ISSN: 2231-3656

International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol. 16 | Issue 3 | Jul – Sep 2026

www.ijpir.com

DOI: https://doi.org/10.61096/ijpir.v16.iss3.2026.953-985

Spirulina + Chlorella vulgaris as Complementary Nutraceutical Microalgae for Antioxidant, Anti-Inflammatory and Metabolic Support

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Published on: 27.07.2026
Published by: Futuristic Publications

Abstract: Chronic non-communicable diseases (NCDs) are multifactorial biological processes that develop due to oxidative stress, low-grade inflammation, and metabolic disorders. The bioactive compounds of edible microalgae are considered to have an impact on all three processes. Spirulina (Limnospira platensis) is a filamentous cyanobacterium that is used as a food additive and pharmaceutical raw material. Chlorella vulgaris is a single-celled green algae species that differs from Spirulina in its cellular structure and biochemical composition. The two microalgae are a rich source of protein, carotenoids, polysaccharides, vitamins, minerals, and polyunsaturated fatty acids. They also contain significant amounts of unique compounds: C-phycocyanin, phycocyanobilin, and γ-linolenic acid in *spirulina* and chlorophyll a and b, lutein, β-carotene, and α-linolenic acid in *chlorella*. The content of many nutrients depends on the habitat and technology of cultivation, which may explain differences in the literature data. In vitro and animal experiments have demonstrated the ability of spirulina and chlorella to neutralise reactive oxygen species by donating electrons and hydrogen atoms, reduce pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and the NF-κB transcription factor, increase glucose uptake and insulin sensitivity, and lower cholesterol levels, especially LDL and triglycerides. Studies of the combined use of chlorella and spirulina have revealed a synergistic effect of the two microalgae on the reduction of oxidative stress and inflammation. However, there is no specific clinical evidence for this synergy. The critical evaluation of the available data on the effects of spirulina and chlorella presented in this article shows that these microalgae can be used as a dietary supplement to improve health status and quality of life. However, it cannot be recommended to use them as a method of treating chronic NCDs since the existing evidence is insufficient in terms of the sample size and experimental design.

Keywords: Spirulina; *Chlorella vulgaris*; phycocyanin; chlorophyll; antioxidant; inflammation; cytokines; glycaemic control; insulin sensitivity; omega fatty acids; cellular health; nutraceutical.

1. INTRODUCTION

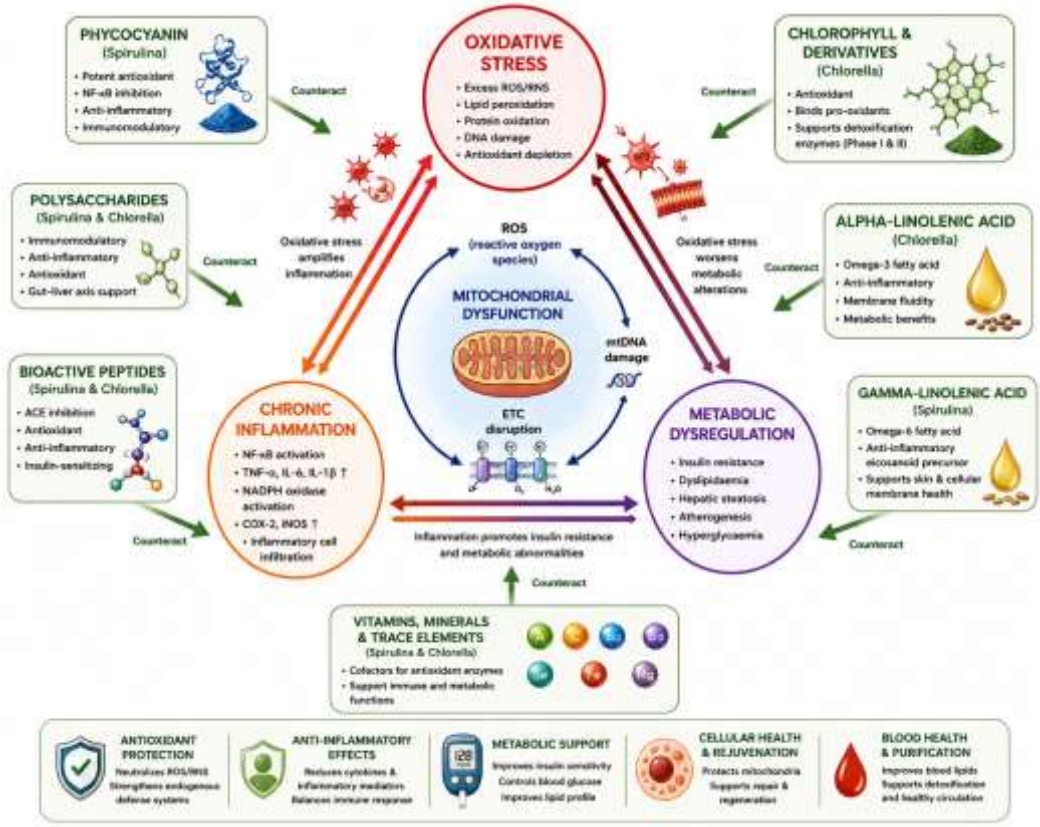

Figure F-1. Conceptual overview of the three converging pathophysiological pillars underlying chronic non-communicable disease. The bidirectional arrows depict reciprocal amplification between oxidative stress, chronic inflammation and metabolic dysregulation; the central mitochondrial-dysfunction loop sustains a self-amplifying cycle of reactive oxygen species production and macromolecular damage [1-4].

1.1 Oxidative stress and chronic disease

Reactive oxygen and nitrogen species are naturally produced in mitochondria during the respiratory chain process and by NADPH oxidase enzymes. At a low level, these species are signalling molecules required for the regulation of physiological processes such as proliferation, differentiation, and cell death [5]. When the production of these species exceeds the neutralising capacity of the cell's antioxidant defence system, oxidative distress occurs. During oxidative distress, reactive oxygen and nitrogen species destroy cellular structures by oxidising polyunsaturated lipids in plasma membranes, causing mitochondrial DNA and respiratory chain protein mutations, altering the function and structure of proteins, and damaging nucleic acids [5-6]. It is the state of oxidative distress that is linked to the aetiology of cardiovascular diseases, diabetes, neurodegenerative and renal diseases, and ageing [5-7]. Additionally, if the production of reactive species is not controlled, it can cause a vicious cycle in the respiratory chain. Since the respiratory chain in mitochondria is responsible for generating most of the body's reactive oxygen species, impairments in its structure and function bring about further production of these species. This vicious cycle is evident in the production of reactive oxygen species, which damages mitochondrial DNA, and the damaged DNA causes further impairments in the respiratory chain [8]. The current understanding of the role of reactive oxygen and nitrogen species in the pathophysiology of chronic degenerative diseases has led to research on the use of antioxidants to suppress the production of these species in the body [6].

1.2 Relationship between oxidative stress and inflammation

Oxidative stress and inflammation are two interconnected processes that aggravate each other. Reactive oxygen species (ROS) can enhance the generation of pro-inflammatory cytokines through the activation of redox-sensitive transcription factors such as NF-κB, the mitogen-activated protein kinase (MAPK) cascade, and Nrf2. Then, these factors initiate the production of TNF-α, IL-6, and IL-1β that in turn lead to neutrophil and macrophage infiltration into the damaged tissue with ROS release [5-6]. This close

connection between ROS and inflammatory signals is the basis of the chronic low-grade inflammatory state associated with metabolic syndrome, atherosclerosis, insulin resistance, and other disorders [7]. Consequently, the reduction of oxidative stress via diminishing ROS production and appropriate regulation of pro-inflammatory cytokines may be an efficient way to prevent chronic disease development [6].

1.3 Nutraceutical microalgae

Microalgae, unlike plants, are single-celled photosynthetic organisms that can accumulate high levels of proteins, pigments, carotenoids, polysaccharides, vitamins, minerals, polyunsaturated fatty acids, and other beneficial substances [5-6]. Spirulina and Chlorella are examples of such microalgae. They are considered functional foods, meaning that they are products from natural sources that may have positive effects on health and contribute to the body's homeostasis [7]. Industrial cultivation allows obtaining microalgae as an ingredient free from the use of arable land and water. It also ensures good qualitative consistency for nutraceutical applications. Thus, this type of food is one of the best sources of food for supplementing a person's diet with antioxidant and anti-inflammatory properties [5, 8].

1.4 Botanical and taxonomic identity

Spirulina (from the Greek spiral) is a filamentous *cyanobacterium* formerly classified in the genus *Arthrospira*; *Arthrospira platensis* is the species most commonly used for human consumption. This microorganism has recently been reclassified as *Limnospira platensis* [5]. *Spirulina* is a Gram-negative prokaryotic organism that performs oxygenic photosynthesis. *Chlorella vulgaris* is a single-celled green alga belonging to the family *Chlorellaceae* and is classified as a eukaryote. It has a thick cell wall made up of cellulose, and its cells contain chloroplasts, which have chlorophyll a and chlorophyll b. [6]. Thus, the two microorganisms have different cellular structures and belong to different classifications in taxonomy. While *Spirulina* does not have a nucleus and other membrane structures, *Chlorella vulgaris* has all the organelles characteristic of eukaryotic cells. The difference in cellular structure and the presence of various membrane-bound organelles can affect the content of biologically active substances in these microorganisms. In addition, the possibility of utilising these products in the human body through the breakdown of cell walls plays a particular role in the digestion and absorption of the obtained substances [6-7].

1.5 Nutritional composition of Spirulina

Spirulina biomass is characterised by a high and balanced content of protein, which accounts for 55–70% of the dry matter on average [5]. This product has a distinctive colour because of the phycobiliproteins, such as C-phycocyanin and allophycocyanin, which are rich in the linear tetrapyrrole chromophore phycocyanobilin, and therefore possess antioxidant properties [6-7]. For instance, phycocyanobilin scavenges intracellular oxygen free radicals by donating an electron or a hydrogen atom to them directly [6]. The antioxidant efficiency of C-phycocyanin was proved in vitro experiments, which demonstrated its ability to remove 89% of DPPH radicals, 11% of superoxide radicals, and reduce more than 85% of the substrate depending on the concentration [6]. Besides, Spirulina contains carotenoids, such as β-carotene, sulfated polysaccharides possessing immunomodulatory properties, vitamins of the B group, mainly B12, iron, and gamma-linolenic acid (GLA; 18:3n-6), among others. High levels of GLA have been especially highlighted by Ötleş and Pire [8], and later they were confirmed in several studies, showing its content in S. platensis biomass to be as high as 1.9 g per 100 g [9]. Thus, the diverse content of Spirulina, including its unique phyco-biliproteins, creates the basis for its antioxidant and anti-inflammatory effects [5, 10].

1.6 Nutritional composition of Chlorella vulgaris

Chlorella vulgaris accumulates large amounts of chlorophyll (*a* and *b*), the carotenoids lutein and β-carotene, proteins and bioactive peptides, intracellular and extracellular polysaccharides, B vitamins, vitamin C, and minerals such as phosphorus, magnesium and iron [5]. Its lipid fraction is dominated by polyunsaturated fatty acids, with α-linolenic acid (ALA, 18:3n-3) identified as the principal ω-3 component in many strains, at reported concentrations of approximately 2.8 g/100 g in *C. vulgaris* [6]. Importantly, the absolute and relative abundance of fatty acids is strain-dependent: a recent comparison of four edible *C. vulgaris* strains demonstrated marked variability in EPA, DHA and PUFA/SFA ratios, with all extracts nevertheless retaining antioxidant and anti-inflammatory activity [7]. The peptide profile is also bioactive — short peptides released during gastrointestinal digestion have been shown experimentally to modulate inflammatory signalling independent of intact protein [5, 8].

Table T-1: Nutritional composition comparison of Spirulina vs Chlorella vulgaris: Comparative nutritional composition of Spirulina platensis and Chlorella vulgaris on a dry-weight basis. The two microalgae exhibit complementary deficiencies: each is rich in pigments and fatty acids absent from the other [5-6, 11-14].

Component	*Spirulina platensis*	*Chlorella vulgaris*	Comment
Protein (% dw)	55–70	50–60	Both complete amino-acid profiles
C-Phycocyanin (% dw)	~10–20	absent	Defines *Spirulina*'s antioxidant pigment
Chlorophyll *a*+*b* (% dw)	trace	1–3	Defines *Chlorella*'s green pigment system
Lutein (mg/100 g)	low–moderate	high	*Chlorella* main carotenoid
β-Carotene (mg/g)	up to ~4	comparable	Singlet-oxygen quenching
γ-Linolenic acid (GLA, 18:3n-6)	~1.9 [9]	negligible	*Spirulina* ω-6 signature
α-Linolenic acid (ALA, 18:3n-3)	low	~2.8	*Chlorella* ω-3 signature
Iron (mg/100 g)	up to 100 (enriched)	moderate	Bioavailable Fe
Vitamin B12 (analogues)	present	low–absent	*Spirulina* unique feature

1.7 Rationale for combining Spirulina and Chlorella

Many researchers warn that one should be careful when choosing *spirulina* and *chlorella* dietary supplements because the amount of lipids and nutrients they contain may vary considerably in different producers and even in the same producer, during different manufacturing processes [4]. However, there is scientific information about the key components of these cyanobacteria that are frequently used in nutraceuticals. It is reported that spirulina is an excellent source of gamma-linolenic acid, phycocyanin, and phycocyanobilin, while chlorella vulgaris contains high concentrations of chlorophylls, lutein, and alpha-linolenic acid [8-9,15]. A composition of *spirulina* and *chlorella* can provide a consumer with a variety of health-promoting molecules: a soluble antioxidant system would be constructed from tetrapyrrols (phycocyanobilin) while a green pigment-based system would be built from chlorophylls [1,6]; anti-inflammatory compounds such as phycobiliproteins and polysaccharides would block the transcription factor NF-κB [10-11,16]; the daily value of omega-3 (mainly ALA) and omega-6 (primarily GLA) polyunsaturated fatty acids would meet the requirements of the human body [8,15,17]; and a combination of vitamins, minerals, and peptides would complement the nutritional composition of the product [1,5]. From the perspective of pharmacology, the mentioned synergistic effects of combined spirulina and chlorella dietary supplements can be useful; thus, it is rational to use this type of nutraceutical rather than separate algae preparations [5,10].

1.8 Aim of the review

This review aimed to evaluate the antioxidant and free-radical scavenging effects, anti-inflammatory properties and effects on cytokine levels, effects on glucose metabolism and insulin sensitivity, omega-balanced fatty acids, and claims about cellular rejuvenation and detoxification of Spirulina and Chlorella vulgaris. The rationale, benefits, and challenges of developing a combined nutraceutical formulation using the two microalgae were discussed, and the differences between individual products were noted, as were the benefits of standardised protocols [9-10].

2. ANTIOXIDANT NATURE AND FREE-RADICAL SCAVENGING

2.1 Reactive oxygen species and cellular damage

ROS and RNS are continuously generated through incomplete reduction of molecular oxygen during mitochondrial respiration, through enzymatic systems such as the NADPH oxidases, xanthine oxidase, uncoupled nitric oxide synthase and cytochrome P450 monooxygenases, and as by-products of peroxisomal β-oxidation [1-2]. Under physiological conditions, low, transient fluxes of superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) act as redox-signalling molecules regulating proliferation, differentiation and apoptosis ("oxidative eustress") [1].

When production exceeds the buffering capacity of cellular antioxidant defences — a state termed "oxidative distress" — biomolecular damage ensues [1-3]. Lipid peroxidation attacks polyunsaturated fatty acids in membrane bilayers, generating reactive aldehydes such as malondialdehyde and 4-hydroxynonenal (4-HNE), the latter a diffusible second messenger that amplifies mitochondrial dysfunction by inhibiting complex I and complex IV and uncoupling oxidative phosphorylation [4]. Protein oxidation produces carbonyl derivatives that misfold or aggregate, while oxidative DNA damage produces 8-oxo-2'-deoxyguanosine and strand breaks that, if unrepaired, drive mutagenesis and mitochondrial DNA depletion [1,4]. These injuries are documented pillars of cardiovascular disease, type 2 diabetes mellitus, neurodegeneration, chronic kidney disease and biological ageing [1-3].

2.2 Antioxidant constituents of Spirulina

Spirulina contains a uniquely diverse antioxidant armamentarium:

- **C-phycocyanin:** A blue phycobiliprotein composing up to ~20% of *Spirulina* biomass, with antioxidant efficacy directly attributable to its covalently bound linear tetrapyrrole chromophore, phycocyanobilin [15-16]. C-PC has been reported to exhibit up to 89% DPPH-radical scavenging activity, ~11% superoxide-scavenging activity and reducing-power efficiencies exceeding 85% *in vitro*, depending on concentration [15].
- **Phycocyanobilin:** A biliverdin-like bilin that circulates after oral C-PC intake and is itself a potent superoxide scavenger, with capacity estimated to be up to 16× that of equimolar ascorbate; PCB additionally can undergo further conversion to phycocyanorubin, an analogue of bilirubin with documented atheroprotective effects [16].
- **β-Carotene:** *Spirulina* biomass contains β-carotene at levels reported up to ~4 mg/g dry weight, contributing to singlet-oxygen quenching and lipid-radical chain-breaking [11,17].
- **Tocopherols:** α-Tocopherol is present at concentrations supporting membrane-radical chain-breaking [11].
- **Phenolic compounds:** Phenolic acids and total phenolics contribute to metal-chelating and free-radical-scavenging activity in *S. platensis* extracts [11,17].
- **Bioactive peptides:** Hydrolysates of *Spirulina* protein release short peptides that retain antioxidant capacity in DPPH and ABTS assays, with greater potency on a per-mass basis than intact protein [11,18].

C-PC safety has been clinically validated: a randomised, double-blind, placebo-controlled study of a C-PC-enriched aqueous extract equivalent to ~1 g/day phycocyanin for 2 weeks showed no anticoagulant activation or platelet dysfunction, with concomitant reductions in AST and ALT [19].

2.3 Antioxidant constituents of Chlorella vulgaris

Chlorella vulgaris complements this profile with a distinct green-spectrum antioxidant system:

- **Chlorophylls *a* and *b*:** Cyclic tetrapyrroles that quench excited-state species and act as electron donors; chlorophyll derivatives retain antioxidant capacity after gastrointestinal digestion [5].
- **Lutein:** A xanthophyll carotenoid concentrated in *C. vulgaris*; lutein is a particularly strong singlet-oxygen quencher and protects PUFAs in photoreceptor outer-segment membranes [5]..
- **β-Carotene:** Reported at levels comparable to those in carrots; chain-breaking antioxidant [5].

- **Phenolic compounds:** Total phenolic content has been correlated with DPPH/ABTS scavenging capacity in *C. vulgaris* extracts, with strain-dependent variation [5,7].
- **Polysaccharides:** Sulphated and non-sulphated polysaccharides of *C. vulgaris* have demonstrable hydroxyl- and superoxide-scavenging activity, and additionally chelate transition metals via uronic acid residues [5,8].
- **Peptides and micronutrients:** Bioactive peptides released during gastrointestinal digestion enhance cellular antioxidant defences [5]; vitamin C and selenium carried by the biomass contribute as cofactors for endogenous antioxidant enzymes [5].

Strain-to-strain variability means absolute antioxidant capacities differ among commercial products, but extracts from all four edible *C. vulgaris* strains tested in one comparative study retained measurable DPPH/ABTS scavenging activity [7].

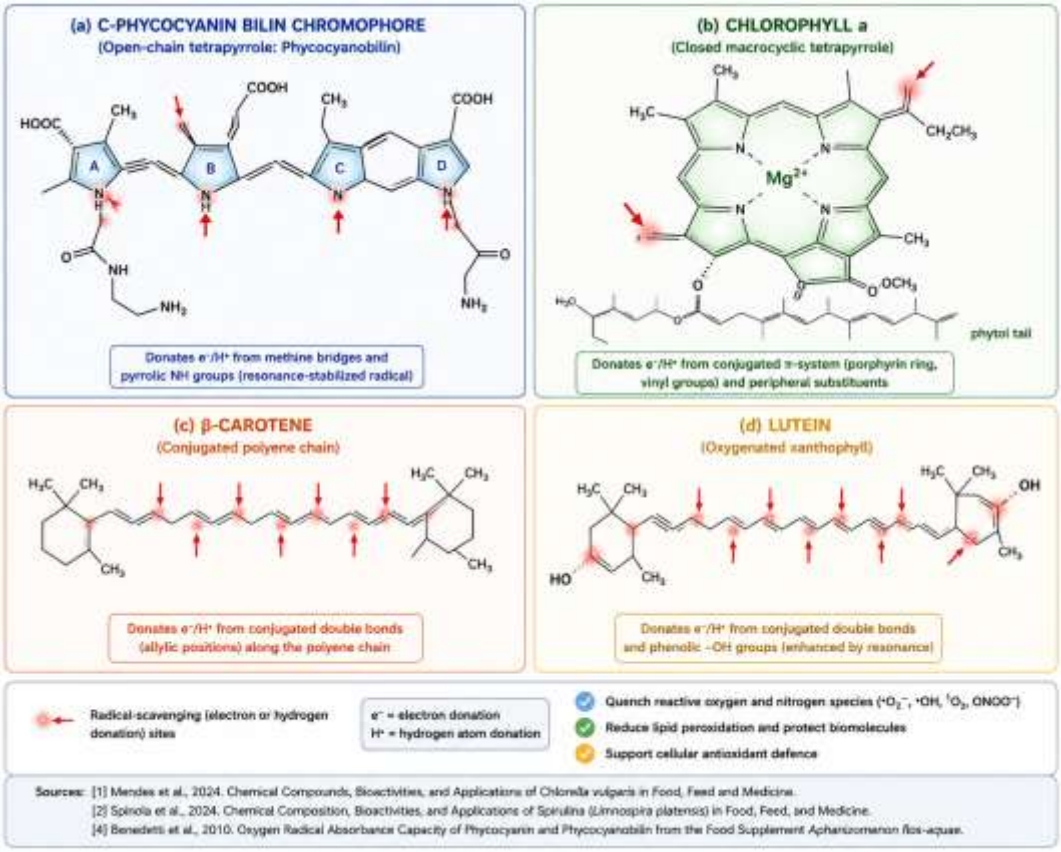

Fig F-2: Structural comparison of the principal pigment antioxidants present in Spirulina platensis and Chlorella vulgaris. (a) Phycocyanobilin is the open-chain linear tetrapyrrole chromophore of C-phycocyanin, donating electrons/hydrogen atoms to neutralise reactive oxygen species. (b) Chlorophyll a is a closed macrocyclic tetrapyrrole with a centrally chelated Mg^{2+} ion and a hydrophobic phytol tail, anchoring it in lipid bilayers. (c) β-Carotene and (d) lutein are long-conjugated-chain carotenoids that quench singlet oxygen and break lipid-radical chain reactions [5, 11, 15-16].

2.4 Direct free-radical scavenging

The most studied mechanism of both microalgae is the direct one-electron- or one-hydrogen-atom-mediated neutralisation of ROS. Dortch, working with well-characterised *S. platensis* preparations, demonstrated that doses of commercial *Spirulina* powder produce a concentration-dependent inactivation of superoxide radicals generated enzymatically by xanthine oxidase, with simultaneous reduction of the metabolic activity of polymorph nuclear neutrophils — an effect relevant to the dampening of respiratory-burst-induced tissue damage [20]. *C. vulgaris* extracts likewise directly neutralise •OH, O_2•$^-$ and DPPH radicals, with the most active fractions typically enriched in carotenoids, phenolics and chlorophyll derivatives [5]. Among the phycobiliproteins, PCB is the principal scavenger of peroxyl radicals in model systems [16], and its supplementation *in vivo* correlates with measurable increases in plasma total

antioxidant capacity — a 42% increase was observed following Se-enriched phycocyanin supplementation in an atherogenic-diet hamster model [21]. The nutraceutical framing of C-PC and PCB in oxidative-stress and inflammation-related pathology is comprehensively reviewed in [22].

2.5 Endogenous antioxidant enzymes

Beyond direct scavenging, *Spirulina* and *Chlorella* preparations have been consistently shown to reinforce the enzymatic phase II antioxidant defence system. Se-fed *S. platensis* supplementation produced a sparing effect on hepatic glutathione peroxidase (~87%) and superoxide dismutase (~56%) activity in hyperlipidaemic hamsters [21]. In a strength-training rat model, *Spirulina* supplementation prevented the training-induced decline in catalase, glutathione peroxidase and glutathione reductase activities, with the 100 mg/kg dose most consistently restoring hepatic and muscle antioxidant enzymes during recovery from physical oxidative stress [23]. *C. vulgaris* similarly upregulates glutathione peroxidase, catalase and SOD in experimental models; the relevant effect is largely mediated by PCB-driven and chlorophyll-driven signalling rather than direct scavenging (see §2.6) [5,8].

Reduced glutathione, the predominant intracellular thiol, is also replenished. In an *in vivo* model of myocardial ischaemia–reperfusion injury, phycocyanin pretreatment restored myocardial GSH concentrations to values significantly above the injury group, alongside parallel increases in SOD, CAT and total antioxidant capacity [24].

2.6 Nrf2-mediated antioxidant defence

A growing body of evidence positions the phycobiliproteins of *Spirulina* — particularly C-PC and its derived peptides — as inducers of the nuclear factor erythroid 2-related factor 2/antioxidant-response element (Nrf2/ARE) pathway. In HepG2 human hepatocytes, a phytocomplex of *Spirulina* induced the expression of ARE/Nrf2-regulated genes while simultaneously providing direct ROS-scavenging capacity, an effect attributable to the protein and pigment fraction of the biomass [25]. Phycocyanin pretreatment dose-dependently upregulated Nrf2 and its downstream cytoprotective targets heme oxygenase-1 and NAD(P)H dehydrogenase quinone 1 in the myocardium of rats subjected to ischaemia–reperfusion injury, and this upregulation was concurrent with NF-κB downregulation [24]. Three novel antioxidant peptides hydrolytically derived from C-PC directly activated the Nrf2 signalling pathway in a zebrafish H_2O_2-induced oxidative-stress model, demonstrating that smaller fragments of C-PC retain and even amplify the parent protein's Nrf2-inducing action [18]. The bilirubin-like activity of PCB is independently corroborated: PCB from *Spirulina* activates atheroprotective HO-1 in endothelial cells, recapitulating the established HMOX-1-inducing effect of its close relative bilirubin [26].

2.7 Inhibition of lipid peroxidation

Because polyunsaturated fatty acids are the principal lipidic substrate of ROS-driven damage, the lipid-peroxidation biomarkers MDA, 4-HNE and F_2-isoprostanes serve as the most clinically relevant indicators of antioxidant efficacy. In the hamsters described in §2.5, supplementation with selenium-enriched *S. platensis* significantly reduced the atherogenic-diet-induced elevation of plasma MDA, paralleled by a 76% reduction in cardiac superoxide production [21]. In rats subjected to intense strength training, *Spirulina* at 50–150 mg/kg prevented the exercise-induced rise in hepatic and muscle MDA [23]. In T2DM patients consuming 20 g/day of *spirulina* sauce (2 g pure spirulina), 2 months of intervention significantly reduced MDA, although no change in serum total antioxidant capacity was detected, suggesting that AT-capacity metrics may lag behind lipid-peroxidation metrics in short-term human studies [27]. For *Chlorella*, although direct mechanistic data on MDA in clinical samples are more limited, animal and *in vitro* evidence converges on the conclusion that chlorophyll- and polysaccharide-rich fractions reduce MDA and raise membrane-stability indices [5].

2.8 Evidence from animal and cell studies

A coherent body of pre-clinical evidence demonstrates that *Spirulina* and *C. vulgaris* counteract oxidative stress through a triad of mechanisms: direct scavenging by pigments and phenolics (§2.4), reinforcement of endogenous antioxidant enzymes (§2.5), and induction of the Nrf2 transcriptional programme (§2.6). The phycobiliproteins of *Spirulina* have been shown to reduce the cardiac expression of the p22phox subunit of NADPH oxidase (by 34% in the SePC group), thereby reducing enzymatic superoxide generation at its source [21]; they are further demonstrated to inhibit LDL oxidation *ex vivo*, a fundamental pro-atherogenic mechanism [28]. *Spirulina* extracts also inhibit H_2O_2-induced DNA damage in cultured lymphocytes and protect rat erythrocytes against t-butyl hydroperoxide-induced lysis — classic *in vitro* models of oxidative injury [23,28].

C. vulgaris extracts reduce intracellular ROS in various cell models, with the effect varying by strain and extraction solvent. Polysaccharide-rich fractions attenuated LPS-induced ROS in macrophages [29]. Peptide hydrolysates of *C. vulgaris* elevate intracellular GSH and decrease ROS in Caco-2 intestinal epithelial cells, consistent with their proposed gastrointestinal bioactivity [5].

2.9 Evidence from experimental and clinical studies

Human trials consistently report reductions in lipid-peroxidation biomarkers but not in all antioxidant metrics:

- **Rezaiyan et al.:** A randomised, double-blind, placebo-controlled trial of 20 g/day *spirulina* sauce (containing 2 g spirulina) in 40 T2DM patients over 2 months observed a significant reduction in MDA but no significant change in total antioxidant capacity — corroborating the dissociation between lipid-peroxidation markers and serum TAC in short interventions [27].
- **Riss et al.:** Se-enriched phycocyanin supplementation in hyperlipidaemic hamsters raised plasma antioxidant capacity by 42% — the human extrapolation of which has been observed in supplementation studies where plasma FRAP rises but TAC can lag [21].
- **Dartsch:** *S. platensis* preparations produced dose-dependent decreases in superoxide generation and neutrophil activation *in vitro*, supporting a plausibility framework for clinical anti-inflammatory effects [20].
- **Brito et al.:** A *Spirulina* dose-response study in rats demonstrated that even at the lowest dose tested (50 mg/kg), the antioxidant enzyme profile was partially restored, indicating a wide effective concentration window [23].

A meta-analysis-level review of 65 articles concluded that *S. platensis* supplementation reduces MDA levels in T2DM patients, alongside favourable effects on FBS, PPBS and HbA1c [30].

2.10 Limitations of antioxidant evidence

Available antioxidant evidence is constrained by several methodological realities:

1. Variable doses and formulations. Effective doses of Spirulina in pre-clinical studies (50–150 mg/kg) translate to widely varying human-equivalent doses (3–9 g/day), while commercial products range from 1–10 g/day. C. vulgaris supplementation has been studied at 1,200 mg/day in NAFLD trials [31] and 3–5% w/w in rodent studies [32].
2. High compositional variability of commercial biomass. Surveys of 17 commercially available *Chlorella* and *Spirulina* biomass samples found substantial inter-batch variability in nutrient and contaminant burden, with several products failing to meet declared specification [9].
3. Short intervention durations. Most clinical trials last 4–16 weeks, which may explain why serum TAC lags behind the earlier-responding MDA endpoint [27].
4. Heterogeneous populations. Healthy volunteers, T2DM patients, NAFLD patients and trained men respond differently; the antioxidant benefits observed in athlete-stressed populations may not generalise [23].
5. Outcome-measure heterogeneity. DPPH/ABTS, FRAP, ORAC, TAC and MDA capture different facets of oxidative status and are not freely interchangeable [1].
6. Limited data on hard clinical endpoints. No trial has yet demonstrated that antioxidant supplementation with *Spirulina*/*Chlorella* reduces hard cardiovascular or mortality endpoints; available data are restricted to surrogate biomarkers.

3. ANTI-INFLAMMATORY EFFECTS AND CYTOKINE REGULATION

3.1 Inflammation and chronic metabolic dysfunction

Chronic metabolic disorders are now understood to share persistent low-grade inflammation — characterised by sustained elevation of circulating cytokines, acute-phase proteins and immune-cell infiltration of metabolic tissues — as a key pathogenic pillar [2-3]. In obesity, hypertrophied adipocytes secrete monocyte chemoattractant protein-1, TNF-α and IL-6, recruiting M1-polarised macrophages to adipose depots [2]. In hepatic steatosis, Kupffer-cell activation and lipid-induced hepatocyte damage sustain the production of IL-1β and TNF-α, contributing to non-alcoholic steatohepatitis [17]. Sustained cytokine

signalling directly impairs insulin-receptor-substrate serine phosphorylation, induces endothelial dysfunction and accelerates atherogenesis [2-3].

3.2 NF-κB signalling

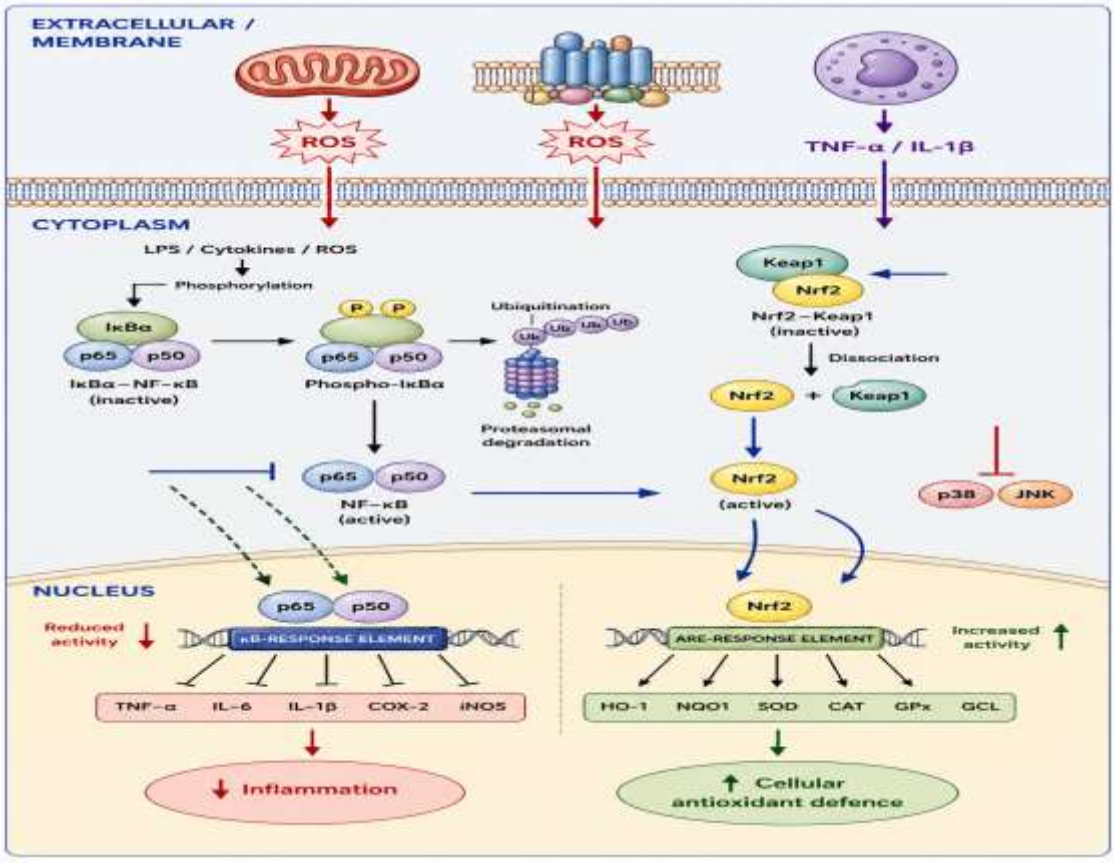

Fig F-3: Complementary molecular mechanisms of antioxidant and anti-inflammatory action. C-phycocyanin and C-PC-derived peptides (from *Spirulina*) activate the Nrf2-ARE programme, inducing phase II cytoprotective enzymes while simultaneously suppressing NF-κB and p38/JNK signalling. *Chlorella vulgaris* exopolysaccharides independently suppress NF-κB target genes and re-programme macrophage polarisation toward a resolution phenotype. Mitochondrial ROS production and NADPH-oxidase expression are reduced [8,17-23].

The NF-κB transcription factor is the master regulator of pro-inflammatory gene expression. Under basal conditions, NF-κB is retained in the cytoplasm by IκB; canonical pathway activation by cytokines, ROS or LPS triggers IκB phosphorylation, ubiquitination and proteasomal degradation, freeing NF-κB to translocate to the nucleus and bind κB-responsive promoters [2]. *Spirulina* extracts (especially C-PC) [23] and *C. vulgaris* polysaccharides [29] suppress this pathway. In a rat myocardial ischaemia–reperfusion model, phycocyanin pretreatment significantly reduced NF-κB protein expression in parallel with Nrf2 upregulation [24]. In a 12-week strength-training rat model, *Spirulina* supplementation reduced NF-κB activation in skeletal muscle and liver dose-dependently [23]. In RAW264.7 macrophages, *Chlorella*-derived exopolysaccharides altered the global transcriptome in the LPS-stimulated state, downregulating NF-κB target genes and upregulating anti-inflammatory mediators [29].

3.3 MAPK signalling

The mitogen-activated protein kinase cascades — p38, ERK1/2 and JNK — integrate oxidative and inflammatory stress signals. p38 and JNK phosphorylate downstream transcription factors that amplify cytokine production; ERK opposes apoptosis and processes repair signals [2]. In experimental work

summarised by Deng and Chow, *S. platensis* extracts suppressed LPS-induced p38 and JNK phosphorylation in macrophages, accompanied by reduced iNOS and COX-2 expression [28]. Although specific human data on MAPK modulation by *Spirulina*/*Chlorella* are limited, the convergent reduction in NF-κB and MAPK targets at the protein-expression level provides a mechanistic basis for the observed cytokine reductions described in §3.4 [23,28-29].

3.4 Pro-inflammatory cytokines

The principal cytokines clinically monitored in chronic low-grade inflammation are TNF-α, IL-6, IL-1β, IL-8 and C-reactive protein (CRP, an IL-6-induced hepatic acute-phase reactant):

- **TNF-α:** *Spirulina* at 50–150 mg/kg significantly reduced TNF-α expression in rat muscle and liver after exhaustive exercise [23]; *Chlorella* polysaccharides dose-dependently suppressed TNF-α secretion in LPS-stimulated macrophages [29].
- **IL-6:** Both microalgae have been reported to reduce IL-6 production in LPS-stimulated macrophages [17,29]; in clinical NAFLD trials, C. vulgaris supplementation reduced serum IL-6 — with one trial of 1,200 mg chlorella/day in NAFLD patients demonstrating significant reductions in hs-CRP and TNF-α alongside metabolic improvements [31].
- **IL-1β:** *Spirulina* and its C-PC reduced IL-1β expression in trained rats [23] and in LPS-stimulated macrophages [28]; in gastrointestinal smooth-muscle models, *C. vulgaris* metabolite-rich fractions attenuated IL-1β signalling [8].
- **IL-8:** Reduced release from LPS-stimulated macrophage models [17].
- **C-Reactive Protein:** Some human trials of *Spirulina* have suggested reductions in hs-CRP, but the evidence is heterogeneous and population-dependent.

Kwak et al. provides the only RCT directly linking *Chlorella* supplementation (5 g/day for 8 weeks) to enhanced NK-cell activity and modulated IFN-γ, IL-1β and IL-12 [33].

Table T-2. Comparative summary of anti-inflammatory and immunomodulatory effects of *Spirulina platensis* (chiefly via C-phycocyanin) and *Chlorella vulgaris* (chiefly via exopolysaccharides and peptides). Arrows indicate direction of effect in the cited models [11,17-19,21,23,28-29].

Pathway / Marker	*Spirulina* / C-PC	*Chlorella vulgaris*
NF-κB activation	↓ (dose-dependent)	↓ (via exopolysaccharides)
Nrf2/ARE activation	↑	not directly demonstrated
p38/JNK MAPK	↓	indirect
TNF-α	↓ (muscle, liver in rats)	↓
IL-6	↓ (athletes, NAFLD)	↓ (macrophages and NAFLD trial)
IL-1β	↓	↓ (gastrointestinal smooth-muscle model)
IL-12	indirect	↑ in NK-cell study
IFN-γ	indirect	↑ in NK-cell study
NK-cell activity	↑ (older adults)	↑ (Kwak 5 g/day × 8 wk)
iNOS / COX-2	↓	not directly demonstrated
M1/M2 macrophage polarisation	not directly demonstrated	↑ M2 markers

3.5 Inflammatory enzymes and mediators

Inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 are the principal enzymatic amplifiers of inflammation. Their induction by LPS and inflammatory cytokines is well-documented to be ROS- and NF-κB-dependent. *S. platensis* water extracts and C-PC markedly inhibit iNOS and COX-2 protein expression in LPS-stimulated RAW264.7 cells, reducing the resulting production of nitric oxide and prostaglandin E_2 (PGE_2) [23,28]. Because iNOS-derived NO and COX-2-derived PGE_2 are independently

pro-atherogenic, their suppression represents a candidate mechanistic link between *Spirulina*/*Chlorella* consumption and reduced lipoprotein oxidation observed in §2.7 [21,28].

3.6 Immunomodulatory activity

Beyond dampening inflammation, *Spirulina* and *C. vulgaris* partially restore appropriate immune function. *Spirulina* preparations have been reported to enhance macrophage phagocytosis *in vitro*, increase natural killer cell activity in older adults, and modulate antibody response to vaccine challenge in animal models — effects attributed to polysaccharides and phycobiliproteins rather than to direct antioxidant action [17]. Transcriptomic analysis of *Chlorella*-derived exopolysaccharides on LPS-stimulated macrophages demonstrated not only suppression of NF-κB-target cytokines but also upregulation of macrophage genes involved in M2-polarisation, repair and wound healing [29]. These dual inflammatory-resolution and immune-repair activities are mechanistically consistent with the oxidative-stress reduction described in §2 [17,29].

3.7 Spirulina phycocyanin and inflammatory regulation

C-PC uniquely combines antioxidant and anti-inflammatory activities via phycocyanobilin. When *Spirulina* is ingested, the C-PC protein is largely hydrolysed to free phycocyanobilin, a biliverdin-like tetrapyrrole [16]. PCB's anti-inflammatory mechanism recapitulates several properties of its close analogue bilirubin: (i) inhibition of NF-κB activation and of NADPH-oxidase-driven superoxide production, (ii) induction of HO-1, and (iii) direct free-radical scavenging [11,26]. Phycocyanin also inhibits microglial activation and modulates the gut microbiota, with secondary effects on systemic inflammation via reduced LPS translocation [17]. These mechanisms are coherent with the observed clinical reductions in CRP and cytokine levels described in §3.4 [17,23].

3.8 Chlorella polysaccharides and immune balance

Chlorella's immunomodulatory activity is largely polysaccharide-driven. Transcriptomic profiling of *Chlorella*-derived exopolysaccharides on RAW264.7 macrophages revealed a pattern of NF-κB-target gene downregulation concurrent with upregulation of genes involved in macrophage polarity induction and cellular repair, consistent with anti-inflammatory and pro-resolution signalling [29]. *C. vulgaris* peptide hydrolysates additionally modulate cytokine balance in gastrointestinal smooth-muscle models — effects relevant to the link between gut-associated immune homeostasis and systemic inflammation [8]. Chlorophyll and its derivatives, prominent in *C. vulgaris* and absent from *Spirulina*, exhibit independent anti-inflammatory activity by inhibiting COX-2 gene expression *in vitro* [5].

A clinical observation in chronic HCV patients receiving microalgal preparations reported lower ALT in 84.6% of patients, and *C. vulgaris* extract inhibited SARS-CoV-2 *in vitro* at 70 µg/mL [34].

3.9 Human clinical evidence

Human anti-inflammatory evidence is more limited than the pre-clinical literature. Selected trials have reported:

- CRP and IL-6 in NAFLD. C. vulgaris supplementation (1,200 mg/day) for 8 weeks in NAFLD patients significantly reduced hs-CRP and TNF-α versus placebo [31].
- **Inflammation in athletes.** *Spirulina* supplementation in trained humans has shown a tendency to reduce CRP and IL-6 over 2–8 weeks, though heterogeneity is high.
- **Heterogeneous outcomes.** Several studies report that *Spirulina* produces clinically meaningful reductions in pro-inflammatory biomarkers only in populations with elevated baseline inflammation (T2DM, NAFLD, obesity), with little effect in healthy controls [17,30].

3.10 Evidence limitations

Several caveats apply:

1. Population dependence. Healthy volunteers show smaller cytokine reductions than populations with pre-existing inflammation [17].
2. Cytokine heterogeneity. Different cytokines reach significance in different studies; a complete profile remains to be assembled [30].

3. Dose and duration. Effective doses for clinical cytokine effects have not been titrated; 2 g/day for 2 months is a common protocol that may not be optimal for chronic inflammation [17,27].
4. Confounding by lipid and metabolic effects. Apparent anti-inflammatory benefit may partially reflect upstream reductions in oxidative stress or ectopic fat [21,28].
5. Lack of hard clinical endpoints. Cancer, MI or mortality endpoints are not yet linked to *Spirulina*/*Chlorella* supplementation [17].

4. GLYCEMIC CONTROL AND INSULIN SENSITIVITY

4.1 Oxidative stress and insulin resistance

Insulin resistance is now understood as a state in which oxidative and inflammatory stress impair insulin-receptor signalling at multiple nodes. ROS directly oxidise and attenuate phosphatidylinositol 3-kinase (PI3K)/Akt activation downstream of the insulin receptor, while pro-inflammatory cytokines promote serine phosphorylation of IRS-1, reducing the capacity for tyrosine phosphorylation [2-3]. This produces reduced GLUT4 translocation to the skeletal-muscle plasma membrane, impaired hepatic glycogen synthesis and unsuppressed hepatic glucose output — the cardinal triad of T2DM pathophysiology [3]. Counteracting ROS production therefore has a mechanistic basis for improving insulin sensitivity, independent of any direct insulinotropic effect [1,3].

4.2 Effects on fasting blood glucose

Clinical evidence for *Spirulina* on fasting blood glucose is mixed but generally favourable:

- In the classic T2DM trial by Parikh et al., 2 g/day *Spirulina* for 2 months produced appreciable decreases in both FBG and postprandial blood glucose in T2DM subjects [35].
- In the add-on metformin trial by Rajabzadeh Karizi et al., 2 g/day Spirulina + standard metformin for 3 months produced a significant mean decrease in FBG (−24.94 mg/dL, $p<0.001$) relative to placebo plus metformin [36].
- Conversely, the Rezaiyan et al. 2023 trial of 20 g/day *spirulina* sauce (containing 2 g pure spirulina) for 2 months produced a non-significant reduction in FBG (−15.3 mg/dL, $p=0.26$) in T2DM patients, indicating that the dose, format or duration may be insufficient [27].

For C. vulgaris, 1,200 mg/day for 8 weeks in NAFLD patients significantly reduced fasting serum glucose and hs-CRP versus placebo, alongside reductions in liver enzymes and body weight [31]. Mizoguchi et al. observed reductions in FBG in subjects with high-risk lifestyle-factor profiles after 16 weeks of C. vulgaris intake [37].

4.3 Effects on glycated haemoglobin

HbA1c reflects integrated glycaemia over ~3 months and is therefore the most clinically meaningful surrogate. Trials of *Spirulina* have produced heterogeneous but generally favourable findings:

- Parikh et al. reported a significant reduction in HbA1c in T2DM patients receiving 2 g/day *Spirulina* [35].
- Rajabzadeh Karizi et al. reported a clinically meaningful and statistically significant decrease (1.43%, $p<0.001$) in HbA1c following add-on Spirulina + metformin for 3 months [36].
- Lee et al. reported no significant change in HbA1c after 12 weeks of *Spirulina* supplementation in T2DM patients, despite favourable effects on triglycerides [38].
- Rezaiyan et al. reported a non-significant difference in HbA1c (mean −0.13%, $p=0.75$) for the 2-month *spirulina*-sauce intervention [27].

This heterogeneity suggests that HbA1c effects are inconsistent between studies — a finding consistent with intervention duration, baseline glycaemia and biomass composition being effect modifiers. HbA1c is a lagging indicator compared to FBG [27,38].

4.4 Effects on insulin concentration

Insulin concentrations are reported variably:

- Rezaiyan et al. noted a non-significant mean reduction in fasting insulin (−1.46 μIU/mL, p=0.28) in T2DM patients after 2 months of *S. platensis* [27].
- Jeong et al. reported that in Goto-Kakizaki diabetic rats, 3% and 5% w/w dietary *Chlorella* for 8 weeks modestly lowered fasting plasma insulin [32].
- *C. vulgaris supplementation studies in NAFLD populations have reported changes in fasting insulin and HOMA indices, though the direction of effect has been inconsistent [31].*

Where no change in plasma insulin occurs, a parallel reduction in FBG suggests improvements in insulin sensitivity rather than increased insulin secretion — in line with the hypothesis of §4.1 that the principal mechanism is oxidative/inflammatory alleviation rather than direct β-cell stimulation [28,32].

4.5 Insulin-resistance markers

The homeostatic model assessment of insulin resistance is a clinically validated surrogate derived from FBG × fasting insulin / 22.5.

- The NAFLD trial by Ebrahimi-Mameghani et al. demonstrated that C. vulgaris 1,200 mg/day for 8 weeks significantly reduced fasting serum glucose and hs-CRP relative to placebo [31].
- Jeong et al. reported reductions in HOMA-index in GK diabetic rats receiving 3% and 5% dietary *Chlorella* [32].
- Rezaiyan et al. reported a non-significant trend (−0.35, p=0.68) toward HOMA-IR reduction, but a significant improvement in the analogous QUICKI marker (+0.025, p=0.03) [27].

Glucose tolerance assessed by oral glucose tolerance tests was not significantly altered in some *Chlorella* supplementation trials in normal rats; however, *in vitro* work confirms *Chlorella* peptides and ACE-inhibitory fractions can act peripherally at the brush border [5,32].

4.6 Regulation of glucose uptake

Mechanisms proposed from pre-clinical work include:

- *Spirulina* polysaccharides have been shown to upregulate GLUT4 translocation in skeletal-muscle cells, increasing peripheral glucose uptake — independent of any direct insulin effect [17,28].
- *C. vulgaris* bioactive peptides may activate AMPK in muscle and liver, increasing GLUT4 trafficking and inhibiting hepatic gluconeogenesis [5].
- Mizoguchi et al. demonstrated in humans that, after 16 weeks of *C. vulgaris* intake, gene-expression profiling of whole-blood RNA revealed altered expression of genes involved in insulin signalling and glucose uptake [37]. These nutrigenomic findings provide direct human evidence complementing the animal and cellular mechanisms.
- Liver glycogen and glucokinase expression were restored in phycocyanin-treated alloxan-injured mice [26], indicating an effect on hepatic glucose handling.

4.7 Effects on carbohydrate digestion

α-Amylase and α-glucosidase are the first-line enzymes that hydrolyse starch and oligosaccharides in the small intestine; their inhibition delays postprandial glucose absorption. *C. vulgaris* has been shown to be a particularly promising dual-inhibitor extract source:

- The ethanolic extract of *C. vulgaris* inhibited human α-amylase by 47.06 ± 8.31% *in vitro* [39].
- A methanolic extract of *C. vulgaris* showed IC_{50} ~2.66 μg/mL against α-amylase *in vitro*, an activity modestly better than that of the standard inhibitor acarbose (IC_{50} ~2.85 μg/mL); molecular docking and dynamics simulations identified polyphenolic ligands responsible for the inhibition [40].
- An aqueous extract of *C. vulgaris* demonstrated potent non-competitive inhibition of α-glucosidase, with the active component characterised as a hydroxybenzene-like compound [41].

These data provide a mechanistic basis for clinical postprandial glycaemia modulation, although the doses tested are typically supra-physiological relative to typical *Chlorella* supplementation amounts.

4.8 Relationship with lipid metabolism

Insulin sensitivity and lipid metabolism are bidirectionally coupled. Ectopic fat accumulation in skeletal muscle and liver impairs insulin signalling via diacylglycerol-induced PKC activation and ceramide-induced apoptosis [3]. Reductions in triglyceride concentrations of 27.3 mg/dL (~22%) [38], 68.6 mg/dL (p<0.001) [27], and 41–42 mg/dL with simultaneous LDL reductions of 33–38 mg/dL [36] across *Spirulina* trials imply that a portion of the observed insulin-sensitising effect is mediated by improved lipid metabolism [21,28,35]. For *C. vulgaris*, multiple clinical studies have reported reductions in TC and TG in NAFLD and hyperlipidaemic populations [5,42].

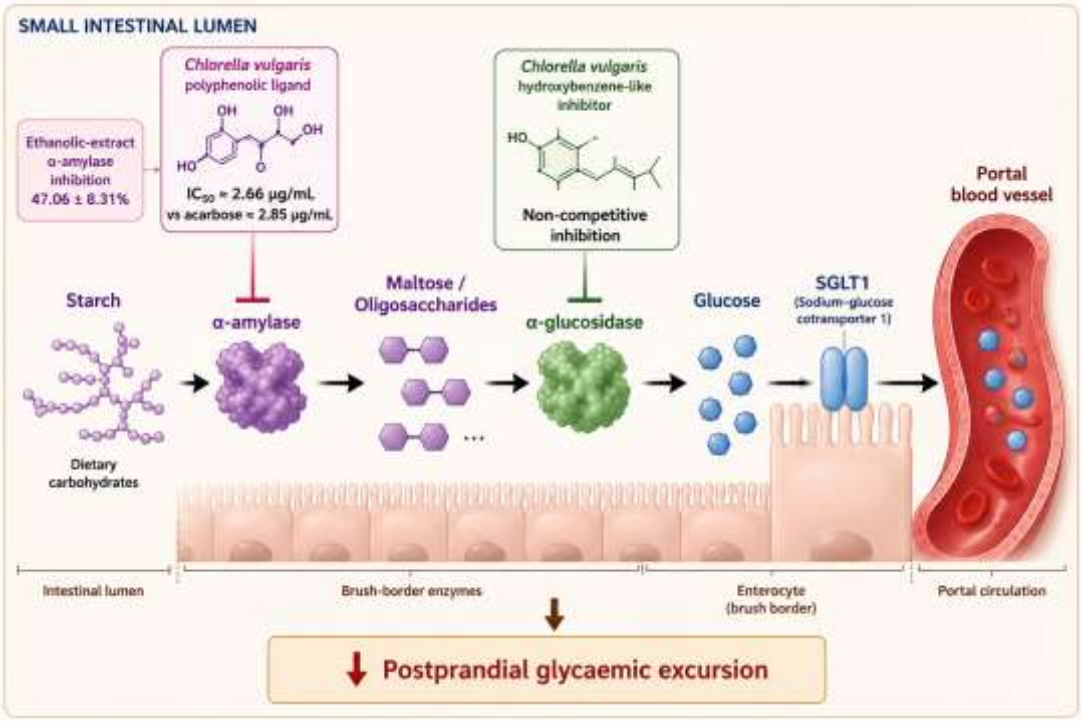

Fig F-4: Schematic of carbohydrate digestion and the dual inhibitory action of *Chlorella vulgaris* bioactives on α-amylase and α-glucosidase. Inhibition of these brush-border enzymes delays starch hydrolysis and reduces the rate of postprandial glucose appearance, providing a plausible mechanism for the modest reductions in fasting blood glucose observed in human supplementation trials [39-41]

4.9 Evidence in type 2 diabetes

Table T-3: Glycaemic and triglyceride outcomes from randomised and add-on clinical trials of *Spirulina* supplementation in type-2 diabetes mellitus. Effect direction and statistical significance are indicated. Heterogeneity across trials reflects variation in dose, format, duration and baseline glycaemia [27,35-36,38].

Study	n	Population	Dose (g/day *Spirulina*)	Duration	FBG	HbA1c	TG
Parikh 2001 [35]	per original	T2DM	2	2 mo	↓	↓	↓
Rajabzadeh Karizi 2023 [36]	per original	T2DM + metformin	2	3 mo	−24.94 mg/dL*	−1.43%*	−41 to −42 mg/dL; LDL −33 to −38 mg/dL
Lee 2008 [38]	n = 37	T2DM	8	12 wk	NC	NS	−27.3 mg/dL (~22%)*
Rezaiyan 2023 [27]	n = 40	T2DM	2 (in sauce)	2 mo	−15.3 mg/dL (NS, p=0.26);	−0.13% (NS, p=0.75)	−68.6 mg/dL (p<0.001)

					QUICKI +0.025*		

The most informative T2DM trials of Spirulina are Parikh et al. and Rajabzadeh Karizi et al. ; both reported reductions in FBG / HbA1c / TG / TC / LDL and marginal HDL increases in T2DM patients. The Lee et al. and Rezaiyan et al. trials reported more modest effects, particularly on HbA1c and insulin, but maintained statistically significant reductions in triglycerides . A recent systematic review concluded that S. platensis supplementation consistently reduces FBS, PPBS, HbA1C, TC, TG and MDA in T2DM, although the strength of evidence varies by outcome and study population [35][36][27,38][30].

For C. vulgaris, the Ebrahimi-Mameghani et al. NAFLD trials showed reductions in fasting glucose, hs-CRP and liver enzymes in the intervention versus placebo arm, suggesting clinically meaningful benefit [31,43]. Data are, however, more limited in T2DM-only populations relative to Spirulina.

4.10 Evidence in metabolic syndrome and fatty liver disease

In NAFLD, C. vulgaris 1,200 mg/day trials have shown multi-faceted improvements: body-weight reduction, serum-glucose lowering, hs-CRP and TNF-α reduction, and improved liver-function tests [31,43]. Finamore et al. and Deng & Chow include metabolic-syndrome-relevant discussion: Spirulina reduces TC, TG and atherogenic index in hyperlipidaemic subjects, and these lipid changes are associated with reductions in systemic oxidative stress and inflammation [17,28]. The Mizoguchi et al. nutrigenomic analysis indicated that several insulin-signalling-pathway genes were upregulated post-Chlorella intake [37]. Panahi et al. provide direct Chlorella + statin RCT data: 600 mg/day C. vulgaris + 20 mg/day atorvastatin for 8 weeks reduced TC, LDL-C and TG similarly to atorvastatin alone, but did not show incremental improvement over the statin [44].

Meta-analytic reinforcement comes from the Mazokopakis NAFLD pilot (6 g/day *Spirulina*) with hepatoprotective and hypolipidaemic effects [45], the Mazloomi *spirulina*-sauce NAFLD cardiometabolic RCT showing improved liver enzymes, oxidative stress, glycaemic profile and lipid profile [46], the Panahi NAFLD combination RCT [47], and the GRADE-assessed *Chlorella* NAFLD meta-analysis (7 RCTs, 375 participants, 8–12 weeks) reporting weight −1.62 kg, FBS −6.25 mg/dL, hs-CRP −1.02 mg/L, LDL −6.45 mg/dL, TC −7.18 mg/dL, ALP −14.84 U/L and AST −4.99 U/L — very-low certainty due to risk of bias [48]. The Yarmohammadi meta-analysis on liver biomarkers reports AST significantly reduced (WMD −9.15 U/L; 95% CI: −16.09, −2.21) and the NAFLD sub-group AST WMD −16.42 U/L [49].

The Bohórquez-Medina meta-analysis [50] updates the *Spirulina* obesity-metabolic-disorder field, van den Driessche et al. [51] adds an explicit intestinal cholesterol-absorption mechanism, and Ngo-Matip et al. [52] provides direct HIV-infected antiretroviral-naïve adult evidence: TC, TG and LDL-C significantly reduced, linked to C-PC-mediated inhibition of LDL oxidation and lipoprotein-lipase upregulation.

4.11 Clinical interpretation

The aggregated evidence base supports a targeted, evidence-based clinical interpretation:

1. *Spirulina* and *C. vulgaris* exert plausible glycaemic effect via a combination of oxidative-stress reduction, anti-inflammatory cytokine modulation, GLUT4/transporter upregulation, hepatic glycogen restoration and α-glucosidase inhibition.
2. The magnitude of effect is highly dependent on baseline metabolic status: effects are clearest in pre-metabolic and metabolic-syndrome populations, less evident in normoglycaemic volunteers.
3. *Spirulina appears more robustly supported than C. vulgaris on hard HbA1c and TG outcomes; C. vulgaris may have a stronger NAFLD evidence base, with trials reporting reductions in fasting glucose, hs-CRP and liver enzymes.*
4. The formulation described in this review should therefore be positioned as supportive nutrition and not as a replacement for antidiabetic medication. Current evidence does not support substitution of supervised pharmacotherapy, but does support adjunctive use for metabolic-syndrome and pre-T2DM populations.

5. OMEGA FATTY ACIDS

5.1 Fatty-acid composition of Spirulina

S. platensis biomass is unusually rich in polyunsaturated fatty acids relative to the saturated-fatty-acid proportion of its total lipid. The principal C18 PUFAs are γ-linolenic acid (GLA; C18:3n-6) and linoleic

acid (LA; C18:2n-6) [6,12], with reported biomass containing approximately 1.9 g GLA per 100 g dry weight — among the highest concentrations in any edible biomass [6]. In a comparison across commercial microalgae, the GLA in *Spirulina platensis* was determined at 1.928% (w/w), markedly higher than in *Chlorella vulgaris* or *Isochrysis galbana* [6]. Saturated fatty acids are dominated by palmitic acid (C16:0), as in most photosynthetic organisms, with minor contributions from myristic (C14:0) and stearic (C18:0) acids [12-13]. An important nuance is that the absolute amount of ALA (C18:3n-3) in most *Spirulina* strains is small relative to GLA, so *Spirulina* is best characterised as an ω-6-rich, GLA-rich microalga rather than as an ω-3 source [12].

Strain- and cultivation-dependent variation introduces considerable batch-to-batch variability. Significant deviations in PUFA profile have been attributed to cultivation temperature, light intensity, salinity, growth phase and nitrogen status [12-13]. A comparative nutrient-profile study showed that *Spirulina platensis* biomass contains a recognisable GLA peak by gas chromatography even after spray-drying and storage, suggesting appreciable robustness of the GLA fraction to processing [13].

5.2 Fatty-acid composition of Chlorella vulgaris

C. vulgaris displays the inverse predominance: the principal omega fatty acid is α-linolenic acid (ALA, C18:3n-3), determined at approximately 2.8 g/100 g dry weight in one comparative nutrient-profile analysis — among the highest concentrations reported in plant-derived biomass [6]. *Chlorella vulgaris* contains a markedly higher proportion of n-3 PUFAs relative to n-6 PUFAs, distinguishing it sharply from *Spirulina* [6,13]. A 2023 multi-strain comparison of four *C. vulgaris* strains identified substantial variability in EPA (C20:5n-3) and DHA (C22:6n-3) content, with at least one strain producing quantifiable EPA peaks and several strains exhibiting distinct omega-3/omega-6 PUFA/SFA ratios [7]. The presence of EPA and DHA is not universally observed; most *C. vulgaris* strains remain dominated by ALA, with EPA emerging only under specific heterotrophic or stress-cultivation regimes [7].

Other principal fatty acids include oleic (C18:1n-9), palmitic (C16:0) and linoleic (C18:2n-6) acids, with the relative proportions varying with strain and growth conditions [12,14].

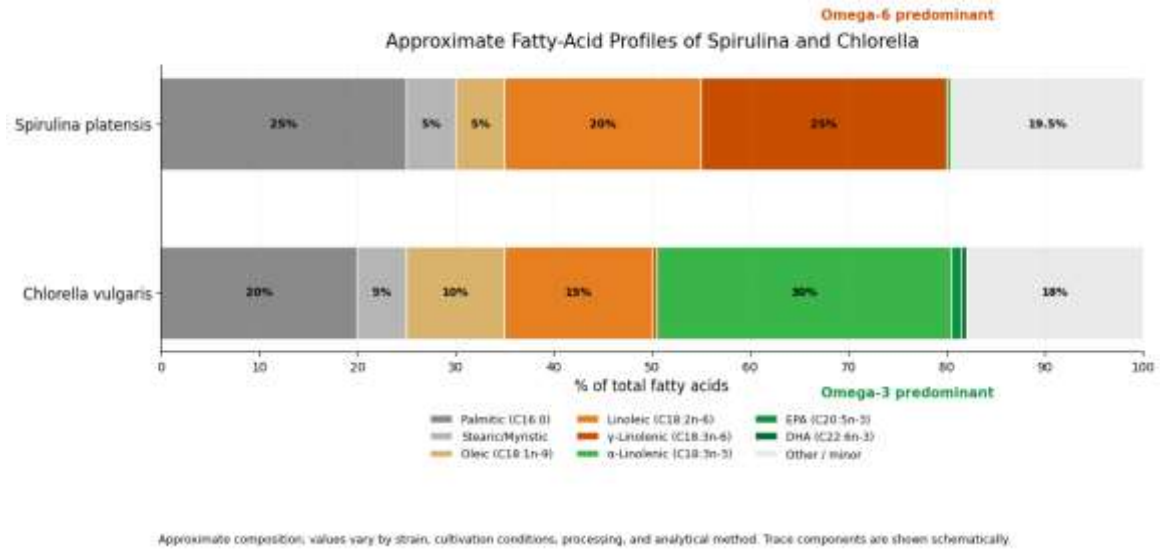

Fig F-5: Comparative stacked-bar representation of the principal fatty-acid profiles of Spirulina platensis and Chlorella vulgaris, illustrating the inverse omega-6 / omega-3 predominance: GLA is concentrated in Spirulina, ALA is concentrated in Chlorella. Strain-dependent variability in EPA and DHA is observed in selected Chlorella strains [12-13].

5.3 Omega-3 and omega-6 balance

The nutritionally meaningful omega profile of a combined product must consider the ratio between ω-6 and ω-3 intake. For the nutraceutical formulation discussed in this review, the intended balance point is:

- *Spirulina* provides the ω-6 backbone (LA + GLA, the latter a key intermediate in prostaglandin E_1 synthesis favouring vasodilation and reduced platelet aggregation) at a recognised ratio. GLA is biologically notable because its conversion to dihomo-γ-linolenic acid — bypassing the rate-limiting

Δ6-desaturase step — directly supports the production of anti-inflammatory eicosanoids such as PGE_1 rather than pro-inflammatory prostaglandins of the n-6 cascade [17,28].

- *C. vulgaris* provides a substantial ω-3 contribution (ALA at 2.8 g/100 g), proximate to the dietary-adequacy targets proposed by expert bodies for vegetarian/vegan populations [6-7].
- The combined formulation therefore contains both rate-limiting and rate-bypassing intermediates of the eicosanoid cascades — a thermodynamic argument supporting complementary activity [12,17,28].

The specific PUFA ratio of any commercial product must be measured by gas chromatography–mass spectrometry rather than inferred from average literature values, because strain and processing effects are substantial.

5.4 Role in cellular membranes

PUFAs are determinants of membrane fluidity, microdomain formation and receptor function. The acyl-chain unsaturation and chain length determine how tightly lipids pack in the bilayer, in turn modulating:

- Membrane fluidity. Increasing ALA and LA content enhances membrane fluidity and curvature stress, which is mechanistically linked to the activity of integral membrane proteins [17,28].
- Receptor function. Membrane fluidity modulates ligand–receptor binding kinetics; insulin-receptor activity and GLUT4 translocation efficiency are sensitive to bilayer physical state [3,28].
- Mitochondrial membranes. PUFA-rich cardiolipins are essential to inner mitochondrial membrane organisation; oxidative depletion of cardiolipin by lipid peroxidation disrupts the respiratory supercomplexes, contributing to mitochondrial dysfunction [4]. Antioxidant preservation of PUFA-rich membranes (§2.7) and the PUFA supply from the diet converge on mitochondrial integrity [1,17,28].
- Nutrient transport. Bilayer fluidity modulates passive and carrier-mediated substrate transport, particularly of long-chain fatty acids and glucose [28].

5.5 Formation of lipid mediators

PUFAs are the substrates for eicosanoid and specialised pro-resolving mediator production. Linoleic acid can be metabolised via cyclooxygenase and lipoxygenase pathways to leukotoxin diols; γ-linolenic acid can be elongated to dihomo-GLA, which is then converted to PGE_1, a vasodilatory anti-platelet, anti-inflammatory prostaglandin [17,28]. α-Linolenic acid, EPA and DHA are the substrates for resolvins, protectins and maresins — specialised lipid mediators that drive the resolution rather than the initiation phase of inflammation [28].

Spirulina supplementation may therefore shift the n-6 PUFA-derived eicosanoid balance toward PGE_1 by providing GLA directly; *C. vulgaris* supplementation may increase the available substrate for resolvin/protectin synthesis via ALA — and to a lesser extent, EPA in selected strains [7,28].

5.6 Inflammatory relevance

The PUFA-dependent shift from pro-inflammatory prostaglandin E_2 / leukotriene B_4 catabolism to anti-inflammatory PGE_1 / resolvin E_1 catabolism offers a plausible lipid-mediator-level explanation for the cytokine changes documented in §3.4 [17,28]. This argument integrates with the parallel antioxidant and NF-κB effects: the antioxidant defence reduces the substrate available for non-enzymatic lipid peroxidation; the substrate shift toward PGE_1 and resolvin-class mediators reduces the enzymatic eicosanoid-based inflammatory load. Importantly, the overall omega-6/omega-3 ratio of the product should be kept within plausible dietary ranges (current typical dietary ratios are 15–20:1; ratios between 4:1 and 10:1 are recommended by some expert bodies), and the formulation should not be claimed to "cure inflammation" on this basis alone [17,28].

5.7 Importance of product-specific fatty-acid analysis

Generic fatty-acid claims should be supported by laboratory analysis of the actual raw-powder biomass and the finished product, because inter-batch variability is high among commercially available microalgal products, particularly where cultivation conditions are not standardised [9]. Quantitative GC-MS analysis of the final formulation is recommended to verify:

- Total lipid content (AOAC 996.06 or equivalent).
- Individual fatty-acid profile and total PUFA / total saturated fatty acid ratio.

- The GLA and ALA peak heights in absolute terms.
- The absence of oxidation markers (peroxide value, anisidine value, TOTOX).
- The absence of contaminants such as microcystins and heavy metals [9].

These measurements should be incorporated into the product specification and Certificate of Analysis.

6. CELLULAR HEALTH, CYTOPROTECTION AND NUTRITIONAL REJUVENATION

6.1 Meaning of cellular rejuvenation

The term "cellular rejuvenation" is not a recognised clinical endpoint in conventional medicine, and should be interpreted within this review as support for the maintenance, repair and resilience of cellular structures and functions through the combined effects of dietary antioxidants, polyphenols, PUFAs, vitamins, minerals and proteins supplied by *Spirulina* and *Chlorella vulgaris*. No clinical trials have demonstrated that any nutraceutical product (including the formulation described here) causes regeneration of tissue, cell-cycle reversion or restoration of damaged parenchymal organs in humans. Cell-rejuvenation language must therefore be used with strict scientific discipline, as discussed in §8.10 [1,17].

6.2 Protection of cellular membranes

Cellular membranes — plasma, organellar and mitochondrial — are the first anatomic substratums of oxidative damage because they are rich in polyunsaturated fatty acids. The lipid-peroxidation evidence in §2.7 supports a clear role of *Spirulina* and *Chlorella* pigments in the preservation of membrane integrity. In hamster models, *Spirulina* Se-supplementation prevented the rise in cardiac superoxide generation and circulating MDA, indicating reduced oxidative damage to cardiomyocyte membranes [21]. Combined with the PUFA supply discussed in §5.4, the dual effect of (i) reduced ROS-driven peroxidation and (ii) increased substrate for membrane renewal provides a coherent framework for membrane-cytoprotective claims [17,28].

6.3 Mitochondrial support

Mitochondrial respiration is the largest single source of intracellular ROS. The Nrf2-activating, glutathione-restoring and NADPH-oxidase-suppressing effects documented in §2.5–2.6 collectively reduce the mitochondrial ROS burden, while PUFA availability supports the renewal of cardiolipin — the inner-mitochondrial-membrane phospholipid essential for cristae organisation and respiratory supercomplex stability [4]. Lipid peroxidation of cardiolipin specifically disrupts respiratory chain complexes I and IV, producing a self-amplifying spiral of mitochondrial damage and ROS overproduction; prevention of this peroxidation is therefore expected to preserve mitochondrial function [4]. Claiming "mitochondrial support" is mechanistically justified at the level of cell biology but remains a surrogate outcome.

6.4 Protection of proteins and DNA

Oxidative damage to protein thiol groups and amino-acid side-chains, and to DNA (oxidative base modifications such as 8-oxo-dG, strand breaks and mitochondrial DNA lesions) are key contributors to cellular ageing and chronic-disease pathogenesis [1]. The C-PC-induced activation of Nrf2/ARE-regulated phase II enzymes (§2.6) — including heme oxygenase-1, NADPH-quinone oxidoreductase 1, glutathione S-transferase, UDP-glucuronosyltransferase, superoxide dismutase, catalase and glutathione peroxidase — is the principal route through which *Spirulina* reduces the oxidative load on cellular proteins and DNA [18,25]. Polysaccharide-rich *Chlorella* extracts attenuate H_2O_2-induced protein oxidation *in vitro*, partially recapitulating *Spirulina*'s Nrf2-mediated effect on a different chemical profile [29]. The bilirubin-like activity of phycocyanobilin may also contribute via biliverdin reductase-mediated gene regulation [16,26].

6.5 Support for cellular repair mechanisms

Nutritionally, both microalgae provide the amino-acid, micronutrient and energy substrate necessary for protein synthesis, enzyme activity and cellular turnover. Their high-quality protein content supports the synthesis of antioxidant enzymes, immune-cell proteins, transport proteins and structural elements of cellular architecture [5,11]. Cellular division (in mitotically competent cell types) is energy-intensive; preserving mitochondrial ATP output indirectly supports nutrient-dependent cellular turnover, although this claim cannot be extrapolated to fixed post-mitotic cells such as cardiomyocytes or neurons [1,4].

6.6 Micronutrient support

Both microalgae are dense sources of:

- Iron. Bioavailable iron from *Spirulina* and *Chlorella* is recognised; *Spirulina* contains ~100 mg Fe/100 g in iron-supplemented cultivation, with high bioavailability from the chlorophyll-protein complexes [5,11].
- Folate / B-complex. Notably B12 analogues in *Spirulina*; folate in both microalgae [5,11].
- β-Carotene and lutein. Major contributors of provitamin A from *Chlorella*; lutein from *Chlorella* supports macular pigment density [5].
- Vitamin C. Present in *Chlorella* and *Spirulina* at concentrations meaningful for cell-protective nutrition [5,11].
- Selenium. Variable depending on cultivation medium, but reported in *Spirulina* up to biologically meaningful concentrations in selenium-enriched preparations [21].

These micronutrients serve as cofactors for the antioxidant defence (Se for Gpx, Fe for catalase, Cu/Zn for SOD) and as direct antioxidants (vitamin C as aqueous-phase chain-breaker; β-carotene as singlet-oxygen quencher) [1,21,28].

6.7 Protein and amino-acid contribution

Both microalgae contain 50–70% protein on dry-weight basis, with essential amino-acid profiles close to those of egg or casein and superior to many plant-derived proteins such as soybean [5,11]. Bioinformatics-validated enzymatic hydrolysates of these proteins release bioactive peptides that retain antioxidant and anti-inflammatory activity and may cross biological barriers in concentrated nutraceutical preparations [11,18].

6.8 Chlorophyll, carotenoids and phycocyanin

These three pigment classes form the principal colour-coded antioxidant system of the two-microalgae combination:

- Phycocyanin (blue; *Spirulina*) — bilin chromophore drives direct scavenging and Nrf2 induction [15,18].
- Chlorophylls *a* and *b* (green; *Chlorella*) — cyclic tetrapyrroles that scavenge radicals and have been associated with reduction in mutagen-inducible oxo-dG formation [5].
- Lutein, β-carotene and mixed carotenoids (yellow-orange; both microalgae) — long-chain conjugated antioxidants that break free-radical chain reactions in membrane lipid bilayers [5,11].

Together, these pigments provide a wide colour-spectrum — and correspondingly wide redox-potential — antioxidant shield, analogous in scope to a multi-antioxidant mixture [1,16].

6.9 Limitations of anti-ageing claims

Anti-ageing claims and cell-rejuvenation language — whether phrased as "regenerates tissues", "reverses ageing", "restores youth", or "rejuvenates cells" — are not supported by direct clinical evidence for either *Spirulina* or *Chlorella vulgaris* [1,17,28]. They should be omitted unless accompanied by product-specific clinical biomarkers of cellular damage (e.g., 8-oxo-dG, mitochondrial membrane potential, MLCL/MCL cardiolipin ratio, protein-carbonyl adducts).

7. BLOOD HEALTH, METABOLIC CLEARANCE AND PHYSIOLOGICAL DETOXIFICATION SUPPORT

7.1 Scientific interpretation of blood purification

"Blood purification" is not a recognised clinical endpoint in modern medicine. In conventional pathophysiology, the analogous concept is hepatic and renal biotransformation of xenobiotics and physiological clearance of reactive carbonyl species, pollutants and metabolic waste by the liver, kidneys, gastrointestinal tract and lungs. Any "blood purification" claim for a nutraceutical formulation must therefore be translated into specific outcomes supported by clinical data: improvement in liver-function biomarkers, reduction in oxidative biomarkers in plasma, enhancement of bowel-toxic-excretion markers, or improvement in urea and creatinine clearance — each of which is independent and supported by specific evidence [9,17].

7.2 Support for healthy blood glucose

The clinical findings summarised in §4.2–4.4 are directly relevant. Trials in T2DM patients receiving Spirulina at 2 g/day for 2-3 months reported reductions in FPG of ~24.9 mg/dL and reductions in HbA1c of 1.43% [35-36]. Chlorella at 1,200 mg/day in NAFLD trials reduced fasting glucose and hs-CRP and significantly decreased liver enzymes (including ALP and ALT) relative to placebo, indicating integrated glycaemic and hepatic impacts [31,43]. These findings are consistent with — but do not establish any generalised concept of "blood purification" [31,35-36].

7.3 Support for healthy blood lipids

Across clinical trials, both microalgae have repeatedly shown favourable shifts in the major lipid fractions:

- *Spirulina* supplementation at 2 g/day significantly reduced TC, TG, LDL-C and VLDL-C, and increased HDL-C in T2DM and dyslipidaemic patients [21,36,38].
- The ~42% increase in plasma total antioxidant capacity recorded alongside reductions in circulating MDA in the Se-enriched phycocyanin hamster model corresponds mechanistically to a reduced circulating oxidative burden on LDL-C [21].
- *Chlorella vulgaris supplementation at doses from 1–3 g/day has shown reductions in TC, TG and LDL-C of variable magnitude, consistent with reduced body fat percentage in NAFLD trials [10,31,42].*

A combined nutraceutical formulation that simultaneously reduces oxidative stress on lipoprotein particles (limiting the formation of oxidised LDL) and reduces their absolute concentration may, in principle, slow atherosclerosis progression — a hypothesis that remains to be tested in hard clinical endpoints (§2.10 and §4.10) [9,21,28].

Meta-analytic cardiovascular context is provided by Pinto-Leite et al. [53] (12 *Chlorella* + 9 *Spirulina* RCTs): *Chlorella* had neutral BP and lipidaemia effects, while *Spirulina* significantly reduced diastolic BP (−0.42, 95% CI: −0.81 to −0.02, p=0.04) with a trend toward TC reduction (p=0.15). The Shiri *Spirulina* BP meta-analysis reports SBP WMD −4.41 mmHg (95% CI: −6.74, −2.07) and DBP WMD −2.84 mmHg (95% CI: −4.65, −1.03) at moderate GRADE quality, strongest at >8 weeks, in hypertensive, age >50 [54]. The Machowiec meta-analysis corroborates: SBP MD −4.59 (95% CI: −8.20, −0.99) and DBP MD −7.02 (95% CI: −8.86, −5.18) in hypertensive subgroup [55]. Casas-Agustench et al. establish the BP-lowering effect of >3 g/day whole edible algae for ≥12 weeks, particularly in those with elevated BP [56]. The Ghaem Far et al. triple-blind RCT of 2 g/day *S. platensis* dressing for 8 weeks reported significant SBP (p=0.02), DBP (p=0.03), TG (p=0.01), TC and LDL reductions in hypertensive patients [57].

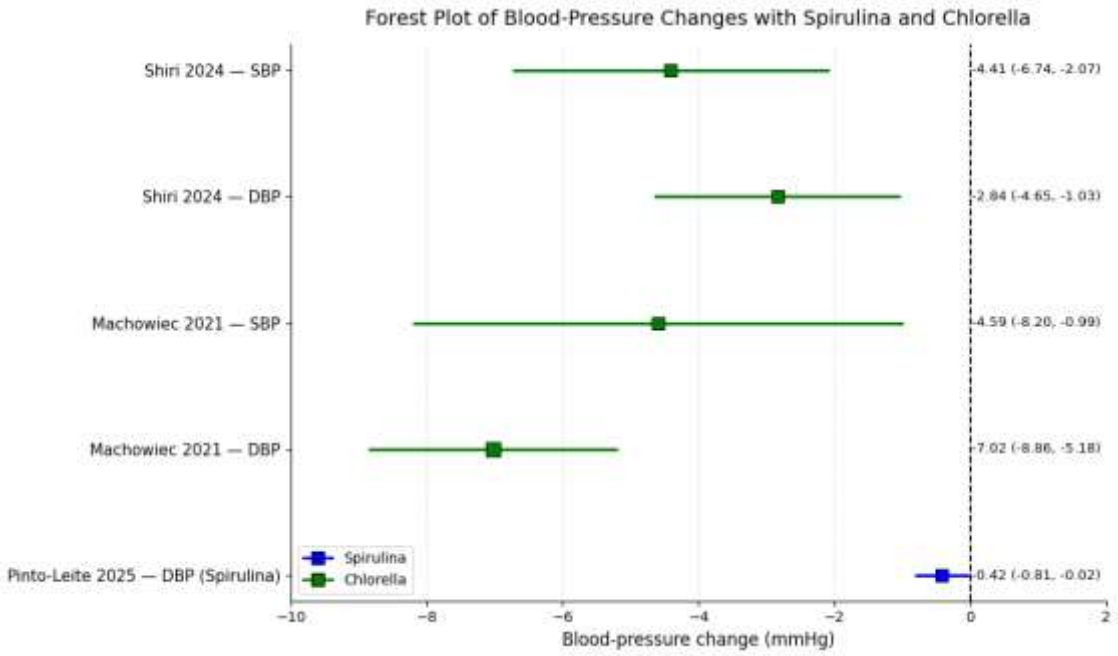

Fig F-6: Forest plot of weighted mean differences and mean differences in systolic and diastolic blood pressure following *Spirulina* supplementation across three independent meta-analyses. Sub-group effects

in hypertensive subjects are presented separately. Pinto-Leite 2025 reported a neutral effect for *Chlorella*. The vertical line at zero represents no effect; values to the left indicate blood-pressure reduction [53-55].

7.4 Oxidative protection of blood components

Lipoproteins, erythrocytes and circulating proteins are major peripheral targets of ROS. Erythrocyte membranes are rich in PUFAs and are particularly vulnerable to lipid peroxidation; oxidised erythrocyte membranes show elevated MDA and decreased membrane fluidity. The lipid-peroxidation biomarkers discussed in §2.7 — plasma MDA, cardiac superoxide production — are biomarkers of oxidation of these compartments [21,28]. *Spirulina*'s antioxidant system has been associated *ex vivo* with reduced LDL oxidation [21,28], a key pro-atherogenic reaction that renders LDL unable to be recognised and cleared by the LDL receptor and instead becomes a substrate for macrophage scavenger-receptor uptake, producing foam cells.

7.5 Haematological and iron-status support

Iron, folate, B12 (analogues), chlorophyll-associated nutrients and protein all theoretically support erythropoiesis at the substrate level. However, chlorophyll does not directly convert into haemoglobin — this is a common medicinal-food myth that must be avoided. Both the chlorophyll-molecular ring structure and the iron required for haeme synthesis (which is independent of dietary chlorophyll intake) must be considered independently. Where patients are iron- or folate-deficient, supplementation with iron or folate directly — rather than chlorophyll ingestion — is the appropriate medical intervention [5,11].

Lacurezeanu & Vodnar [58] consolidate the iron-status evidence: *A. platensis* enhanced erythropoietic activity (↑ Hb, ↑ serum ferritin, ↑ RBC); *C. vulgaris* increased SOD/GPx and decreased lipid peroxidation. Both algae decreased hepcidin (a pro-inflammatory cytokine that inhibits iron absorption), supporting improved iron balance. Gao et al. [59] report the direct quantitative value: *Spirulina* Hb-regeneration efficiency = 54.8%, significantly higher than $FeSO_4$ (47.2%) and ferric citrate (34.7%), via intestinal nanosized-iron delivery. Gunawan et al. [60] report a pooled mean-difference of Hb in pregnant women receiving *Spirulina* = +1.81 g/dL (95% CI 0.24, 3.39; p=0.024).

Table T-4. Iron-status and haematological outcomes from human and animal studies of *Spirulina* and *Chlorella vulgaris*. Both microalgae enhance erythropoiesis-related biomarkers and decrease hepcidin, suggesting improved iron balance [58-63].

Evidence	***Spirulina / A. platensis***	***Chlorella vulgaris***
Hb in cyclists (6 g/day × 14 d)	+3.4%; no VO_2max benefit	not assessed
Hb in cyclists (6 g/day × 21 d)	+6%	not assessed
Hb (21-day, 6 g/day)	not assessed	+4%
Iron-deficiency anaemia (rat model): Hb-regeneration efficiency	54.8% (vs $FeSO_4$ 47.2%; ferric citrate 34.7%)	not assessed
Hb in pregnant women (meta-analysis): pooled mean-difference	+1.81 g/dL (95% CI 0.24, 3.39; p = 0.024)	not assessed
Serum ferritin, RBC count	↑	↑
Erythrocyte SOD/GPx activity	stabilised	↑
Lipid peroxidation in iron-deficiency context	↓	↓
Hepcidin	↓	↓

7.6 Liver-associated metabolic clearance

The liver performs physiological detoxification through:

- Phase I (cytochrome P450-mediated oxidation/reduction).
- Phase II (glutathione conjugation, glucuronidation, sulphation, acetylation).

- Biliary elimination of conjugated products.

Spirulina has been reported to support hepatic function in animal models of hepatotoxicity at doses comparable to the human equivalent, demonstrating reduction in ALT, AST, GGT and ROS [17]. Chlorella supplementation has been associated with reduced ALT in NAFLD trials, providing the most clinically applicable hepatoprotective correlate [31,43]. These observations are consistent with a glutathione-replenishing and Nrf2-inducing effect on phase II conjugation enzymes (described in §2.6). The Sun et al. [64] review explicitly integrates HACCP/ISO standards and the requirement for verified formulations in microalgae-based liver-health claim substantiation.

7.7 Gastrointestinal elimination

Both microalgae provide dietary fibre and contribute to a healthy gut microbiota composition. Algal polysaccharides and chlorophyll-associated derivatives can support bacterial growth and short-chain-fatty-acid production (butyrate, propionate) — metabolites that epigenetically regulate colonocyte proliferation and immune function. Rigorous clinical evidence for the magnitude of these effects is still emerging, and a "blood purification" framing is best avoided in this context [9,17].

7.8 Evidence concerning heavy metals and environmental contaminants

There is some evidence that *Chlorella vulgaris* can bind heavy metals (lead, cadmium, arsenic, mercury) and that *Spirulina* contributes antioxidant protection against hepatotoxicity during metal exposure in rats. Kyratzopoulou et al. [65] provides 2025 *in vitro* bioremediation data on *C. vulgaris* heavy-metal (Ni/Pb/Zn/Cd/Cu) mono-component removal of 65–99% on day 3 and 72–99% on day 7 (multi-component 49–99% at day 3 and 62–99% at day 7) — confirming the *in vitro* bioremediation capacity applicable only to industrial processing streams, not a clinical detoxification claim. However:

- The in vivo evidence derives predominantly from animal studies at supra-physiological doses.
- The chelating effect of dropped heavy metals is achieved through thiol-containing molecules, not by generic antioxidant action.
- A combined nutraceutical formulation should not be claimed to "remove heavy metals from the body" on the basis of these data alone [9,17].

7.9 Limits of detoxification claims

In summary, the formulation must not be claimed to:

- Purify or cleanse the blood.
- Remove all toxins from the body.
- Replace medical chelation in acute poisoning.
- Treat chronic metal-burden disease.

The product may legitimately be positioned as supportive of normal physiological detoxification through micronutrient, antioxidant and anti-inflammatory mechanisms and the provision of dietary fibre — but this language must remain conservative.

8. PRODUCT DISCUSSION

8.1 Rationale for the combined formulation

The complementary phytochemical profile of *S. platensis* (phycocyanin + phycocyanobilin + GLA-rich PUFA) and *Chlorella vulgaris* (chlorophylls + lutein + ALA-rich PUFA + peptides) is the central rationale for combining them in a single nutraceutical formulation. Each component contributes one or more distinct functional features:

- Phycocyanin and PCB provide anti-inflammatory tetrapyrrole activity and Nrf2 induction [15-16,18].
- Chlorophylls provide antioxidant and chemopreventive protection of gastrointestinal mucosa [5].
- β-Carotene and lutein provide singlet-oxygen quenching and photoprotection [5,11].

- *Chlorella* peptides and proteins provide antioxidant and anti-inflammatory peptides after digestion [5,18].
- Polysaccharides provide immune-modulating and gut-supportive effects [17,29].

8.2 Proposed complementary mechanisms

The combined formulation is hypothesised to support:

1. Antioxidant defence via combined tetrapyrrole and xanthophyll/β-carotene spectra plus induction of Nrf2-regulated genes.
2. Inflammatory balance via NF-κB suppression, MAPK modulation, and the dual eicosanoid-resolving effect of GLA (pro-PGE_1) and ALA (pro-resolving mediators).

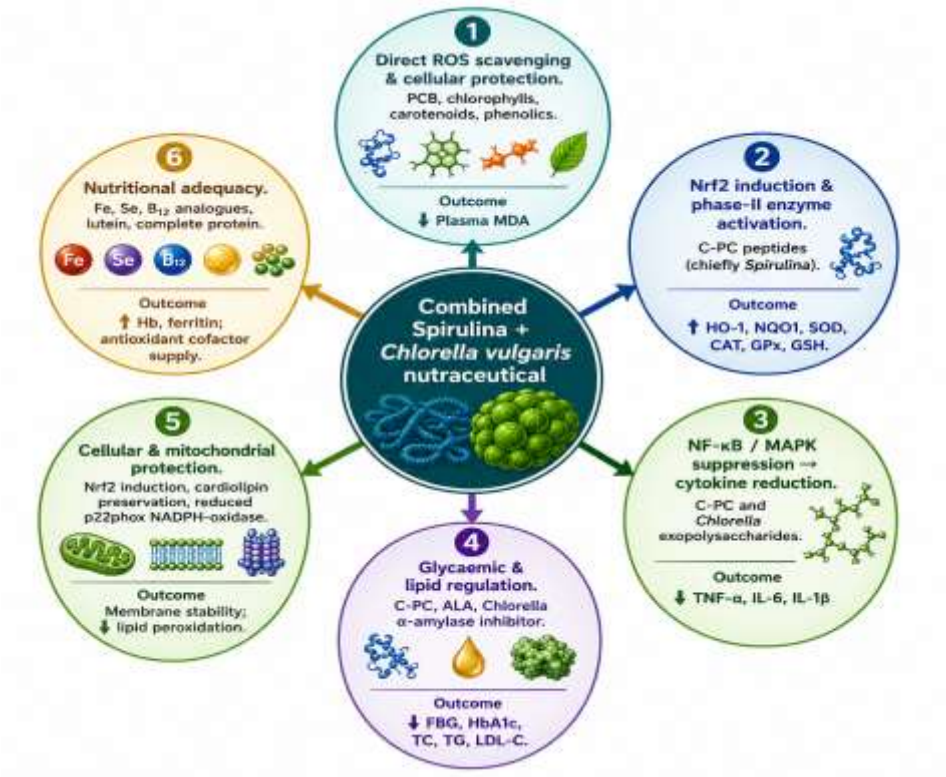

Fig F-7: Integrated hub-and-spoke mechanism by which the combined *Spirulina* + *Chlorella vulgaris* nutraceutical formulation is hypothesised to exert multiple, complementary bioactivities. Each spoke denotes a primary mechanistic axis; outcome biomarkers are shown alongside. The colour convention (blue for *Spirulina*-dominant; green for *Chlorella*-dominant; purple for shared) reflects the complementary phytochemical architecture of the two microalgal biomasses [8-9,11,16-23,25,27,38,40,47-48,66].

3. Glucose and lipid metabolism via fasting-glucose and LDL-C reductions (see §4 and §7.3).
4. Cellular membrane nutrition via supply of PUFAs and protection against lipid peroxidation (see §5.4).
5. Mitochondrial protection via Nrf2 induction, reduced cardiolipin peroxidation and reduced mitochondrial superoxide generation (see §6.3).
6. General nutritional adequacy via dense protein, vitamin and mineral supply.

8.3 Possible advantages of combining both microalgae

Combining both microalgae provides:

- Broader nutrient diversity (wider vitamin, mineral and pigment coverage).
- Compensating strains: if the *Spirulina* batch is low in carotenoids, *Chlorella* compensates, and vice versa.

- A more balanced ω-6 / ω-3 profile than can be achieved by either species alone (GLA + ALA) [7,12].
- Equalising antioxidant systems across the aqueous and lipid phases: PCB and chlorophyll operate in different cellular compartments and provide complementary protection.

8.4 Product composition

Table T-5. Product details of the combined Spirulina–Chlorella vulgaris nutraceutical (Biotex Life Solutions Pvt. Ltd.).

Parameter	Details
Product category	Nutraceutical dietary supplement (combined edible microalgae)
Composition per serving	Spirulina (Limnospira platensis) — 250 mg; Chlorella vulgaris — 250 mg
Spirulina: Chlorella ratio	1:1
Total microalgal biomass per serving	500 mg
Physical form	Whole biomass powder; Chlorella as broken-cell-wall powder
Recommended use	One serving per day
Key actives — Spirulina	C-phycocyanin, phycocyanobilin, γ-linolenic acid (GLA, 18:3n-6)
Key actives — Chlorella	Chlorophyll a and b, lutein, β-carotene, α-linolenic acid (ALA, 18:3n-3)
Additional nutrients	Protein, carotenoids, polysaccharides, B-group vitamins, vitamin C, iron and minerals
Positioning	Daily nutritional supplement providing antioxidant pigments, essential fatty acids and micronutrients
Manufacturer	Biotex Life Solutions Pvt. Ltd., Hyderabad, Telangana, India

Fig F-8: Spirulina+Chlorella by Biotex Life Solutions.

The combined nutraceutical developed by Biotex Life Solutions Pvt. Ltd., is a dietary supplement formulated from two edible microalgae. Each serving provides *Spirulina* (*Limnospira platensis*) and *Chlorella vulgaris* in a fixed 1:1 ratio — 250 mg of each — giving 500 mg of total microalgal biomass, and is taken as one serving per day. Both are supplied as whole biomass powders, with the *Chlorella* provided as a broken-cell-wall powder to aid digestion and nutrient absorption. The formulation brings

together the characteristic actives of each organism: from *Spirulina*, C-phycocyanin, phycocyanobilin and γ-linolenic acid (GLA); and from *Chlorella*, chlorophyll a and b, lutein, β-carotene and α-linolenic acid (ALA). These are accompanied by the broader nutrient content of the two microalgae, including protein, carotenoids, polysaccharides, B-group vitamins, vitamin C, iron and other minerals. At 500 mg per serving, the product is positioned as a daily nutritional supplement that supplies antioxidant pigments, essential fatty acids and micronutrients.

8.5 Raw-material standardisation

A quality product should specify:

- Species and strain identity (e.g., *Limnospira platensis* PCC 9108 and *Chlorella vulgaris* SAG 211-12 or equivalent).
- Cultivation conditions (photobioreactor, freshwater/marine water medium, temperature regime).
- *Chlorella* cell-wall-disruption method (high-pressure homogenisation, enzymatic lysis, pulsed electric field).
- Phycocyanin content of *Spirulina* (e.g., ≥10% C-PC by spectrophotometry).
- Chlorophyll content of *Chlorella* (e.g., ≥1% total chlorophyll).
- Carotenoid content (e.g., ≥5 mg/g total carotenoids).
- Protein content (≥50% w/w by Dumas or Kjeldahl).
- Fatty-acid profile (PUFA/SFA ratio ≥1.0, with declared GLA and ALA peaks) [6-7,9,13].

8.6 Quality-control requirements

Mandatory analyses include:

- **Microbial contamination:** Total plate count, yeast/mould, coliforms, *E. coli*, *Salmonella*, *Staphylococcus aureus*.
- **Heavy metals:** Lead, arsenic, cadmium, mercury — below Pharmacopoeia limits.
- **Pesticides:** Multi-residue screening.
- **Cyanotoxins:** Microcystin-LR, nodularin, anatoxin-a, cylindrospermopsin — below WHO drinking-water thresholds (1 µg/L for microcystin-LR).
- **Residual solvents:** If extracts are used, verify that no toxic solvent residue remains.
- **Aflatoxins:** B1, B2, G1, G2 — below 5 µg/kg.
- **Stability:** Moisture, water activity, peroxide value, microbial stability at 6/12/24 months.
- **Nutrient content:** Periodical reassessment of phycocyanin, chlorophyll, carotenoid, fatty acid and protein at batch level [9].

Table T-6. Mandatory quality-control test matrix for the combined Spirulina + Chlorella vulgaris nutraceutical product, including microbial, heavy-metal, pesticide, cyanotoxin, residual-solvent, mycotoxin, stability and nutrient-content parameters with their acceptance criteria [9,65,67-68].

Test category	Parameter/target	Acceptance limit
Microbial	Total plate count, yeast/mould, coliforms, *E. coli*, *Salmonella*, *S. aureus*	Pharmacopoeia limits
Heavy metals	Lead, arsenic, cadmium, mercury	Pharmacopoeia limits
Pesticides	Multi-residue screen	LOQ-level compliance
Cyanotoxins	Microcystin-LR, nodularin, anatoxin-a, cylindrospermopsin	<1 µg/L
Residual solvents	(only if extracts used)	ICH Q3C limits
Mycotoxins	AFB1, AFB2, AFG1, AFG2	<5 µg/kg total
Stability	Moisture, water activity, peroxide value, microbial stability at 6 / 12 / 24 months	Per protocol
Nutrient content (per-batch CoA)	Phycocyanin, chlorophyll, carotenoids, fatty-acid profile, protein	Per CoA specification

8.7 Safety and tolerability

Most published clinical studies report good tolerability. Adverse events typically include:

- Mild gastrointestinal discomfort.
- Nausea.
- Bloating.
- Temporary stool discolouration (greenish-blue, attributable to algal pigments).
- Headache (rare).
- Allergic reactions (especially in individuals known to be sensitive to algae or with histamine intolerance) [9,11,17].

Slow titration from a low daily dose (e.g., 1 g/day for one week, then 2 g/day) is recommended to mitigate early gastrointestinal effects.

8.8 Clinical precautions

Clinical caution and medical advice should be sought before use in:

- **Pregnancy and breastfeeding.** Data in these populations are limited; standard dietary supplements are generally considered acceptable but the formulation should be discussed with the treating physician.
- **Children.** No long-term paediatric safety studies are available.
- **Autoimmune disorders.** The immunomodulatory effects of *Spirulina* and *Chlorella* (see §3.6) are theoretically beneficial but could theoretically perturb immunosuppressive therapy — medical advice is required.
- **Phenylketonuria.** Not a contraindication, but standard practice should be applied concerning protein-intake accounting.
- **Liver or kidney disease.** Although generally beneficial, dose adjustment may be required.
- **Patients using antidiabetic medicines.** Because the formulation reduces fasting glucose, HbA1c and HOMA-IR, additive effect with hypoglycaemic medications is possible — careful monitoring is recommended (consult §4.11).
- **Patients using anticoagulants or antiplatelet drugs.** Theoretical considerations regarding GLA's effect on platelet function call for caution.
- **Patients receiving immunosuppressive therapy.** Immunomodulatory effects may interfere with therapy.

8.9 Ergogenic and immunostimulatory adjunctive evidence

A growing body of RCTs evaluates the ergogenic and immunostimulatory potential of the two microalgae, mostly at 2–8 g/day dosing in trained populations.

Table T-7. Randomised controlled trials of *Spirulina* and *Chlorella* as ergogenic and immunostimulatory adjuncts. Doses, durations, populations and key outcomes are summarised to facilitate cross-trial comparison [33,61-63,69-70].

Study	Microalga	Dose	Duration	Population	Key effect
Ali, Aubeeluck, Gurney 2023 [61]	*Spirulina*	6 g/day	14 d	Recreational cyclists	**Hb +3.4%**; no VO_2max benefit
Gurney, Brouner, Spendiff 2022 [62]	*Spirulina*	6 g/day	21 d	Trained cyclists	↓ submaximal HR; ↓ lactate; **Hb +6%**; ↑ peak/avg repeated-sprint power
Gurney & Spendiff 2022 (review) [69]	*Spirulina*/*Chlorella*	—	—	—	Mechanistic alignment with antioxidant hypothesis
Gurney, Brouner, Spendiff 2023 [63]	*Chlorella*	6 g/day	21 d	Trained cyclists	**Hb +4%**; ↓ HR; ↓ lactate; ↑ peak/avg sprint power
White & Gurney	*Chlorella*	6	2 d	Young healthy	↓ lactate; ↑ O_2 pulse; no

2024 [70]		g/day		adults	VO_2max effect
Kwak et al. 2012 [33]	*Chlorella*	5 g/day	8 wk	Healthy adults	↑ NK-cell activity; modulated IFN-γ, IL-1β, IL-12

8.9.1 Spirulina

- Ali, Aubeeluck & Gurney [61] demonstrated a 3.4% increase in Hb after 14 days of 6 g/day *Spirulina* in recreationally active cyclists; no VO_2max benefit.
- Gurney, Brouner & Spendiff [62] recorded significant reductions in submaximal HR and blood lactate, and significant augmentations in peak/average power during repeated-sprint bouts after 21 days of 6 g/day *Spirulina*; Hb +6%.
- Gurney & Spendiff [69] provides a comprehensive mechanistic review aligning with the antioxidant-driven hypothesis of §2.

8.9.2 Chlorella

- White & Gurney [70]: Two-day supplementation (6 g/day) lowered lactate and increased O_2 pulse during submaximal and maximal exercise; no VO_2max effect.
- Gurney, Brouner & Spendiff [63]: 21-day *Chlorella* (6 g/day) produced a 4% Hb increase; ↓ submaximal HR & lactate; ↑ peak and average power during sprint testing.
- Kwak et al. [33]: 8-week 5 g/day *Chlorella* significantly enhanced NK-cell activity and modulated serum IFN-γ, IL-1β and IL-12 in healthy adults — the only direct RCT linking *Chlorella* to cytokine-mediated immunostimulation.

8.9.3 Integration

The ergogenic and immunostimulatory responses to both algae appear to be mechanistically distinct: *Spirulina*-related lactate/HR effects are consistent with the antioxidant + glycerol-evaporative cooling hypothesis, while *Chlorella*-related NK-cell and cytokine effects are consistent with the polysaccharide-driven immune-balance mechanism discussed in §3.6 [23,33].

8.10 Evidence-based product positioning

Legitimate positioning language includes:

- "Provides antioxidant cellular protection" (consistent with §2).
- "Supports a healthy inflammatory balance" (consistent with §3).
- "Supports metabolic wellness" (consistent with §4).
- "Provides nutritional support for energy" (consistent with §6).
- "Supports healthy glucose and lipid metabolism" (consistent with §4 and §7.3).
- "Provides general cellular nutrition" (consistent with §6).

Claims that should be avoided include:

- "Cures diabetes" or "replaces metformin" — not supported by clinical data (§4).
- "Removes toxins" or "purifies blood" — not a recognised clinical outcome (§7).
- "Reverses ageing" or "regenerates youth" — not supported by clinical data (§6.9).
- "Rejuvenates damaged organs" — not a recognised clinical outcome.
- "Treats inflammatory diseases" — anti-inflammatory claims must be limited to biomarker support, not therapeutic effect.
- "Prevents cardiovascular disease" — claim not supported by RCT endpoint data (§2.10 and §7.3).
- "Replaces medicines" — never appropriate for nutraceuticals.

8.11 Research gaps

Open research questions for the field and for this product specifically include:

- Randomised controlled trials using the exact product described in §8.4, with the exact strain and cultivation background of the manufacturer.
- Dose-response studies in T2DM, NAFLD and metabolic-syndrome populations.
- Long-term (≥12-month) safety studies in healthy and at-risk populations.
- Product-specific biomarker analysis correlated with clinical outcomes.
- Standardisation of cultivation strains and post-harvest processing across batches.
- Head-to-head comparison with *Spirulina* alone, *Chlorella* alone, and placebo in clinically relevant compounds.

Qin et al. [67] covers EU New Resource Food Approval Regulation (EU 2015/2283) and Chinese national standards for *Arthrospira*, *Chlorella*, *Haematococcus pluvialis* and *Dunaliella salina* as novel food or additives. Vigani et al. [68] provides EU-level regulatory analysis confirming the restriction imposed by food-safety and novel-food regulations on microalgal products. Pereira et al. [71] addresses regulatory considerations for antioxidant-labelling claims under EFSA and FDA frameworks.

9. CONCLUSION

Limnospira platensis and *Chlorella vulgaris* are two of the most thoroughly studied edible microalgae. They exhibit complementary protein, pigment, polysaccharide, micronutrient and fatty-acid profiles, with *Spirulina* distinguished by its high GLA and C-PC content, and *Chlorella* distinguished by its chlorophylls, lutein and ALA-rich lipid fraction. Mechanistically, both — alone and in combination — show consistent:

- Direct free-radical scavenging and Nrf2-ARE-mediated induction of endogenous antioxidant enzymes *in vitro* and *in vivo*.
- Suppression of NF-κB and MAPK signalling, accompanied by reduced TNF-α, IL-6, IL-1β and CRP.
- Reductions in fasting plasma glucose, HbA1c and HOMA-IR in T2DM and NAFLD trials.
- Reductions in TC, TG and LDL-C and increases in HDL-C across multiple lipid-lowering studies.
- Membrane and mitochondrial protection through combined antioxidant, PUFA and chlorophyll mechanisms.

Based on these consistent observations, the combination of *Spirulina* and *Chlorella vulgaris* in a single nutraceutical formulation is scientifically plausible as an adjunctive nutritional support for cellular antioxidant defence, inflammatory balance, glucose metabolism, lipid metabolism and general cellular nutrition. However:

- Direct evidence of synergy between the two species in the exact combination discussed in §8 is limited. Existing clinical data derive overwhelmingly from single-species supplementation.
- Hard clinical endpoints (cardiovascular events, cancer incidence, mortality, hospitalisation) have not yet been examined for the combined product and remain absent for the single-species products.
- Claims of cell rejuvenation, blood purification or treatment of metabolic disease must be avoided unless supported by product-specific clinical evidence.

Future research should prioritise standardised trials using the exact combination, dose-response designs, long-term safety assessments and head-to-head comparisons with the individual species, in clearly characterised populations with measurable metabolic and immune dysregulation.

10. DECLARATIONS

10.1 Ethics approval and consent to participate

Not applicable. This review did not involve human participants, human data or experimental animals.

10.2 Consent for publication

Not applicable.

10.3 Availability of data and materials

All information evaluated in this review was obtained from published scientific literature cited in the manuscript. No new datasets were generated or analysed.

10.4 Competing interests

The authors are associated with Biotex Life Solutions Pvt. Ltd. involved in the development or commercialisation of the nutraceutical formulation discussed in this review. This relationship is disclosed for transparency.

10.5 Funding

Biotex Life Solutions Pvt. Ltd. supported this review. The sponsor did not influence the selection or interpretation of the scientific literature. This research received no external academic funding.

10.6 Authors' contributions

All authors contributed to the conception and design of the review. They conducted the literature search and drafted the manuscript. Authors critically reviewed the scientific content. All authors read and approved the final manuscript.

10.7 Acknowledgements

The authors acknowledge Biotex Life Solutions Pvt. Ltd. for technical, editorial or administrative support.

10.8 Use of artificial intelligence

Artificial-intelligence-assisted language tools were used for linguistic refinement and organisational support. All scientific statements, references and interpretations were reviewed and approved by the authors, who take full responsibility for the final manuscript.

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