costs could be reduced (US $3.05 kg^{-1}) if biomass was produced using waste water as a nutrient source [116]. A biomass production costs of US$ 4.92 kg^{-1} would be acceptable if biomass was used for the production of high-value products (processed biomass value, US $123 kg^{-1}) [117]; β-carotene (from *Dunaliella* spp.) and astaxanthin (from *Haematococcus* spp.) command a market value of $100–1000 kg^{-1} [117]. Moreover, the demand for astaxanthin, β-carotene and lutein is now emerging in the market. The global carotenoid market was valued at €1.3 billion in 2017 and is projected to reach €1.8 billion by 2022 [7]. Other high-value compounds such as antioxidant enzymes could make *Dunaliella* biotechnologies economically profitable. The current biomass productivity of *D. salina* lies between 0.75 g m^{-2} d^{-1} and 3.0 g m^{-2} d^{-1} (ash-free dry weight, AFDW) [118], depending on seasonality and other cultivation factors.

Variation of growth parameters disturbs intracellular metabolism via photosynthesis and photorespiration, which results in an imbalance between the generation and detoxification of ROS, ultimately stimulating enzymatic and non-enzymatic antioxidant responses. Therefore, modifications of cultivation factors may enhance antioxidants levels, making *Dunaliella* a competitive candidate for antioxidant (enzyme and non-enzyme) production.

4. Tuning Antioxidant Enzyme Activity in *Dunaliella*

There are two ways of inducing stress in *Dunaliella* during growth: biotic and abiotic manipulation [119]. Due to a lack of control over biotic stress, abiotic stress strategies are more sustainable. Abiotic stress conditions are classified as the nutrimental and physical conditions [105,120,121], which are introduced either during the inoculation of cells or during cell growth. Nutrimental factors include:

i.   Concentration and source nutrients (e.g., carbon, nitrogen, phosphorus, iron)
ii.   Concentration of trace elements (e.g., Zn^{2+}, Cu^{2+})
iii.   Chemicals (e.g., phenol, H_2O_2)
iv.   Heavy metals (e.g., Hg^{2+}, Cd^{2+})

Physical factors include:

i.   Irradiation level
ii.   Types of light (direct sunlight, artificial light sources (white, red, blue))
iii.   Temperature
iv.   pH
v.   Salinity

vi.   Size of inoculation

4.1. Salinity

Salinity induces osmotic stress, which has been found to affect antioxidant enzyme activity in *Dunaliella* species. Osmotic stress is divided into two types based on the salinity of the medium: hyposaline (salinity < 0.5 M) and hypersaline (salinity > 0.5 M).

CAT and APX activities were found to be highest when the cells were grown at a low salinity level (>0.5 M), with the activity decreasing with increased salinity. In contrast, SOD activity increased twofold at 1 M and threefold at 2 M compared to the activity at 0.5 M NaCl [122]. However, above 2 M NaCl, a decrease in the SOD activity was observed [122]. Above 2 M NaCl, accumulation of glycerol acts as an osmolyte for balancing high salinity-induced osmotic stress [123], which may be the reason for reduced enzymatic antioxidant activity. Similarly, CAT, APX, and GPX activity were found to decrease in *D. tertiolecta* concurrently with an increase in salt concentrations [124], perhaps due to the participation of glutathione (GSH) and ascorbate in the scavenging of H_2O_2 [124,125]. A higher GR activity in *Dunaliella* species (*salina and tertiolecta*) was observed at a low salt concentration (0.1 M NaCl) [124] which would increase the ratio of NADP +/NADPH, thereby ensuring the availability of NADP + to accept electrons resulting in a reduced flow of electrons to O_2 for generation of ROS [126].

On the contrary, a study using *D. tertiolecta* demonstrated that CAT, SOD, DHAR, and GTX activity were unaltered under a wide range (0.05–3.00 M NaCl) of salinities, whilst APX activity increased by 200%, and MDHAR activity by 300% relative to the optimal growth salinity (0.5 M NaCl) [125]. Increased level of enzymatic activity in hypersaline condition may be due to the increase in the formation of ROS (peroxides) or increases in ATP synthesis via the Mehler-peroxidase reaction (which generates ATP by substituting dioxygen for carbon as the electron acceptor) [127]. Also, *Dunaliella* can retain a higher level of Mehler-peroxidase reaction at higher saline conditions [125].

The data above indicates that high salinity-induced osmotic stress is not an effective way to activate all antioxidant enzymes in *Dunaliella* strains. The above investigations also suggest that different *Dunaliella* strains, when exposed to external salinity, show different antioxidant enzyme activity due to differences in resistance and response. Hence, consideration of strain selection must be given if this approach is chosen.

4.2. Light Irradiation

D. salina exposed to UV-B irradiation enhanced CAT, SOD, POD activities, reaching maximum levels within three days after they levelled off [128], suggesting that CAT, SOD, POD activity could be part of the cell's short-term adaptation against UV-B irradiation. Micosporine-like amino acids (shinorine, porphyra, and palythinol) could act as part of a supportive antioxidants network to protect cells against UV-B irradiation or ROS [129]. The synthesis of these compounds could be the reason for the levelling off enzymatic antioxidant response after three days of growth [128]. *D. tertiolecta* exposed to static and fluctuating natural UV radiation (400–700 nm) for short-term (1–3 days) or long-term (4–7 days) showed that only short-term irradiation significantly increased SOD and GR activity compared to non-UV irradiated cells. Glutathione content was reduced under both short and long-term irradiation, whilst APX activity did not significantly change, indicating that natural UV radiation-induced $O_2 \cdot^-$ was mitigated by SOD, and that H_2O_2 was detoxified by the consumption of glutathione [130]. In contrast, *D. salina* treated with UV-B irradiation (irradiated cultures for 4 h) and cultured for 15 days increased its CAT, POD, and SOD activity compared to untreated cells [34]. Moreover, APX activity (140 μmol ascorbate mg^{-1} protein h^{-1}) was found to increase in *D. bardawil* cells exposed to UV-A radiation as an addition to cultivation under high light energy (150 μmol photons $m^{-2} s^{-1}$) compared to cells cultivated at low light intensities (35 μmol photons $m^{-2} s^{-1}$). Increased APX activation under high light with UV-A radiation may be due to the direct effect of UV-A irradiance on the photosynthetic reactions

that produce increased ROS within the chloroplast and leads to the increased enzymatic activity [131].

Exposing *D. salina* to extremely high light intensities (1000 μmol photons $m^{-2} s^{-1}$) causes excess electron flow in electron transport chains of the photosystem, which leak electrons onto O_2, thereby generating superoxide ion ($O_2^{\bullet -}$) and results in enhancing SOD enzyme activity as the expression level of the antioxidant genes (SOD gene, Fe-SOD) increases [132]. No study has yet shown the effect of high light (>920 μmol photons $m^{-2} s^{-1}$) on CAT activity in *Dunaliella* strains. Perhaps CAT is not involved in mitigating oxidative stress generated during acclimation of *Dunaliella* under high light intensities. Rather, peroxidases or other small antioxidant molecules (carotenoid) have been suggested to participate in the detoxification of ROS, as β-carotene content was found to increase when the algal was cultivated under increasing light intensities [133]. Therefore, CAT, POD, SOD and APX activity in *Dunaliella* can be improved by growing cells under UV radiation combined with a wide range of light irradiation (150–920 μmol photons $m^{-2} s^{-1}$). However, further studies are needed to evaluate the effect of extreme high light-induced (e.g., 1500 μmol photons $m^{-2} s^{-1}$) oxidative stress on antioxidant enzyme response.

4.3. Temperature

Temperature affects the microalgal growth rate and the biochemical compositions as the cells' ability to assimilate nutrients are reduced [134], which can cause intracellular stress.

Cultivation at low temperature (13 °C) together with low light irradiation (20 μmol photons $m^{-2} s^{-1}$) was found to enhance SOD, APX, MDHAR, DHAR activity in *D. salina* compared to the unstressed controls [135], whilst cultivation at a very low temperature (5 °C) decreased CAT activity [136]. Less carotenoid and ascorbate contents was also observed when the cells were cultivated at extremely low temperatures [135]. *D. salina* exposed to low temperature and cultivated under a high light intensity (100–1200 μmol photons $m^{-2} s^{-1}$) was found to increase SOD, MDHAR, GR, APX and POD activity relative to unstressed cells. High light intensities provide saturated CO_2 absorption and the low temperature decreased the rate of CO_2 absorption, which induced photo-oxidative stress and resulted in increased enzymatic activity [35]. SOD, APX, DHAR, activity were increased in *D. salina* (IR-1) when cells were grown for 2 days under high temperature (28 °C) under light irradiation ranging between 100–1200 μmol photons $m^{-2} s^{-1}$ compared to a different strain (Gh-U) [35]. The different oxidative stress responses between two strains could result from variations in resistance to low temperature and high light. Only a few studies describe the effect of temperature on antioxidant enzyme responses in *Dunaliella,* and further investigations are required to explore the effect of temperature on antioxidant enzyme activity.

4.4. Nutrients

Deprivation of nitrogen, sulphur, or phosphorous in the culture medium was found to enhance CAT, SOD, and APX activity compared to unstressed *Dunaliella* cells [37,137]. Deprivation of nitrogen reduces chlorophyll content and the synthesis of chloroplastic proteins, whereas deprivation of sulphur decreased the generation of carotenoid and increased ROS levels, which further increased antioxidant enzyme activity. Deprivation of Mn, Zn, Fe was also found to produce an oxidative stress response in *D. salina* with overexpressed SOD isoenzymes [36]. Nitrogen limitation in the growth medium elevated CAT, SOD, and APX levels in *D. salina* [138], indicating that depletion (limitation) of nitrogen also can play a pivotal role as an oxidative stressor for improving antioxidant enzyme activity. Research has also demonstrated that the deprivation of any single nutrient was more effective in enhancing antioxidant enzyme activity relative to combined nutrients deprivation conditions [137].

4.5. Metals

Heavy metals can induce oxidative stress in microalgae, by generating ROS by auto-oxidation, blocking essential functional groups in biomolecules, or substituting essential metal ions [139]. When *D. tertiolecta* was cultured with silver nanoparticle (AgNPs), CAT, SOD, and POD activity increased initially and then decreased over time [140], which indicate that antioxidant enzymes act as a first line of defence to protect the cells by mitigating metal-induced oxidative stress. Nonetheless, antioxidant enzyme activity could not eliminate the metal toxicity during prolonged exposure of cells [140].

Short-term exposer of Hg^{2+} increased APX activity in *D. tertiolecta* compared to un-stressed cells. Hg^{2+} binds to a sulfhydryl group and disturbs the protein functions leading to stress conditions [141]. In contrast, long-term exposer to Hg^{2+} increased β-carotenoid content in *Dunaliella* cells. β-carotenoid acts as a supportive antioxidant. Pre-treatment of *Dunaliella* with Zn followed by cultivation in the presence of ROS generating agents (e.g., H_2O_2, paraquat) was found to decrease carotenoids level, CAT and APX activities [142], as cells were unable to enhance antioxidants levels. Moreover, the enhanced growth rate indicated that heavy metal toxicity in Zn-treated cells was detoxified by other antioxidants systems (either chelation reactions (metal to PC) or by displacements of Zn from a PC-Zn complex).

Small quantities of Cu^{2+} are essential for growing cells. During cultivation with Cu^{2+} (>5 μM $CuCl_2$) oxidative stress was induced in *Dunaliella* cells [143]. *D. salina* and *D. tertiolecta* exposed to Cu^{2+} in the growth medium had enhanced APX activity. A higher enzymatic activity was measured in *D. tertiolecta* compared to *D. salina*, which could be due to the generation of lower amounts of carotenoid in *D. tertiolecta* [143]. Metals such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn) are also essential micronu-trients required for various biochemical and physiological functions during microalgae growth [144]. However, non-essential heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are systematic toxicants [145]. Epidemiological and experimental studies have shown that these metals have adverse effects on health in humans, including cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders, diabetes, hearing loss, hematologic and immunologic disorders, and various types of cancer, an association between exposure and cancer incidence in humans and animals [146]. Using heavy metals enriched biomass in the food industry or as a feed in the aquaculture industry is not a viable strategy to induce stress in algae cultures.

4.6. Chemicals

Chemicals modulate cellular metabolism and produce oxidative stress resulting in higher antioxidant levels in cells [147]. Culturing *D. bardawil* with a mixture of two surfactants (sodium dodecylbenzene sulfonate (SDBS) and cetyl trimethyl ammonium chloride (CTAC)) induced oxidative stress, increasing CAT and SOD activity compared to untreated cells [148].

The exposure of *D. salina* to 2-chlorophenol increased CAT, SOD, and GST activities to mitigate oxidative stress, which was produced by protein carbonyls formation through metal catalyzed oxidation reaction [149]. However, APX activity decreased when *D. salina* was cultured with 2-chlorophenol. Increased level of GST activity may be due to the ability of GST to catalyze the reduced glutathione in the presence of phenol [149]. Moreover, GST can also reduce lipid hydroperoxides to the corresponding alcohols [150]. Investigation of the effect of two pesticides on the antioxidant response in the culture of *Dunaliella* demon-strated that both pollutants (trichlorfon and dimehypo) at a low concentration induced oxidative stress that stimulated the activity of CAT compared to untreated cells [151]. CAT activity in *D. salina* was improved when cells were grown with herbicides (methylene blue and norflurazon) under low-temperature conditions [152]. Herbicides suppressed the synthesis of chlorophyll and decreased the carotenoid biosynthesis leading to an increase

in CAT activity. Therefore, phenolic compounds or herbicides as stressors during *Dunaliella* culturing could be an effective strategy for enhancing enzyme activity.

4.7. Combination of Abiotic Factors on Antioxidant Enzyme Activity

Most research on antioxidant enzyme activity in *Dunaliella* focuses on only one factor, with a few studies assessing the combined effect of two factors. The studies indicate that a combination of factors is more effective in producing enzymatic antioxidant responses in *Dunaliella* compared to exposure to a single stressor [37,103,137]. Factors that can be combined to produce an antioxidant response include UV-B radiation + high salinity + nitrogen deficiency (enhanced CAT, SOD, POD activities).

UV-B radiation combined with high salinity and nitrogen deficiency enhanced CAT, SOD, POD activity relative to the non-radiated cells grown under normal growth conditions [34]. The combined effect of UV-B irradiation and nitrogen starvation can produce oxidative stress, which was eliminated by enhanced SOD, APX, and GPX activities relative to untreated *D. salina* cells, whilst GR activity did not significantly change. However, the combined effect on antioxidant enzyme activity was significantly lower relative to the effect of either UV-B radiation or nitrogen deprivation [37]. Therefore, the combination of UV-B irradiation and nitrogen deprivation may not be effective in enhancing antioxidant enzyme activity in *Dunaliella*. Salinity (2–3 M NaCl) associated with propyl gallate can enhance CAT and APX activity, whilst SOD activity was unaltered in *D. salina* compared to unstressed cells [152] suggests that salinity associated with other chemical-induced stress may increase in antioxidant enzyme activity in *Dunaliella*. There is a need for further research into this are to develop a deeper understanding of the 'tuning parameters' for enhanced enzymatic activity. The effects of abiotic factors on antioxidant enzyme activity are summarised in Table 4.

Table 4. Antioxidant enzymatic response of *Dunaliella* exposed to different abiotic exogenous stress.

	Stress Conditions	Strains	Optimum Light (μmol Photon m^{-2} s^{-1})	Salinity (NaCl) (M)	Temperature (°C)	Antioxidant Enzymatic Response	References
Salinity	0.05–3.00 M NaCl	*D. tertiolecta* (UTEX999)	150 Continuous light	0.1–0.5	26	No effect on SOD and CAT, GTR activities APX increased (2-fold) at high salinities (0.2–3 M)	[125]
	0.5–5.5 M NaCl	*D. salina*	150 Light: dark cycle (12:12 h)	1.5	(25 ± 2)	CAT activity decreases with salinity SOD increased (0.5–2 M) and then decrease APX decreases (0.5–2 M) and then constant	[122]
	0.05–4.0 M NaCl	*D. salina* and *D. tertiolecta*	150 Light: dark cycle (12:12 h)	1.25	(25 ± 2)	CAT and SOD activities increase at hypo saline condition in *D. tertiolecta* APX activity in *D. tertiolecta* higher at hypo saline and lower at hyper saline condition GPX activity decreased in *D. tertiolecta* at higher saline condition (>1.25 M)	[124]

Table 4. *Cont.*

Stress Conditions	Strains	Optimum Light (μmol Photon m^{-2} s^{-1})	Salinity (NaCl) (M)	Temperature (°C)	Antioxidant Enzymatic Response	References
Light	1–4.0 M NaCl	*D. salina*	54 (Continuous)	2	22	APX increase (171%) at 4 M compared to 2 M	[153]
	Outdoor with natural UVR	*D. tertiolecta*	250 Light: dark cycle (12:12 h)	-	20	SOD activity slightly changed in short and unaltered for long term exposure but no change in APX and GTX activity	[130]
	UV-B for 6 days	*D. salina*	60–80 Light: dark cycle (12:12 h)	-	20	CAT activity increased on 3rd day SOD activity increased on 4th day POD activity increased on 3rd day	[128]
	UV-B and UV-C for 24 h	*D. bardawil* and *D. salina*	150 UV-A	-	26	APX increased by UV -A	[131]
	UV-B for 4 h	*D. salina*	920	-	25	CAT, SOD and POD activity increased	[34]
Nutrients	Depletion of N, P, S, NP, NS, NPS	*D. salina*	60 (Continuous)	-	30	SOD and CAT activity increased	[137]
	KNO$_3$ (0.05, 0.5, 5 mM)	*D. salina*	150 Continuous light	-	25	CAT, SOD and APX activity decreased with increased nitrogen concentration	[138]
Light and nutrients	Light intensity and depletion of Mn, Zn, Fe, N	*D. salina*	70 Light: dark cycle (16:8 h)		20	Mn-SOD overexpressed	[36]
	UV-B 2 h day^{-1} for 3 days and deprivation of nitrogen	*D. salina*	85 Continuous light		25±5	POD and APX activity increased	[37]
Light and temperature	Low temperature and low light for 24 h	*D. salina* (Gh-U)	100 Light: dark (16:8 h)		28 ± 0.5	SOD activity increased APX activity increased at low light level GR, MDHAR increased at low temperature	[135]
	Low temperature and medium light for 24 h	*D. salina* (IR-1 and Gh-U)	100 Light: dark (16:8 h)		28 ± 0.5	POD activity increased, GTX increased in strain Gh-U but not in IR-1 at low temperature GR, DHAR activity increased in Gh-U than IR-1	[35]
Metals	Cu^{2+} (1–20 μM) for 24–72 h	*D. salina* and *D. tertiolecta*	54 Continuous light		22	APX activity increased	[143]
	Hg^{2+} (0–40 μM) for 48 h and 28 days	*D. tertiolecta*	54 (Continuous light)		(22 ± 2)	APX activity increased	[141]

Table 4. *Cont.*

Stress Conditions	Strains	Optimum Light (μmol Photon m^{-2} s^{-1})	Salinity (NaCl) (M)	Temperature (°C)	Antioxidant Enzymatic Response	References
Chemicals	*D. viridis*	117 (Light: dark) (12:12 h)		(28 ± 2)	Pigment production decreased	[154]
	D. salina and D. tertiolecta	46 White fluorescent light		25	SOD activity decreased Chlorophyll 'a' content decreased with increase in the exposure time	[155]
	(*D. tertiolecta*)	100 Continuous light		25	CAT activity increased until 6 days then decreased, SOD activity increased up to 4 days, POD activity increased until 6 days	[140]
	D. tertiolecta (CCAP19/6B)	58 Continuous		(24 ± 1)	Increased carotenoid	[156]
	D. bardawil	144 (Light: dark) (14:10 h)		26	CAT and SOD activity increased	[148]
	D. salina	54 (Light: dark) (14:10 h)		26	CAT activity increased	[151]
	D. salina	100 (Continuous daylight)		(25 ± 1) °C	SOD, CAT, GTX activity increased APX activity decreased	[149]
Chemical and salinity	*D. salina* (UTEX 200)	70 Light: dark (16:8 h)		25	Maximum CAT activity at 2 M NaCl and no change in SOD activity at 1 and 3 M NaCl Minor increase in APX activity at 3 M NaCl	[152]
Chemical and temperature	*D. salina* (IPASS D-294)	73.6 White fluorescent light		27	CAT activity increased Chlorophyll and carotenoid content decreased with temperature	[136]

Note: Rows also show stress condition descriptions in the first column:
- Cd^{2+} (0–20 μg L^{-1}) for 24, 48,72, and 96 h
- Cr^{6+} (40 ppm) for 24 h
- Silver nanoparticles (10, 50, 100 and 200 mg L^{-1} Ag-NPs) for 8 days
- Carbamazepine (CBZ) (0–200 mg L^{-1}) 24, 48,72, and 96 h
- SDBS and CTAC for 48 h
- Trichlorfon and dimehypo
- 2-chlorophenol for 24, 48, and 96 h
- (1, 2, 3 M NaCl) and PG (propyl gallate) for 48 h
- 10 °C and 5 °C, Methylene blue and norflurazon

5. Culturing Systems

Commercially, large-scale microalgae biomass is produced in open ponds, raceway ponds, enclosed photobioreactors (PBRs), or a combination of hybrid systems [157]. The cultivation of *Dunaliella* also employs these various production systems [40,106,158–161] depending on the location and the desired end-products.

The PBR is considered a suitable production method for microalgae (*Dunaliella*) cultivation to enhance antioxidant production with biomass productivity in a 'monoculture' and to maintain culturing reproducibility. This method is flexible, controllable, and able to provide sustainable biomass production [105,162]. In open pond systems, the cell concentration is lower compared to that obtained during PBR cultivation as external factors (light irradiation, temperature, evaporation, contamination, aeration) are difficult to control. However, PBRs are not yet outperforming open pond systems in production volume and costs [40]. To the best of knowledge, no studies so far have reported the use of specific PBRs to culture *Dunaliella* strains to enhance enzymatic antioxidants and non-enzymatic

antioxidants. The effect of the production system on antioxidants needs to be studied further.

6. Post-Culture Downstream Processing

High-cost downstream processing is one of the major challenges to making microalgae bio-refinery economically profitable [10,163]. Technological developments in harvesting, post-harvest storage, cell disruption, extraction, and possibly fractionation are needed. Harvesting the cells from the growth medium at the end of the culturing process is one of the most critical and challenging steps in mass *Dunaliella* culture [164].

6.1. Harvesting

The selection of appropriate harvesting techniques is dependent on the characteristics of the microalgae. Considerable challenges in harvesting *Dunaliella* cells are the lack of a rigid cell wall, its small size (length 5–25 µm), the low cell density (0.1%) in the culture medium, and the high salinity (2–3 M NaCl) of growth medium. Traditionally, harvesting procedures to concentrate microalgae cells are filtration, sedimentation, flocculation, flotation, centrifugation [163–165]. Several authors have extensively reviewed the harvesting of microalgae, and in the following section, a few key points are made with particular reference to *Dunaliella* [164,166–168].

6.2. Post-Harvesting Storage Processing

For *Dunaliella* to be a viable and sustainable natural antioxidant enzyme source, suitable drying and storage of biomass must be established. Optimized post-harvest storage conditions can minimise the loss of antioxidant enzyme activity and increase these valuable compounds' shelf life. Cold storage is commonly used to preserve chemical, nutritional, and sensory properties of post-harvest processed living cells as respiration and other metabolic reactions are reduced at lower temperatures [169]. Biomass can be prepared for cold storage via a dry or a wet route. In the dry route, *Dunaliella* biomass is freeze-dried or air-dried, then stored at cold temperature. In contrast, in a wet route, biomass is preserved at cold temperature straightway [25]. Several reports demonstrate that antioxidant stability depends on the type of biomass, type of chemical components, length of storage time, storage temperature, and drying method [170–173]. Roy et al., (2020) compared the storages of both wet and freeze-dried algal biomass and suggested that CAT and SOD activity in freeze-dried *D. tertiolecta* biomass can be retained for eight months when stored $-20\,^{\circ}$C, whereas activities in wet biomass or crude extract remain unchanged for four months when stored at $-80\,^{\circ}$C [173–175]. Therefore, using wet biomass, either fresh or frozen, as feedstock may be an economically feasible method for antioxidant enzyme storage compared to dried cell storage. Extraction procedure of antioxidants from algae biomass depends on the desired products. For example, lipid-soluble antioxidants (e.g., carotenoids, tocopherol, flavonoids, ascorbate) are extracted by organic solvents (e.g., hexane, heptane, methyl tertiary burate, methanol, ethanol) with a mechanical treatment (e.g., ultrasonication, microwave, high pressure) [2,10,16,174]. Water-soluble antioxidants or antioxidant enzymes are recovered from the algae biomass by aqueous solvent or buffer systems together with mechanical assistance [37,128,138,175,176]. Recently, green solvents (e.g., cyclopentyl methyl ether (CPME), dimethyl carbonate (DMC)), ionic liquids (e.g., ethanol containing 1-n-butyl-3-methylimidazolium) and supercritical CO_2—based extraction have been used for the recovery of carotenoids (e.g., β-carotene, astraxanthin) [7,177].

7. Challenges and Future Opportunities

Despite the potential of *Dunaliella* as a source for high-value compounds including antioxidants, the main challenges are as follows:

i.   Low growth rate and productivity of *Dunaliella* strain [4,28,174] relative to other sources (such as fungus, yeast) [178–181] and selection of a suitable strain;

ii.   Transferring lab-scale optimised abiotic stress strategies to a commercial scale;

iii. Seasonal and environmental variation in biomass production, and the control of competitors in open ponds cultivation systems;

iv. Engineering developments of sustainable large-scale technologies (open raceways ponds and PBR) for *Dunaliella* and microalgae in general for efficient biomass and enzymatic antioxidant production;

v. Developing sustainable downstream technologies (harvesting, extraction, and purification) for antioxidant enzymes production at commercial scale;

vi. Establishing tools for proteomics, genomics, and metabolomics, and implement them to develop strains for the accumulation of intracellular antioxidant enzymes;

vii. Stabilisation of antioxidant enzymes in food ingredients and extension of their shelf life;

viii. Algae extracts can be used as taste enhances in foods; however, some strains are associated with a bitter or astringent taste;

ix. Introducing a general platform to solve regulatory and labelling issues, as this varies from country to country.

Based on the reviewed literature, emphasis has been on carotenoid production from *Dunaliella*, limiting the exploration of *Dunaliella* biomass to produce other high-value compounds such as antioxidant enzymes. The tolerance of *Dunaliella* to extreme environments (such as high salinity and high light levels) makes these strains versatile. It enables them to survive in stressful environments, providing opportunities to explore these strains as natural antioxidant enzyme sources either as a main product or by-products. In addition, there is an opportunity to improve technological developments in harvesting biomass by employing cost-effective processes (such as flocculation or microbubbles techniques). Due to the lack of a rigid cell wall, the extraction process is cheaper relative to other algae cells or plants, which will reduce downstream processing costs. As antioxidant enzymes in wet *Dunaliella* biomass can be retained for one month when stored at $-20\,^{\circ}\mathrm{C}$ [175]. The development of an extraction procedure for antioxidant enzymes from wet biomass can save the energy costs required for drying the biomass.

Techno-economic assessment can be conducted to assure the profitability of developed cultivation and downstream processing systems to produce specific antioxidants enzymes or biorefinery strategies. There is also an opportunity to explore novel applications of *Dunaliella*-derived antioxidant enzymes in the pharmaceuticals, nutraceuticals, agricultural and cosmetic industries. Growing end-user applications for antioxidant enzymes and increasing end-user awareness may enhance the demand in the global market.

8. Conclusions

Dunaliella can accumulate a wide range of molecules, with a high value in the global food, aquaculture, animal feed, pharmaceutical, and nutraceutical industry. Currently, β-carotene production from *D. salina* is commercially cost-effective. High biomass production costs; lack of optimal cultivation conditions and culturing systems; technical difficulties in scaling–up; lack of cost-effective downstream processing technologies; and instability of antioxidant enzymes at room temperature are the major constraints to the commercialisation of *Dunaliella*-based antioxidant enzyme production. Tuning abiotic stress factors during cultivation can activate different antioxidant systems in *Dunaliella*. A multiple factors-based stress strategy is more effective relative to a single inducing stress factor during growth in a photobioreactor (considered monoculture) system, giving design flexibility and controllability.

Author Contributions: Conceptualisation U.K.R., B.V.N., J.J.M.; Writing—original manuscript preparation U.K.R.; Writing—review and editing U.K.R., B.V.N. and J.J.M. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hadi, M.R.; Shariati, M.; Afsharzadeh, S. Microalgal biotechnology: Carotenoid and glycerol production by the green algae Dunaliella isolated from the Gave-Khooni salt marsh, Iran. *Biotechnol. Bioprocess Eng.* **2008**, *13*, 540–544. [CrossRef]
2. Michalak, I.; Chojnacka, K. Algae as production systems of bioactive compounds. *Eng. Life Sci.* **2015**, *15*, 160–176. [CrossRef]
3. Camacho, F.; Macedo, A.; Malcata, F. Potential industrial applications and commercialization of microalgae in the functional food and feed industries: A short review. *Mar. Drugs* **2019**, *17*, 312. [CrossRef] [PubMed]
4. Ahmed, R.A.; He, M.; Aftab, R.A.; Zheng, S.; Nagi, M.; Bakri, R.; Wang, C. Bioenergy application of *Dunaliella salina* SA 134 grown at various salinity levels for lipid production. *Sci. Rep.* **2017**, *7*, 1–10. [CrossRef]
5. Gujar, A.; Cui, H.; Ji, C.; Kubar, S.; Li, R. Development, Production and Market Value of Microalgae Products. *Appl. Microbiol.* **2019**, *5*, 1–9. [CrossRef]
6. Goiris, K.; Muylaert, K.; De Cooman, L. Microalgae as a Novel Source of Antioxidants for Nutritional Applications. *Handb. Mar. Microalgae Biotechnol. Adv.* **2015**, *10*, 160–180. [CrossRef]
7. Marino, T.; Casella, P.; Sangiorgio, P.; Verardi, A.; Ferraro, A.; Hristoforou, E.; Molino, A.; Musmarra, D. Natural beta-carotene: A microalgae derivate for nutraceutical applications. *Chem. Eng. Trans.* **2020**, *79*, 103–108. [CrossRef]
8. Barkia, I.; Saari, N.; Manning, S.R. Microalgae for high-value products towards human health and nutrition. *Mar. Drugs.* **2019**, *17*, 304. [CrossRef]
9. Raja, R.; Coelho, A.; Hemaiswarya, S.; Kumar, P.; Carvalho, I.S.; Alagarsamy, A. Applications of microalgal paste and powder as food and feed: An update using text mining tool. *Beni-Suef Univ. J. Basic Appl. Sci.* **2018**, *7*, 740–747. [CrossRef]
10. Khanra, S.; Mondal, M.; Halder, G.; Tiwari, O.N.; Gayen, K.; Bhowmick, T.K. Downstream processing of microalgae for pigments, protein and carbohydrate in industrial application: A review. *Food Bioprod. Process.* **2018**, *110*, 60–84. [CrossRef]
11. Figueira, F.D.S.; Crizel, T.D.M.; Silva, C.R.; Salas-Mellado, M.d.l.M. Elaboration of gluten-free bread enriched with the microalgae Spirulina platensis. *Braz. J. Food Technol.* **2011**, *14*, 308–316.
12. Gouveia, L.; Oliveira, A.C. Microalgae as a raw material for biofuels production. *J. Ind. Microbiol. Biotechnol.* **2009**, *36*, 269–274. [CrossRef] [PubMed]
13. Fradique, M.; Batista, A.P.; Nunes, M.C.; Gouveia, L.; Bandarra, N.M.; Raymundo, A. Incorporation of Chlorella vulgaris and Spirulina maxima biomass in pasta products. Part 1: Preparation and evaluation. *J. Sci. Food Agric.* **2010**, *90*, 1656–1664. [CrossRef] [PubMed]
14. Rumin, J.; Nicolau, E.; de Oliveira, R.G.; Fuentes-Grünewald, C.; Picot, L. Analysis of Scientific Research Driving Microalgae Market Opportunities in Europe. *Mar. Drugs* **2020**, *18*, 264. [CrossRef] [PubMed]
15. Transparency Market Research. Microalgae—Based Products Market (Product: Spirulina, Chlorella, Astaxanthin, β-Carotene, PUFA Fatty Acid, Lutein, and Others; Food and Beverages, Personal Care, Nutraceuticals and Dietary Supplements, Pharmaceuticals, Chemicals, Fuel, Animal Feed)—Global Industry Analysis, Sixe, Share, Growth, Trends, and Forecast, 2020–2030. 2020. Available online: https://www.transparencymarketresearch.com/about-us.html (accessed on 26 April 2021).
16. Maadane, A.; Merghoub, N.; Ainane, T.; El Arroussi, H.; Benhima, R.; Amzazi, S.; Bakri, Y.; Wahby, I. Antioxidant activity of some Moroccan marine microalgae: Pufa profiles, carotenoids and phenolic content. *J. Biotechnol.* **2015**, *215*, 13–19. [CrossRef]
17. Gangadhar, K.N.; Pereira, H.; Rodrigues, M.J.; Custódio, L.; Barreira, L.; Malcata, F.X.; Varela, J. Microalgae-based unsaponifiable matter as source of natural antioxidants and metal chelators to enhance the value of wet *Tetraselmis chuii* biomass. *Open Chem.* **2016**, *14*, 299–307. [CrossRef]
18. Assuncao, M.F.G.; Amaral, R.; Martins, C.B.; Ferreira, J.D.; Ressurreicao, S.; Santos, S.D.; Varejão, J.M.T.B.; Santos, L.M.A. Screening microalgae as potential sources of antioxidants. *J. Appl. Phycol.* **2017**, *29*, 865–877. [CrossRef]
19. García, J.L.; de Vicente, M.; Galán, B. Microalgae, old sustainable food and fashion nutraceuticals. *Microb. Biotechnol.* **2017**, *10*, 1017–1024. [CrossRef] [PubMed]
20. Gangl, D.; Zedler, J.A.Z.; Rajakumar, P.D.; Martinez, E.M.R.; Riseley, A.; Włodarczyk, A.; Purton, S.; Sakuragi, Y.; Howe, C.J.; Jensen, P.E.; et al. Biotechnological exploitation of microalgae. *J. Exp. Bot.* **2015**, *66*, 6975–6990. [CrossRef]
21. Villarruel-López, A.; Ascencio, F.; Nuño, K. Microalgae, a Potential Natural Functional Food Source—A Review. *Polish J. Food Nutr. Sci.* **2017**, *67*, 251–263. [CrossRef]
22. Chen, H.; Jiang, J.G. Osmotic responses of Dunaliella to the changes of salinity. *J. Cell. Physiol.* **2009**, *219*, 251–258. [CrossRef]
23. Ben-Amotz, A.; Avron, M. The biotechnology of cultivating the halotolerant alga Dunaliella. *Trends Biotechnol.* **1990**, *8*, 121–126. [CrossRef]
24. Del Campo, J.A.; García-González, M.; Guerrero, M.G. Outdoor cultivation of microalgae for carotenoid production: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 1163–1174. [CrossRef] [PubMed]

25. Shariati, M.; Hadi, M.R. Microalgal Biotechnology and Bioenergy in Dunaliella. In *Progress in Molecular and Environmental Bioengineering—From Analysis and Modeling to Technology Applications*; Carpi, A., Ed.; Intechopen: Rijeka, Croatia, 2011. Available online: https://www.intechopen.com/books/progress-in-molecular-and-environmental-bioengineering-from-analysis-and-modeling-to-technology-applications/microalgal-biotechnology-and-bioenergy-in-dunaliella (accessed on 26 April 2021). [CrossRef]

26. Chen, J.; Wang, Y.; Benemann, J.R.; Zhang, X.; Hu, H.; Qin, S. Microalgal industry in China: Challenges and prospects. *J. Appl. Phycol.* **2016**, *28*, 715–725. [CrossRef]

27. Sui, Y.; Vlaeminck, S.E. Dunaliella Microalgae for Nutritional Protein: An Undervalued Asset. *Trends Biotechnol.* **2020**, *38*, 10–12. [CrossRef]

28. Liang, M.H.; Xue, L.L.; Jiang, J.G. Two-stage cultivation of Dunaliella tertiolecta with glycerol and triethylamine for lipid accumulation: A viable way to alleviate the inhibitory effect of triethylamine on biomass. *Appl. Environ. Microbiol.* **2019**, *85*, 1–10. [CrossRef] [PubMed]

29. Bhalamurugan, G.L.; Valerie, O.; Mark, L. Valuable bioproducts obtained from microalgal biomass and their commercial applications: A review. *Environ. Eng. Res.* **2018**, *23*, 229–241. [CrossRef]

30. Church, J.; Hwang, J.H.; Kim, K.T.; McLean, R.; Oh, Y.K.; Nam, B.; Joo, J.C.; Lee, W.H. Effect of salt type and concentration on the growth and lipid content of Chlorella vulgaris in synthetic saline wastewater for biofuel production. *Bioresour. Technol.* **2017**, *243*, 147–153. [CrossRef] [PubMed]

31. Widowati, I.; Zainuri, M.; Kusumaningrum, H.P.; Susilowati, R.; Hardivillier, Y.; Leignel, V.; Bourgougnon, N.; Mouget, J.L. Antioxidant activity of three microalgae *Dunaliella salina*, *Tetraselmis chuii* and *Isochrysis galbana* clone Tahiti. In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bali, Indonesia, 2017; Volume 55, p. 012067.

32. Singh, R.; Kumar, M.; Mittal, A.; Kumar, P. Microbial enzymes: Industrial progress in 21st century. *3 Biotech* **2016**, *6*, 1–15. [CrossRef]

33. Raveendran, S.; Kuruvilla, A.; Rebello, S. Applications of Microbial Enzymes in Food Industry. *Food Technol. Biotechnol.* **2018**, *56*, 16–30. [CrossRef]

34. Hanaa, H.; El Baz, F.K.; El-Baroty, G.S. Production of Antioxidant by the Green Alga *Dunaliella salina*. *Int. J. Agric. Biol.* **2004**, *6*, 49–57.

35. Haghjou, M.M.; Shariati, M.; Smirnoff, N. The effect of acute high light and low temperature stresses on the ascorbate-glutathione cycle and superoxide dismutase activity in two *Dunaliella salina* strains. *Physiol. Plant.* **2009**, *135*, 272–280. [CrossRef] [PubMed]

36. Saha, S.K.; Moane, S.; Murray, P. Effect of macro- and micro-nutrient limitation on superoxide dismutase activities and carotenoid levels in microalga *Dunaliella salina* CCAP 19/18. *Bioresour. Technol.* **2013**, *147*, 23–28. [CrossRef]

37. Al-Rashed, S.A.; Ibrahim, M.M.; El-Gaaly, G.A.; Al-Shehri, S.; Mostafa, A. Evaluation of radical scavenging system in two microalgae in response to interactive stresses of UV-B radiation and nitrogen starvation. *Saudi J. Biol. Sci.* **2016**. [CrossRef] [PubMed]

38. Singh, P.; Baranwal, M.; Reddy, S.M. Antioxidant and cytotoxic activity of carotenes produced by *Dunaliella salina* under stress. *Pharm. Biol.* **2016**, *54*, 2269–2275. [CrossRef]

39. Mirshekari, M.; Einali, A.; Valizadeh, J. Metabolic changes and activity pattern of antioxidant enzymes induced by salicylic acid treatment in green microalga *Dunaliella salina* under nitrogen deficiency. *J. Appl. Phycol.* **2019**, *31*, 1709–1719. [CrossRef]

40. Hosseini Tafreshi, A.; Shariati, M. Dunaliella biotechnology: Methods and applications. *J. Appl. Microbiol.* **2009**, *107*, 14–35. [CrossRef]

41. Rahman, K. Studies on free radicals, antioxidants, and co-factors. *Clin. Interv. Aging* **2007**, *2*, 219–236.

42. Krishnamurthy, P.; Wadhwani, A. Antioxidant Enzymes and Human Health. In *Antioxidant Enzyme*; Intech: Rijeka, Croatia, 2012; Volume 1, pp. 3–18. Available online: https://www.intechopen.com/books/antioxidant-enzyme/antioxidant-enzymes-and-human-health (accessed on 26 April 2021). [CrossRef]

43. Rahal, A.; Kumar, A.; Singh, V.; Yadav, B.; Tiwari, R.; Chakraborty, S.; Dhama, K. Oxidative stress, prooxidants, and antioxidants: The interplay. *Biomed. Res. Int.* **2014**, *2014*, 761264. [CrossRef]

44. Panda, S.K. Assay Guided Comparison for Enzymatic and Non-Enzymatic Antioxidant Activities with Special Reference to Medicinal Plants. In *Antioxidant Enzyme*; Intech: Rijeka, Croatia, 2012; Volume 14, pp. 381–400. Available online: https://www.intechopen.com/books/antioxidant-enzyme/assay-guided-comparison-for-enzymatic-and-non-enzymatic-antioxidant-activities-with-special-referenc (accessed on 26 April 2021). [CrossRef]

45. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [CrossRef]

46. Mtaki, K.; Kyewalyanga, M.S.; Mtolera, M.S.P. Assessment of antioxidant contents and free radical-scavenging capacity of chlorella vulgaris cultivated in low cost media. *Appl. Sci.* **2020**, *10*, 8611. [CrossRef]

47. Rezayian, M.; Niknam, V.; Ebrahimzadeh, H. Oxidative damage and antioxidative system in algae. *Toxicol. Rep.* **2019**, *6*, 1309–1313. [CrossRef] [PubMed]

48. Nimse, S.B.; Pal, D. Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv.* **2015**, *5*, 27986–28006. [CrossRef]

49. Kaushal, J.; Mehandia, S.; Singh, G.; Raina, A.; Arya, S.K. Catalase enzyme: Application in bioremediation and food industry. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 192–199. [CrossRef]

50. Gopal, R.K.; Elumalai, S. Industrial Production of Superoxide Dismutase (SOD): A Mini Review. *J. Probiotics Health* **2017**, *5*. [CrossRef]

51. Pandey, V.P.; Awasthi, M.; Singh, S.; Tiwari, S.; Dwivedi, U.N. A Comprehensive Review on Function and Application of Plant Peroxidases. *Biochem. Anal. Biochem.* **2017**, *6*, 1–16. [CrossRef]

52. Keokamnerd, T.; Acton, J.C.; Han, I.Y.; Dawson, P.L. Effect of commercial rosemary oleoresin preparations on ground chicken thigh meat quality packaged in a high-oxygen atmosphere. *Poult. Sci.* **2008**, *87*, 170–179. [CrossRef] [PubMed]

53. Hamid, M. Khalil-ur-Rehman Potential applications of peroxidases. *Food Chem.* **2009**, *115*, 1177–1186. [CrossRef]

54. Canter, N. Use of antioxidants in automotive lubricants. *Tribol. Lubr. Technol.* **2008**, *64*, 12.

55. Jegannathan, K.R.; Nielsen, P.H. Environmental assessment of enzyme use in industrial production-a literature review. *J. Clean. Prod.* **2013**, *42*, 228–240. [CrossRef]

56. Godic, A.; Poljšak, B.; Adamic, M.; Dahmane, R. The role of antioxidants in skin cancer prevention and treatment. *Oxid. Med. Cell. Longev.* **2014**, *2014*, 860479. [CrossRef]

57. Allemann, I.B.; Baumann, L. Antioxidants used in skin care formulations. *Ski. Ther. Lett* **2008**, *13*, 5–9.

58. Stephenie, S.; Chang, Y.P.; Gnanasekaran, A.; Esa, N.M.; Gnanaraj, C. An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. *J. Funct. Foods* **2020**, *68*, 103917. [CrossRef]

59. Bafana, A.; Dutt, S.; Kumar, S.; Ahuja, P.S. Superoxide dismutase: An industrial perspective. *Crit. Rev. Biotechnol.* **2011**, *31*, 65–76. [CrossRef]

60. Beach, D. Antioxidants (Natural, and Synthetic) Market for Pharmaceuticals, Food & Beverages Sector, Feed Additives, Cosmetics Industry, and Other Applications: Global Industry Perspective, Comprehensive Analysis, and Forecast, 2014–2020, Marrket Research Store. 2016. Available online: http://www.marketresearchstore.com/report/antioxidants-market-for-pharmaceuticals-food-z38119 (accessed on 26 April 2021).

61. Sasse, A.; Colindres, P.; Brewer, M.S. Effect of natural and synthetic antioxidants on the oxidative stability of cooked, frozen pork patties. *J. Food Sci.* **2009**, *74*, 1–6. [CrossRef]

62. Schillaci, C.; Nepravishta, R.; Bellomaria, A. Antioxidants in food and pharmaceutical research. *Albanian J. Pharm. Sci.* **2014**, *1*, 15–25.

63. Ito, N.; Hirose, M.; Fukushima, S.; Tsuda, H.; Shirai, T.; Tatematsu, M. Studies on antioxidants: Their carcinogenic and modifying effects on chemical carcinogenesis. *Food Chem. Toxicol.* **1986**, *24*, 1071–1082. [CrossRef]

64. Thompson, D.C.; Trush, M.A. Studies on the mechanism of enhancement of butylated hydroxytoluene-induced mouse lung toxicity by butylated hydroxyanisole. *Toxicol. Appl. Pharmacol.* **1988**, *96*, 122–131. [CrossRef]

65. Silaghi-dumitrescu, L.; Loghin, F. Evaluation of the possible endocrine disruptive effect of butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate in immature female rats. *Farmacia* **2013**, *61*, 202–211.

66. Thorat, I.D.; Jagtap, D.D.; Mohapatra, D.; Joshi, D.C.; Sutar, R.F.; Kapdi, S.S. Antioxidants, their properties, uses in food products and their legal implications. *Int. J. Food Stud.* **2013**, *2*, 81–104. [CrossRef]

67. El Shafey, H.M.; Bahashwan, S.A.; Alghaithy, A.A.; Ghanem, S. Microbial superoxide dismutase enzyme as therapeutic agent and future gene therapy. *Curr. Res. Technol. Educ. Top. Appl. Microbiol. Microb. Biotechnol.* **2010**, *1*, 435–443.

68. Surai, P.; Surai, P.F. Antioxidant Systems in Poultry Biology: Superoxide Dismutase. *Anim. Nutr.* **2015**, *1*, 1–17. [CrossRef]

69. Gridley, D.S.; Green, L.M.; Nelson, G.A.; Pecaut, M.J.; Slater, J.M. Therapeutic Utilities of SOD Mimetics: Cancer, Radiotherapy and SOD Mimetics. In *Madame Curie Bioscience Database [Internet]*; Landes Bioscience: Austin, TX, USA, 2013. Available online: https://www.ncbi.nlm.nih.gov/books/NBK6099/ (accessed on 26 April 2021).

70. Miłek, J.; Wójcik, M.; Verschelde, W. Thermal stability for the effective use of commercial catalase. *Polish J. Chem. Technol.* **2014**, *16*, 75–79. [CrossRef]

71. Morón, Ú.M.; Castilla-Cortázar, I. Protection Against Oxidative Stress and "IGF-I Deficiency Conditions". In *Antioxidant Enzyme*; Intechopen: Rijeka, Croatia, 2012. Available online: https://www.intechopen.com/books/antioxidant-enzyme/protection-against-oxidative-stress-and-igf-i-deficiency-conditions- (accessed on 26 April 2021). [CrossRef]

72. Chenevix Trench, G.; Young, J.; Coggan, M.; Board, P. Glutathione S-transferase M1 and T1 polymorphisms: Susceptibility to colon cancer and age of onset. *Carcinogenesis* **1995**, *16*, 1655–1657. [CrossRef] [PubMed]

73. Hashizume, E.; Chan, G.P. Skin-whitening and skin-condition-improving effects of topical oxidized glutathione: A double-blind and placebo-controlled clinical trial in healthy women. *Clin. Cosmet. Investig. Dermatol.* **2014**, 267–274. [CrossRef]

74. Barrita, J.; Sánchez, M. Antioxidant Role of Ascorbic Acid and His Protective Effects on Chronic Diseases. *Oxidative Stress Chronic Degener. Dis. A Role Antioxid.* **2013**, 450–484. [CrossRef]

75. Borek, C. Antioxidants and the prevention of hormonally regulated cancer. *J. Men's Health Gend.* **2005**, *2*, 346–352. [CrossRef]

76. Constantinou, C.; Papas, A.; Constantinou, A.I. Vitamin E and cancer: An insight into the anticancer activities of vitamin E isomers and analogs. *Int. J. Cancer* **2008**, *123*, 739–752. [CrossRef]

77. Petrulea, M.; Muresan, A.; Duncea, I. Oxidative Stress and Antioxidant Status in Hypo- and Hyperthyroidism. In *Antioxidant Enzyme*; Intechopen: Rijeka, Croatia, 2012. Available online: https://www.intechopen.com/books/antioxidant-enzyme/oxidative-stress-and-antioxidant-status-in-hypo-and-hyperthyroidism (accessed on 26 April 2021). [CrossRef]

78. Kaliora, A.C.; Dedoussis, G.V.Z.; Schmidt, H. Dietary antioxidants in preventing atherogenesis. *Atherosclerosis* **2006**, *187*, 1–17. [CrossRef]

79. Sak, K. Cytotoxicity of dietary flavonoids on different human cancer types. *Pharmacogn. Rev.* **2014**, *8*, 122–146. [CrossRef] [PubMed]

80. Vinayagam, R.; Xu, B. Antidiabetic properties of dietary flavonoids: A cellular mechanism review. *Nutr. Metab.* **2015**, *12*, 1–20. [CrossRef] [PubMed]

81. Jiang, W.; Wei, H.; He, B. Dietary flavonoids intake and the risk of coronary heart disease: A dose-response meta-analysis of 15 prospective studies. *Thromb. Res.* **2015**, *135*, 459–463. [CrossRef] [PubMed]

82. Hertog, M.G.L.; Feskens, E.J.M.; Kromhout, D.; Hertog, M.G.L.; Hollman, P.C.H.; Hertog, M.G.L.; Katan, M.B. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007–1011. [CrossRef]

83. Yaakob, Z.; Ali, E.; Zainal, A.; Mohamad, M.; Takriff, M.S. An overview: Biomolecules from microalgae for animal feed and aquaculture. *J. Biol. Res.* **2014**, *21*, 6. [CrossRef]

84. Martins, D.A.; Custódio, L.; Barreira, L.; Pereira, H.; Ben-Hamadou, R.; Varela, J.; Abu-Salah, K.M. Alternative sources of n-3 long-chain polyunsaturated fatty acids in marine microalgae. *Mar. Drugs* **2013**, *11*, 2259–2281. [CrossRef] [PubMed]

85. Harris, W.S.; Mozaffarian, D.; Rimm, E.; Kris-Etherton, P.; Rudel, L.L.; Appel, L.J.; Engler, M.M.; Engler, M.B.; Sacks, F. Omega-6 fatty acids and risk for cardiovascular disease: A science advisory from the American Heart Association nutrition subcommittee of the council on nutrition, physical activity, and metabolism; council on cardiovascular nursing; and council on epidem. *Circulation* **2009**, *119*, 902–907. [CrossRef] [PubMed]

86. Lundebye, A.-K.; Hove, H.; Måge, A.; Bohne, V.J.B.; Hamre, K. Levels of synthetic antioxidants (ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole) in fish feed and commercially farmed fish. *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* **2010**, *27*, 1652–1657. [CrossRef]

87. Dawidowicz, A.L.; Olszowy, M.; Jóźwik-Dolęba, M. Antagonistic Antioxidant Effect in Butylated Hydroxytoluene/Butylated Hydroxyanisole Mixture. *J. Food Process. Preserv.* **2015**, *39*, 2240–2248. [CrossRef]

88. Mittler, R.; Vanderauwera, S.; Gollery, M.; Van Breusegem, F. Reactive oxygen gene network of plants. *Trends Plant Sci.* **2004**, *9*, 490–498. [CrossRef]

89. Cirulis, J.T.; Scott, J.A.; Ross, G.M. Management of oxidative stress by microalgae. *Can. J. Physiol. Pharmacol.* **2013**, *91*, 15–21. [CrossRef]

90. Kaushik, D.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, 53. [CrossRef]

91. Bak, S.; Beisson, F.; Bishop, G.; Hamberger, B.; Höfer, R.; Paquette, S.; Werck-Reichhart, D. Cytochromes P450. *Arab. B* **2011**, *9*, e0144. [CrossRef] [PubMed]

92. Martin-Montalvo, A.; Sun, Y.; Diaz-Ruiz, A.; Ali, A.; Gutierrez, V.; Palacios, H.H.; Curtis, J.; Siendones, E.; Ariza, J.; Abulwerdi, G.A. Cytochrome b5 reductase and the control of lipid metabolism and healthspan. *NPJ Aging Mech. Dis.* **2016**, *2*, 16006. [CrossRef] [PubMed]

93. Pruchniak, M.P.; Araźna, M.; Demkc, U. Biochemistry of oxidative stress. *Adv. Exp. Med. Biol.* **2016**, *878*, 9–19. [CrossRef]

94. Barbusiński, K. Controversy Over Fenton Mechanism. *Ecol. Chem. Eng. S* **2009**, *16*, 347–358.

95. Tatsumi, H.; Nakase, H.; Kano, K.; Ikeda, T. Mechanistic study of the autoxidation of reduced flavin and quinone compounds. *J. Electroanal. Chem.* **1998**, *443*, 236–242. [CrossRef]

96. Vašková, J.; Vaško, L.; Kron, I. Oxidative Processes and Antioxidative Metaloenzymes. In *Antioxidant Enzyme*; Intechopen: Rijeka, Croatia, 2012; Volume 2, pp. 19–58. Available online: https://www.intechopen.com/books/antioxidant-enzyme/oxidative-processes-and-antioxidative-metaloenzymes (accessed on 26 April 2021). [CrossRef]

97. Gupta, V.K.; Sharma, S.K. Plants as Natural Antioxidants. *Indian J. Nat. Prod. Resour.* **2006**, *5*, 326–334.

98. Sofo, A.; Scopa, A.; Nuzzaci, M.; Vitti, A. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *Int. J. Mol. Sci.* **2015**, *16*, 13561–13578. [CrossRef]

99. Sharma, P.; Jha, A.B.; Dubey, R.S.; Pessarakli, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, *2012*, 217037. [CrossRef]

100. Mallick, N.; Mohn, F.H. Reactive oxygen species: Response of algal cells. *J. Plant Physiol.* **2000**, *157*, 183–193. [CrossRef]

101. Marí, M.; Morales, A.; Colell, A.; García-Ruiz, C.; Fernández-Checa, J.C. Mitochondrial glutathione, a key survival antioxidant. *Antioxid. Redox Signal.* **2009**, *11*, 2685–2700. [CrossRef]

102. Jajic, I.; Sarna, T.; Strzalka, K. Senescence, Stress, and Reactive Oxygen Species. *Plants* **2015**, *4*, 393–411. [CrossRef]

103. Juneja, A.; Ceballos, R.M.; Murthy, G.S. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies* **2013**, *6*, 4607–4638. [CrossRef]

104. Ben-Amotz, A.; Polle, J.E.W.; Rao, D.V.S. *The Alga Dunaliella*; CRC Press: Boca Raton, FL, USA, 2009; ISBN 1578085454.

105. Perez-Garcia, O.; Escalante, F.M.E.; de-Bashan, L.E.; Bashan, Y. Heterotrophic cultures of microalgae: Metabolism and potential products. *Water Res.* **2011**, *45*, 11–36. [CrossRef] [PubMed]

106. Benavente-Valdes, J.R.; Aguilar, C.; Contreras-Esquivel, J.C.; Méndez-Zavala, A.; Montañez, J. Strategies to enhance the production of photosynthetic pigments and lipids in chlorophycae species. *Biotechnol. Rep.* **2016**, *10*, 117–125. [CrossRef] [PubMed]

107. Gupta, P.L.; Lee, S.M.; Choi, H.J. Integration of microalgal cultivation system for wastewater remediation and sustainable biomass production. *World J. Microbiol. Biotechnol.* **2016**, *32*. [CrossRef] [PubMed]

108. Kharati-Koupaei, M.; Moradshasi, A. Effects of sodium nitrate and mixotrophic culture on biomass and lipid production in hypersaline microalgae Dunaliella viridis Teod. *Braz. Arch. Biol. Technol.* **2016**, *59*, 1–10. [CrossRef]

109. Chen, C.Y.; Yeh, K.L.; Aisyah, R.; Lee, D.J.; Chang, J.S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresour. Technol.* **2011**, *102*, 71–81. [CrossRef] [PubMed]

110. Gibor, A. The culture of brine algae. *Biol. Bull.* **1956**, *111*, 223–229. [CrossRef]

111. Gladue, R.M.; Maxey, J.E. Microalgal feeds for aquaculture. *J. Appl. Phycol.* **1994**, *6*, 131–141. [CrossRef]

112. Ramos, A.A.; Polle, J.; Tran, D.; Cushman, J.C.; Jin, E.; Varela, J.C. The unicellular green alga *Dunaliella salina* Teod. as a model for abiotic stress tolerance: Genetic advances and future perspectives. *Algae* **2011**, *26*, 3–20. [CrossRef]

113. Borowitzka, M.A.; Borowitzka, L.J. *Micro-Algal Biotechnology*; Cambridge University Press: Cambridge, UK, 1988; ISBN 0521323495.

114. Herrero, C.; Cid, A.; Fabregas, J.; Abalde, J. Yields in biomass and chemical constituents of four commercially important marine microalgae with different culture media. *Aquac. Eng.* **1991**, *10*, 99–110. [CrossRef]

115. Cifuentes, A.S.; Gonzalez, M.A.; Parra, O.O. The effect of salinity on the growth and carotenogenesis in two Chilean strains of *Dunaliella salina* Teodoresco. *Biol. Res.* **1996**, *29*, 227–236. [PubMed]

116. Ibañez, E.; Cifuentes, A. Benefits of using algae as natural sources of functional ingredients. *J. Sci. Food Agric.* **2013**, *93*, 703–709. [CrossRef]

117. Tredici, M.R.; Rodolfi, L.; Biondi, N.; Bassi, N.; Sampietro, G. Techno-economic analysis of microalgal biomass production in a 1-ha Green Wall Panel (GWP®) plant. *Algal Res.* **2016**, *19*, 253–263. [CrossRef]

118. Kumar, D.; Singh, B. Algal biorefinery: An integrated approach for sustainable biodiesel production. *Biomass Bioenergy* **2019**, *131*, 105398. [CrossRef]

119. Harvey, P.J.; Ben-Amotz, A. Towards a sustainable *Dunaliella salina* microalgal biorefinery for 9-cis β-carotene production. *Algal Res.* **2020**, *50*, 102002. [CrossRef]

120. Suzuki, N.; Rivero, R.M.; Shulaev, V.; Blumwald, E.; Mittler, R. Abiotic and biotic stress combinations. *New Phytol.* **2014**, *203*, 32–43. [CrossRef] [PubMed]

121. Fu, W.; Paglia, G.; Magnúsdóttir, M.; Steinarsdóttir, E.A.; Gudmundsson, S.; Palsson, B.Ø.; Andrésson, Ó.S.; Brynjólfsson, S. Effects of abiotic stressors on lutein production in the green microalga *Dunaliella salina*. *Microb. Cell Fact.* **2014**, *13*, 3. [CrossRef] [PubMed]

122. Minhas, A.K.; Hodgson, P.; Barrow, C.J.; Adholeya, A. A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. *Front. Microbiol.* **2016**, *7*, 1–19. [CrossRef]

123. Mishra, A.; Jha, B. Antioxidant response of the microalga *Dunaliella salina* under salt stress. *Bot. Mar.* **2011**, *54*, 195–199. [CrossRef]

124. Mishra, A.; Mandoli, A.; Jha, B. Physiological characterization and stress-induced metabolic responses of *Dunaliella salina* isolated from salt pan. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 1093–1101. [CrossRef] [PubMed]

125. Tammam, A.A.; Fakhry, E.M.; El-sheekh, M. Effect of salt stress on antioxidant system and the metabolism of the reactive oxygen species in *Dunaliella salina* and Dunaliella tertiolecta. *J. Biotechnol.* **2011**, *10*, 3795–3808. [CrossRef]

126. Jahnke, L.S.; White, A.L. Long-term hyposaline and hypersaline stresses produce distinct antioxidant responses in the marine alga Dunaliella tertiolecta. *J. Plant Physiol.* **2003**, *160*, 1193–1202. [CrossRef] [PubMed]

127. Reddy, A.R.; Chaitanya, K.V.; Vivekanandan, M. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* **2004**, *161*, 1189–1202. [CrossRef]

128. Asada, K. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Biol.* **1999**, *50*, 601–639. [CrossRef]

129. Tian, J.; Yu, J. Changes in ultrastructure and responses of antioxidant systems of algae (*Dunaliella salina*) during acclimation to enhanced ultraviolet-B radiation. *J. Photochem. Photobiol. B Biol.* **2009**, *97*, 152–160. [CrossRef]

130. Shick, J.M.; Dunlap, W.C. Mycosporine-like amino acids and related gadusols: Biosynthesis, acumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.* **2002**, *64*, 223–262. [CrossRef]

131. Janknegt, P.J.; De Graaff, C.M.; Van De Poll, W.H.; Visser, R.J.W.; Helbling, E.W.; Buma, A.G.J. Antioxidative responses of two marine microalgae during acclimation to static and fluctuating natural uv radiation. *Photochem. Photobiol.* **2009**, *85*, 1336–1345. [CrossRef]

132. White, A.L.; Jahnke, L.S. Contrasting effects of UV-A and UV-B on photosynthesis and photoprotection of beta-carotene in two Dunaliella spp. *Plant Cell Physiol.* **2002**, *43*, 877–884. [CrossRef]

133. Park, S.; Polle, J.E.W.; Melis, A.; Lee, T.K.; Jin, E. Up-regulation of photoprotection and PSII-repair gene expression by irradiance in the unicellular green alga *Dunaliella salina*. *Mar. Biotechnol.* **2006**, *8*, 120–128. [CrossRef] [PubMed]

134. Lamers, P.P.; Van De Laak, C.C.W.; Kaasenbrood, P.S.; Lorier, J.; Janssen, M.; De Vos, R.C.H.; Bino, R.J.; Wijffels, R.H. Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. *Biotechnol. Bioeng.* **2010**, *106*, 638–648. [CrossRef] [PubMed]

135. Ras, M.; Steyer, J.P.; Bernard, O. Temperature effect on microalgae: A crucial factor for outdoor production. *Rev. Environ. Sci. Biotechnol.* **2013**, *12*, 153–164. [CrossRef]

136. Haghjou, M.M.; Shariati, M.; Pozveh, M.H. The effect of low light intensities on oxidative stress induced by short-term chilling in *Dunaliella salina* teod. *Pak. J. Biol. Sci.* **2006**, *9*, 2048–2054. [CrossRef]

137. Alizadeh, G.I.; Aliev, I.I.; Magerramova, K.K.; Galandarli, I.Z.; Dibirova, G.H.; Jalilova, A.R. The response reaction of Dunaliella cells against the influence of Methylene blue and Norflurazon under the low temperature stress conditions. *Eur. J. Biotechnol. Biosci.* **2015**, *3*, 7–10.

138. Lv, H.; Cui, X.; Wahid, F.; Xia, F.; Zhong, C.; Jia, S. Analysis of the physiological and molecular responses of *Dunaliella salina* to macronutrient deprivation. *PLoS ONE* **2016**, *11*, e0152226. [CrossRef] [PubMed]

139. Yilancioglu, K.; Cokol, M.; Pastirmaci, I.; Erman, B.; Cetiner, S. Oxidative Stress Is a Mediator for Increased Lipid Accumulation in a Newly Isolated *Dunaliella salina* Strain. *PLoS ONE* **2014**, *9*, e91957. [CrossRef]

140. Michalak, a Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. *Plant Cell* **2006**, *15*, 523–530.

141. Hazani, A.A.; Ibrahim, M.M.; Shehata, A.I.; El-Gaaly, G.A.; Daoud, M.; Fouad, D.; Rizwana, H.; Moubayed, N. Ecotoxicity of Ag-nanoparticles on two microalgae, Chlorella vulgaris and Dunaliella tertiolecta. *Arch. Biol. Sci.* **2013**, *65*, 1447–1457. [CrossRef]

142. Zamani, N.; Rasekh, F.; Moradshahi, A.; Kholdebarin, B. Physiological responses of Dunaliella tertiolecata to Hg^{2+}-induced oxidative stress. *Iran. J. Sci. Technol.* **2009**, *33*, 65–74.

143. Tsuji, N.; Hirayanagi, N.; Okada, M.; Miyasaka, H.; Hirata, K.; Zenk, M.H.; Miyamoto, K. Enhancement of tolerance to heavy metals and oxidative stress in Dunaliella tertiolecta by Zn-induced phytochelatin synthesis. *Biochem. Biophys. Res. Commun.* **2002**, *293*, 653–659. [CrossRef]

144. Nikookar, K.; Moradshahi, A.; Hosseini, L. Physiological responses of *Dunaliella salina* and Dunaliella tertiolecta to copper toxicity. *Biomol. Eng.* **2005**, *22*, 141–146. [CrossRef] [PubMed]

145. Liang, Y.; Sarkany, N.; Cui, Y. Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol. Lett.* **2009**, *31*, 1043–1049. [CrossRef] [PubMed]

146. Sharma, S.S.; Dietz, K.J. The relationship between metal toxicity and cellular redox imbalance. *Trends Plant Sci.* **2009**, *14*, 43–50. [CrossRef]

147. Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K.; Sutton, D.J. Heavy metal toxicity and the environment. *Mol. Clin. Environ. Toxicol.* **2012**, *101*, 133–164. [CrossRef]

148. Yu, X.; Chen, L.; Zhang, W. Chemicals to enhance microalgal growth and accumulation of high-value bioproducts. *Front. Microbiol.* **2015**, *6*, 1–10. [CrossRef]

149. Qv, X.Y.; Jiang, J.G. Toxicity evaluation of two typical surfactants to Dunaliella bardawil, an environmentally tolerant alga. *Environ. Toxicol. Chem.* **2013**, *32*, 426–433. [CrossRef] [PubMed]

150. Mofeed, J.; Abdel-aal, E.I. Effect of Phenol on Some Antioxidant Enzymes in the Marine Microalga *Dunaliella salina*. *J. Environ. Sci. (JOESE 5)* **2015**, *44*, 185–196.

151. Tappel, A.L. Glutathione peroxidase and hydroperoxides. *Methods Enzymol.* **1978**, *52*, 506–513.

152. Chen, H.; Jiang, J.G. Toxic effects of chemical pesticides (trichlorfon and dimehypo) on *Dunaliella salina*. *Chemosphere* **2011**, *84*, 664–670. [CrossRef]

153. Einali, A.; Valizadeh, J. Propyl gallate promotes salt stress tolerance in green microalga *Dunaliella salina* by reducing free radical oxidants and enhancing β-carotene production. *Acta Physiol. Plant.* **2015**, *37*, 1–11. [CrossRef]

154. Nikookar, K.; Moradshahi, A.; Kharati, M. Influence of Salinity on the Growth, Pigmentation and Ascorbate Peroxidase Activity of Dunaliella Lake in Shiraz. *Iran. J. Sci. Technol.* **2004**, *28*, 117–125.

155. Marcano, L.B.C.; Carruyo, I.M.; Montiel, X.M.; Morales, C.B.; De Soto, P.M. Effect of cadmium on cellular viability in two species of microalgae (Scenedesmus sp. and Dunaliella viridis). *Biol. Trace Elem. Res.* **2009**, *130*, 86–93. [CrossRef]

156. Arun, N.; Vidyalaxmi, V.; Singh, D.P. Chromium (VI) induced oxidative stress in halotolerant alga *Dunaliella salina* and D. tertiolecta isolated from sambhar salt lake of Rajasthan (India). *Cell. Mol. Biol.* **2014**, *60*, 90–96. [CrossRef] [PubMed]

157. Tsiaka, P.; Tsarpali, V.; Ntaikou, I.; Kostopoulou, M.N.; Lyberatos, G.; Dailianis, S. Carbamazepine-mediated pro-oxidant effects on the unicellular marine algal species Dunaliella tertiolecta and the hemocytes of mussel Mytilus galloprovincialis. *Ecotoxicology* **2013**, *22*, 1208–1220. [CrossRef] [PubMed]

158. Novoveská, L.; Ross, M.E.; Stanley, M.S.; Pradelles, R.; Wasiolek, V.; Sassi, J.F. Microalgal carotenoids: A review of production, current markets, regulations, and future direction. *Mar. Drugs* **2019**, *17*, 640. [CrossRef] [PubMed]

159. Sierra, E.; Acién, F.G.; Fernández, J.M.; García, J.L.; González, C.; Molina, E. Characterization of a flat plate photobioreactor for the production of microalgae. *Chem. Eng. J.* **2008**, *138*, 136–147. [CrossRef]

160. García-González, M.; Moreno, J.; Manzano, J.C.; Florencio, F.J.; Guerrero, M.G. Production of *Dunaliella salina* biomass rich in 9-cis-??-carotene and lutein in a closed tubular photobioreactor. *J. Biotechnol.* **2005**, *115*, 81–90. [CrossRef]

161. Zhu, Y.H.; Jiang, J.G. Continuous cultivation of *Dunaliella salina* in photobioreactor for the production of β-carotene. *Eur. Food Res. Technol.* **2008**, *227*, 953–959. [CrossRef]

162. Kleinegris, D.M.M.; Janssen, M.; Brandenburg, W.A.; Wijffels, R.H. Continuous production of carotenoids from *Dunaliella salina*. *Enzym. Microb. Technol.* **2011**, *48*, 253–259. [CrossRef]

163. Ndukwe, G.I.; Mustapha, K.B.; Ayo, R.I. Constraints to large scale algae biomass production and utilization. *J. Algal Biomass Util.* **2012**, *3*, 14–32.

164. Kim, J.; Yoo, G.; Lee, H.; Lim, J.; Kim, K.; Kim, C.W.; Park, M.S.; Yang, J.W. Methods of downstream processing for the production of biodiesel from microalgae. *Biotechnol. Adv.* **2013**, *31*, 862–876. [CrossRef] [PubMed]

165. Milledge, J.J.; Heaven, S. A review of the harvesting of micro-algae for biofuel production. *Rev. Environ. Sci. Biotechnol.* **2013**, *12*, 165–178. [CrossRef]

166. Sharma, K.K.; Garg, S.; Li, Y.; Malekizadeh, A.; Schenk, P.M. Critical analysis of current Microalgae dewatering techniques. *Biofuels* **2013**, *4*, 397–407. [CrossRef]

167. Japar, A.S.; Takriff, M.S.; Yasin, N.H.M. Harvesting microalgal biomass and lipid extraction for potential biofuel production: A review. *J. Environ. Chem. Eng.* **2017**, *5*, 555–563. [CrossRef]

168. Roy, M.; Mohanty, K. A comprehensive review on microalgal harvesting strategies: Current status and future prospects. *Algal Res.* **2019**, *44*, 101683. [CrossRef]

169. Najjar, Y.S.; Abu-Shamleh, A. Harvesting of microalgae by centrifugation for biodiesel production: A review. *Algal Res.* **2020**, *51*, 102046. [CrossRef]

170. Gao, S.; Yan, R.; Chen, F. Effects of Different Storage Temperatures and Times on Germination and Antioxidant Responses of *Jatropha curcas* L. Seeds. *J. Agric. Sci. Technol.* **2015**, *17*, 1619–1628.

171. Ahmed, F.; Li, Y.; Fanning, K.; Netzel, M.; Schenk, P.M. Effect of drying, storage temperature and air exposure on astaxanthin stability from Haematococcus pluvialis. *Food Res. Int.* **2015**, *74*, 231–236. [CrossRef]

172. Trung, T.S.; Huyen, N.T.K.; Minh, N.C.; Le Trang, T.T.; Han, N.T. Optimization of harvesting of microalgal Thalassiosira pseudonana biomass using chitosan prepared from shrimp shell waste. *Asian J. Agric. Res.* **2016**, *10*, 162–174. [CrossRef]

173. Oliveira, L.S.; Rufino, M.S.; Moura, C.F.; Cavalcanti, F.R.; Alves, R.E.; Miranda, M.R. The influence of processing and long-term storage on the antioxidant metabolism of acerola (*Malpighia emarginata*) purée. *Rev. Bras. Frutic.* **2011**, *30*, 398–408. [CrossRef]

174. Mediani, A.; Abas, F.; Tan, C.; Khatib, A. Effects of Different Drying Methods and Storage Time on Free Radical Scavenging Activity and Total Phenolic Content of Cosmos Caudatus. *Antioxidants* **2014**, *3*, 358–370. [CrossRef]

175. Roy, U.K.; Nielsen, B.V.; Milledge, J.J. Effect of post-harvest conditions on antioxidant enzyme activity in Dunaliella tertiolecta biomass. *Biocatal. Agric. Biotechnol.* **2020**, *27*, 101661. [CrossRef]

176. Xu, Y.; Ibrahim, I.M.; Wosu, C.I.; Ben-Amotz, A.; Harvey, P.J. Potential of new isolates of *Dunaliella salina* for natural β-carotene production. *Biology* **2018**, *7*, 14. [CrossRef] [PubMed]

177. Kumar, R.R.; Rao, P.H.; Subramanian, V.V.; Sivasubramanian, V. Enzymatic and non-enzymatic antioxidant potentials of Chlorella vulgaris grown in effluent of a confectionery industry. *J. Food Sci. Technol.* **2014**, *51*, 322–328. [CrossRef] [PubMed]

178. Miękus, N.; Iqbal, A.; Marszałek, K.; Puchalski, C.; Świergiel, A. Green chemistry extractions of carotenoids from daucus carota L.-Supercritical carbon dioxide and enzyme-assisted methods. *Molecules* **2019**, *24*, 4339. [CrossRef] [PubMed]

179. Cavka, A.; Jornsson, L.J. Comparison of the growth of filamentous fungi and yeasts in lignocellulose-derived media. *Biocatal. Agric. Biotechnol.* **2014**, *3*, 197–204. [CrossRef]

180. Gomez, J.A.; Höffner, K.; Barton, P.I. From sugars to biodiesel using microalgae and yeast. *Green Chem.* **2016**, *18*, 461–475. [CrossRef]

181. Munir, N.; Sharif, N.; Naz, S.; Farkhanda, M. Algae: A potent antioxidant source. *Sky J. Microbiol. Res.* **2013**, *1*, 22–31.