ISSN 2468-8827

World NCD Federation

IJNCD

International Journal of Noncommunicable Diseases

An Official Publication of World NCD Federation
Volume 6 / Special Issue / November 2021
www.ijncd.org

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Original Article

Spirulina: A daily support to our immune system

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ABSTRACT

In recent years, the various health benefits of Cyanobacteria microalgae – such as *Arthrospira platensis*, commonly called Spirulina, an edible blue-green algae – have attracted scientific attention including micro-level examinations of its bioactive components. As a whole food and nutritional supplement, it serves as a plant protein source, which has shown positive effects across a wide range of human health concerns, from malnutrition to metabolic syndrome. Spirulina bioactives, such as essential amino acids, phycocyanin, polysaccharides, carotenoids, and chlorophyll, and essential vitamins and trace minerals, are responsible for its holistic actions against oxidative stress and inflammation, and its antiviral, antibacterial, and immune-modulating effects. Various *in vitro*, *in vivo*, and *ex vivo* experiments have established Spirulina's mechanism of action and its effect on immunity as a proof of concept. The phenolic compounds and extracellular metabolites released from Spirulina whole food after digestion are postulated to strengthen the epithelial lining with antibacterial effects against pathogenic bacteria, adding to its prebiotic effect on the gut microbiota (like Bifidobacterium and Lactobacillus) due to its fiber content. In this study, the digestibility of Spirulina was assessed by the determination of free amino acids and peptide release during the each phase of digestion in a simulated static digestive model system. The hypothesis bridging poor gut health to low-level inflammation and metabolic syndrome, and the potential to address those issues with nutritional supplementation, such as with Spirulina, could also be beneficial in the long run to reduce comorbid illnesses, such as those associated with the currently prevailing coronavirus disease 2019 pandemic.

Keywords: Algal proteins, amino acids, antioxidants, *Arthrospira platensis*, bioactive, chronic disease, digestion, gut health, immune system, metabolic syndrome, microbiome, peptides, spirulina

Introduction

Nutraceutical – the term itself is indicative of its nutritional and supportive action. The consumption of nutraceuticals is increasing worldwide due to the bourgeoning incidence of chronic diseases and increasing health consciousness. This has led to rising demands for nutritional supplements among consumers. And now, due to the current coronavirus disease 2019 (COVID-19) pandemic, the consumption of nutraceuticals and dietary supplements in the United States has trended even higher, with an annual sales growth of $345 million in 2020; similar trends have been observed globally, in countries such as India, China, New Zealand, and France.[1]

Purpose

To provide an overview of the scientific and clinical literature on the general health benefits of Spirulina, with a focus on its gut health, immunomodulatory, and antiviral effects, linked to its nutritional profile and antioxidant properties. Then to assess the digestibility of Spirulina using a static *in vitro* digestive model system. High

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Access this article online	
Website: www.ijncd.org	Quick Response Code
DOI: 10.4103/2468-8827.330650	

How to cite this article: Vasudevan SK, Seetharam S, Dohnalek MH, Cartwright EJ. Spirulina: A daily support to our immune system. Int J Non-Commun Dis 2021;6:S47-54.

Submitted: 30-Jul-2021 **Revised:** 15-Aug-2021
Accepted: 15-Sep-2021 **Published:** 19-Nov-2021

digestibility and bioavailability of the bioactive nutrients in Spirulina may explain its many positive physiological effects.

Background on spirulina

Spirulina *(Arthrospira platensis)*, belonging to the Cyanobacteria family and commonly called blue-green algae, has a rich nutrient profile yielding many promising health benefits.[2,3] Reports reveal extensive growth in the global Spirulina market size from $393.6 million in 2019, which is predicted to reach $897.61 million by 2027.[4] Hence, production of Spirulina is an important industry globally.

Spirulina has been deemed a superfood owing to its nutrient-dense biomass.[5-7] Spirulina is a plant protein enriched with phytopigments, including carotenoids, chlorophyll, and phycocyanin, and is generally regarded as safe to consume.[8,9] Phycocyanin, a light-harvesting pigment protein derived from Spirulina, has gained traction in the food industry as a bright blue natural colorant additive, which also shares value in medicine due to its multiple pharmacological actions proven in numerous preclinical studies. Spirulina is composed mainly of proteins, along with all the essential amino acids, and fatty acids, vitamins (302,000 IU/100 g Vitamin A as carotenes; 26 mg/100 g Vitamin B complex), and minerals (3.67 g/100 g).[10,11] Its antioxidant properties are attributed to the presence of phenolics, tocopherols, β-carotenes (0.29 g/100 g), chlorophyll a (1 g/100 g), gamma linolenic acid (1.1 g/100 g), polysaccharides (4.6 g/100 g), and crude phycocyanin (14 g/100 g).[12]

General health benefits

Based on a systematic review in 2018 by de la Jara *et al*., Spirulina has proven benefits in dyslipidemia, diabetes, hypertension, immune function, inflammation, and viral infections. The studies reviewed for the analysis were classified based on the clinical outcomes and the dosage used (ranging between 2 and 20 g/day), enabling the reader to interpret the data individually.[13] A similar meta-analysis in 2019 by Hamedifard *et al*. showed that the effectiveness of Spirulina in diabetes and dyslipidemia was due to its rich content of protein, polysaccharides, and carotenoids.[14] The quality protein stimulates the beta cells of the pancreas to secrete insulin and reduces interleukin (IL)-6 levels, which are more pronounced in abdominal fat, responsible, in part, for insulin resistance. The fiber content is postulated to reduce glucose and fat absorption in the small intestine, increase plasma cholesterol esterification by lecithin-cholesterol acyltransferase, and increase high-density lipoprotein cholesterol levels.[15] These are a few of the mechanistic actions of Spirulina for improving insulin sensitivity, metabolic syndrome, and cholesterol homeostasis.[16-20] These metabolic changes, along with Spirulina's various other physiological effects, can influence gut health, the immune response, and antiviral activities in the human body.

Gut health

Although the expression "gut health" lacks a clear definition in medicine, multiple data have shown the molecular links between effective gastrointestinal function to generalized wellbeing. Animal studies [Table 1] and some human data have demonstrated the importance of food digestion and the utilization of the digested ingredients to the gut microbiota and local immunity that regulates local and systemic inflammation. Thereby, the defined criterion for a healthy gastrointestinal system initiates from digestion and absorption of nutrients to a state of overall wellbeing.[26-28] In a review article by Ercolini and Fogliano, food that is partially digested with reduced bioavailability for absorption in the small intestine is utilized by large intestinal gut microbiota, which helps to maintain microbial diversity. In contrast, the easily digested nutrients with higher bioavailability from Western diets serve as calories and are not available for colonic fermentation or as a food source for the colonic microflora.[29] As this era has witnessed plenty of diseases attributed to changes in dietary patterns and early adoption of Western lifestyles, the impact on gut microbiota leads to gut dysbiosis, low-level inflammation, nonalcoholic steatohepatitis/nonalcoholic fatty liver disease, and metaflammation.[26,23]

To address the rising concerns of lifestyle diseases, the design of food ingredients and nutraceuticals that are nutritious to the host as well as beneficial to gut microbiota by enabling the body's innate capabilities in handling the oxidative stress due to infections or inflammation, are the need for hour. Spirulina microalgae rich in protein has been proposed to play an important role in gut health. Research has included *in vitro*, animal, and human studies on the mechanism of action and role of Spirulina with regard to increased gut microbial diversity and enhanced immunity.[21,22,24,25,30]

Immune-modulating effects

Various experiments have demonstrated that Spirulina possesses immunostimulatory and immunomodulatory effects by blocking the allergen-induced release of cytokines, like IL-4, IgE, leukotrienes, prostaglandins, and chemoattractants. An experiment in rats showed that

Table 1: Spirulina studies in various animal models

Effect on Microbial diversity and consequences	Reference
In old mice, spirulina exhibited antimicrobial action against pathogenic gut bacteria, thereby altering the bacteroides/ fermicutes ratio, with increased levels of roseburia and lactobacillus (otherwise seen in lower quantities with consumption of a high-fat diet) leading to reduced hepatic inflammation and NASH progression	Neyrinck *et al*., 2017[25]
In rats fed a high-fat diet, spirulina improved lipid metabolism and reduced fatty liver changes by upregulating AMPK-α and downregulating HMG-CoA pathway, by increasing specific beneficial bacteria like prevotella and SCFA producers in colon	Li *et al*., 2018[30]
In a mouse model of intestinal inflammation (colitis), spirulina displayed dose-dependent anti-inflammatory mechanisms in the colon and prevented damage in intestinal epithelial cell lining by modulating the cytokines IFN-γ and TNF-α	Garcia *et al*., 2020[21]
In rats fed a high-fat diet, spirulina supplementation increased the expression of tight junction proteins ZO-1, occludin, and claudin-1, maintaining gut epithelial lining and gut homeostasis and preventing chronic metabolic inflammation	Yu *et al*., 2020[22]
In healthy male mice, spirulina displayed dose-dependent positive effects on microbial diversity by changing a few physiological aspects such as oxidative stress, lipid profiles (leptin levels), and appetite control	Hu *et al*., 2019[24]

NASH – Nonalcoholic steatohepatitis, AMPK-α – Activated protein kinase-alpha, HMG-CoA – 3-hydroxy-3-methylglutaryl-coenzyme a reductase, SCFA – Short-chain fatty acid, TNF-α – Tumor necrosis factor-alpha, IFN-γ – Interferon gamma

Spirulina can inhibit allergen-induced histamine release and tumor necrosis factor-alpha (TNF-α) production by mast cells. Higher doses of Spirulina can even prevent anaphylaxis and animal death.[31,32] In another rat experiment, Spirulina extract could reduce acute inflammation and edema induced by carrageenan, as well as chronic inflammation and granuloma formation induced by cotton pellet implantation, at a dose comparable to standard indomethacin treatment. Spirulina extract also significantly reduced levels of TNF-α, IL-1 β, and IL-6.[33] In addition, Spirulina was reported to increase IgA levels in human saliva. IgA is a significant antibody in saliva, which has anti-adhesion, antibacterial, and antiviral effects.[34] Spirulina-sourced phycocyanin has also shown anti-inflammatory effects by reducing peroxidase-induced inflammatory edema in mouse paw by scavenging hydrogen peroxide and peroxyl radicals, both of which are responsible for local tissue damage.[35]

In a clinical study of 150 participants, 2000 mg per day of Spirulina given for 6 months significantly reduced allergic rhinitis symptoms compared to placebo.[36] A similar study done by Mao *et al*. in 36 participants with allergic rhinitis showed that the same dose of 2000 mg Spirulina per day for 12 weeks reduced serum levels of IL-4 in peripheral blood mononuclear cells.[37]

Anemia and immunosenescence are common in the elderly. In a study conducted by Selmi *et al*., Spirulina supplementation for 12 weeks improved anemia and immune health in a patient population aged 50 years and above with a history of anemia. Mean corpuscular hemoglobin levels and complete blood counts steadily increased during the course of the study, indicating a high level of iron bioavailability in the Spirulina. Moreover, after 6 and 12 weeks of Spirulina supplementation, the majority of subjects showed increased activity of indoleamine 2,3-dioxygenase enzyme, an important regulator of the immune system used as a surrogate measurement of immune function, along with increased white blood cell counts.[38]

Although various studies indicate molecular-level mechanistic changes and downstream regulation in JAK-STAT and nuclear factor kappa B pathways in cell line models, rigorous clinical studies are needed to validate the potential immunomodulatory effects of Spirulina and its extracts. The use of Spirulina as a supportive therapy for immunotherapeutic drug regimens may be justifiable based on its antioxidant and anti-inflammatory activities. In a recently published review article from 2020, McCarty and DiNicolantonio proposed that the use of nutraceuticals might function to counteract the oxidative stress produced by conditions like COVID-19, and promote early recovery by preventing further damage to the lung parenchyma induced by the pro-inflammatory cytokine cascade of the immune system in response to viral infections.[39]

Antiviral effects

Spirulina extracts have known *in vitro* and *ex vivo* benefits against viruses, such as herpes simplex virus (HSV), influenza virus, and human immunodeficiency virus (HIV), as demonstrated by reduced rates of viral infection and transmission in animal cell plaque assays by blocking syncytium formation and inhibiting hemagglutination.[40,41] The effective inhibitory dose (ED_{50}) of Spirulina against HSV-2 is 50%, and <20% for adenovirus.[42] The polysaccharides found in Spirulina extract can act as effective antiviral agents, have low anticoagulation activity, a long half-life in blood (approximately 150 min), and dose-dependent bioactivity.[43]

In a 6-month study of HIV-1-infected patients, 82 participants received 10 g of Spirulina per day, along with antiretroviral therapy (ART) and balanced nutrition, compared with 87 participants who received ART

and balanced nutrition alone. All were monitored for 12 months for disease progression, CD4 + T-cell counts, and viral loads. The Spirulina-treated group showed significant decreases in p24 antigen ($P = 0.001$), indicating lower viral loads, and a significant increase in $CD4^+$ T-cells ($P = 0.001$) compared to the group receiving the standard of care. Notably, there was an improvement in hemoglobin levels in some participants in Spirulina arm, further indicating its high iron bioavailability.[44]

In a recently published study from 2020, phycocyanin extracted from Spirulina was predicted to bind to the active site of the nsp12 viral polymerase of COVID-19, which is required for viral replication, and as such, is a potential drug target. In an *in silico* docking study, the most energetically favorable binding pose of phycocyanin in the active site of the nsp12 RNA-dependent RNA polymerase enzyme was similar to that of the natural substrate, adenosine triphosphate. The average binding energy of phycocyanin from 1000 poses was–4 kcal/mol. The negative values of the docking energies suggest that phycocyanin binds spontaneously without consuming energy. Although these docking experiments were computer simulated using complex mechanisms, the putative ability of phycocyanin to bind to this viral protein essential for COVID-19 replication and transmission represents a compelling prospect worthy of further investigation.[45]

An investigation in to the inherent digestibility of Spirulina can provide an understanding of the relevance of this microalgae to support gut health, and can add to our understanding of opportunities to address the global concern for lifestyle diseases and enhance the immune system with this important plant protein source. Thus, a study was undertaken to assess the digestibility of Spirulina and to identify characteristics of the breakdown products from *in vitro* digestion of this microalgae.

Materials and Methods

Study design

In vitro digestion

The *in vitro* protein and algal cell breakdown of Parry™ Organic Spirulina (Parry Nutraceuticals, Chennai, Tamil Nadu, India) was assessed using a static digestion model in experiments conducted at NIZO Food Research (NIZO Food Research BV, Ede, The Netherlands). Intact protein, and peptide and amino acid fractions were assessed after static oral, gastric and intestinal digestion phases. Sampling was done at 0-time (t = 0); after 60 min of simulated gastric digestion (G60); and after an additional 90 min of simulated digestion in the upper gastrointestinal tract (I90). Protein determination, peptide profiling and free amino acid determination were performed at specific timepoints during the static digestion experiments. In addition, a visual analysis was done. Pea protein isolate was used as a comparator, and whey protein isolate was used as a standard positive control for protein and amino acid analysis. The ingredient samples for testing were supplied by Parry Nutraceuticals, Chennai, India.

The ingredient powders were dissolved in reverse osmosis purified water at a concentration of 3% w/w of protein [Table 2] and subjected to the static digestion model as described by Wijayanti e*t al*. in 2019,[45] and shown in Figure 1. After the addition of artificial saliva (α-amylase in simulated salivary buffer), samples were incubated at 37°C for 5 min (simulated oral cavity, t = 0). Then, the pH was decreased to pH 2 over a period of 15 min by gradual addition of 1M HCl, followed directly by the addition of simulated gastric fluid (porcine pepsin and amino lipase A in a simulated gastric buffer) and incubated for 60 min (simulated gastric digestion, G60). Subsequently, the pH was increased to pH 6 by gradual addition of 5M NaOH over a period of 5 min, followed directly by the addition of simulated intestinal fluid (porcine pancreatin and bovine bile in a simulated pancreatic buffer) for 90 min of incubation (simulated intestinal digestion, I90). The digestion was ended by flash freezing the samples. Samples were taken at different stages during the *in vitro* digestion procedure: before digestion (t = 0), after the gastric phase (G60), and after the intestinal phase (I90). All samples were run in duplicate.

Visual appearance

Pea protein isolate and Parry Organic Spirulina samples taken from the start of the *in vitro* digestion experiment (t = 0), at the end of the gastric phase (G60), and at the end of the intestinal phase (I90) were photographed and analysed in a Hunter Colorimeter to determine Hunter $L^*a^*b^*$ color parameters.

Measurement of free amino acid levels

Samples were analysed by LC-MS/MS (EZfaast) to determine the free amino acid content, and values were corrected for the blank containing digestive solutions without added protein as a negative control. Based on this, an estimated percentage of protein breakdown into free amino acids in the samples was calculated, along with that for the positive control, whey protein isolate.

Table 2: Protein specifications and weights of protein powder samples to obtain a 250 g solution containing 3% w/w protein content

Sample	Protein content (g/100 g powder)	Weight of powder (g)	Weight of water (g)	Total weight (g)	Final protein concentration (% w/w)
Organic spirulina	63.5	12.5	237.5	250	3.17
Pea protein isolate	72.0	10.8	239.2	250	3.11
Whey protein isolate	88.6	8.8	241.2	250	3.13

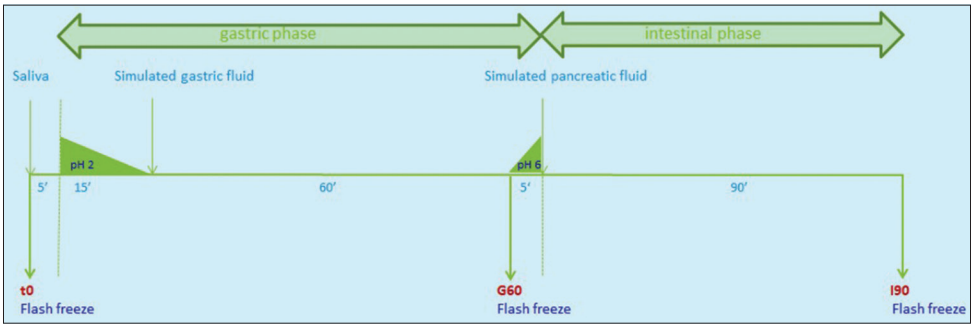

Figure 1: Schematic overview of static digestive model

Peptide profiling

A Spirulina sample, taken at the end of the intestinal phase and then freeze dried, was analyzed and compared to the starting material using QTOF/LC-MS for the purpose of peptide profiling. The retention time, mass and peak height of the detected peptides, within the detection limits of the equipment, are reported.

Results

Photographs of samples taken before *in vitro* digestion, after the gastric phase, and after the intestinal phase for the Spirulina and pea protein powders are shown in Figure 2a and b, respectively.

Hunter L*a*b* color analysis assesses three parameters of a sample: L* is an assessment of lightness. The scale ranges from 0 for totally black to 100 for totally white. Hunter a* color analysis is an assessment of color along the red-green axis. Positive (+) values are red and negative (−) values are green. Hunter b* color analysis is an assessment of color on the blue-green axis. Positive values (+) are yellow and negative values (−) are blue.

As shown in Figure 2a, Spirulina is dark green before digestion, green-yellow after the gastric phase and yellow-green after the intestinal phase confirmed by the L*a*b* color results which shows a decrease in green (a*) and an increase in yellow (b*) during digestion. Lightness (L*) increases, meaning samples become lighter, but the difference is minimal [Table 3].

For pea protein isolate [Figure 2b], the sample is middle gray (L* =50) at t = 0, and becomes darker with digestion. Hunter red-green color, a*, for pea protein isolate remains red with digestion (a* is negative), and more blue color is evident in the isolate sample at the end of simulated intestinal digestion [Hunter blue-yellow axis, b*, becomes negative; Table 3].

Spirulina samples had 3% free amino acids at t = 0, and 3% and 15% free amino acids after gastric phase digestion (G60) and intestinal phase digestion (I90), respectively. Baseline undigested pea protein isolate (t = 0) and samples taken after the gastric phase (G60) and intestinal phase (I90) had 0%, 0%, and 10% breakdown, respectively, into free amino acids. In comparison, for the internal reference, whey protein isolate, had 0% free amino acids at baseline (t = 0), and samples after G60 and I90 had 0%, and 17% breakdown, respectively, into free amino acids [Table 4].

The peptide profile of Spirulina was determined using QTOF/LC-MS before digestion and after the gastric and intestinal phases. Molecular weight distribution of peptides was determined from the retention time and the mass and peak heights of the detected peptides.

As shown in Figure 3, the total amount of peptides increased as digestion progressed and showed a shift from larger to smaller peptides as digestion progressed. In total 162, 860, and 1278 compounds were detected for the t = 0, G60 and I90 samples, respectively [Figure 3]. This shows that the number of low-molecular-weight peptides increases at the end of the intestinal phase, indicating that these peptides could be utilized by the host, which likely, at least in part, serves as the source of the health benefits Spirulina is known for. Further experiments may be required to show

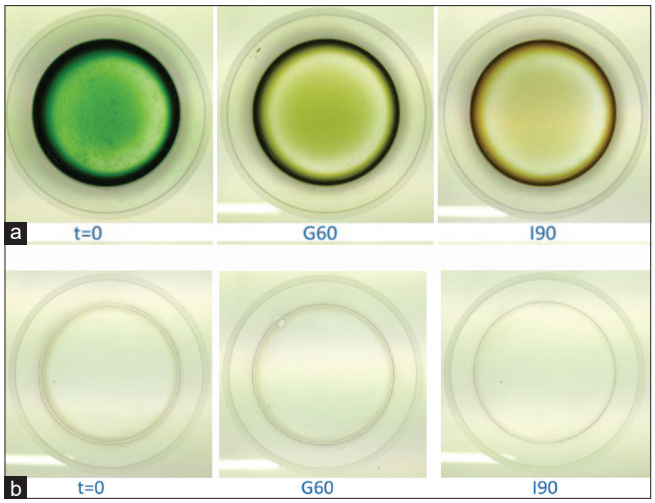

Figure 2: Visual appearance of Spirulina (a) and pea protein isolate (b) before (t = 0) and after gastric (G60) and intestinal phase (I90) digestion

the level of gastrointestinal absorption and the specific dynamic action of these peptides at a cellular level, as well as the utilization of broken cell walls by the gut microbiota.

Conclusions

Dietary support programs have been successful in strengthening medical care and health outcomes. Sustaining a high level of antioxidants in the body is essential for effective immune system function, especially in the case of a chronic disease burden.

To the best of our knowledge this is the first study to assess digestion of Spirulina protein and observe the release of amino acids and peptides at each stage of digestion. The results also demonstrate that Spirulina whole cell plant protein is comparable in protein content to other protein isolates. In addition, it is a better source of free amino acids at all stages of digestion. It is well known that nutraceuticals and food supplements are vital for the maintenance of homeostasis and healthy living, and microalgae has found its place in the human food chain to deliver nutrients for the host and the gut bacteria. The link between gut microbial metabolite release and cardiovascular health, liver health, and mental health has attracted researchers to design food around the maintenance of microbial diversity.

Along with various meta-analyses, review papers and proof of concept studies, Spirulina and its extracts, both of which contain high levels of phycocyanin, have shown proven health benefits due to their antioxidant properties, immune-modulating mechanisms, antigenic inhibition, and anti-fatigue effects. However, being naturally grown in open pond culture, the inherent

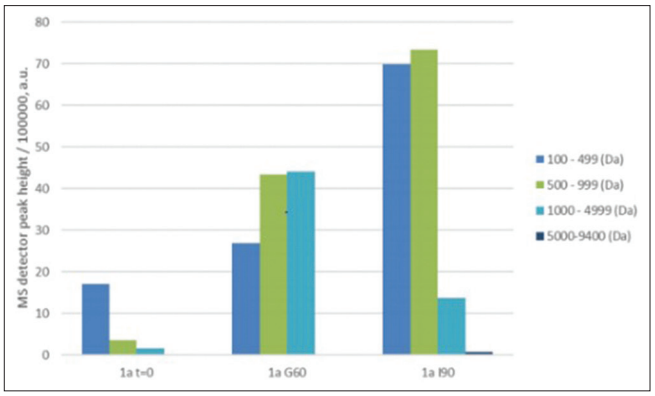

Figure 3: Molecular weight distribution of Spirulina peptides measured at t = 0, G60, and I90, corrected for the blank containing solutions without added protein

Table 3: Hunter L*a*b* color analysis results for spirulina and pea protein isolate

Sample (color)	T=0	G=60	I=90
Spirulina (L*)	4.24	7.06	5.17
Pea protein isolate (L*)	52.07	46.26	29.33
Spirulina (a*)	−0.06	−1.22	0.21
Pea protein isolate (a*)	−3.85	−4.94	−3.09
Spirulina (b*)	4.05	8.45	5.74
Pea protein isolate (b*)	0.89	−5.83	−4.11

Table 4: Percent breakdown into free amino acids for samples indicated. Values corrected for the blank containing digestive solutions without added protein

Sample	T=0	G=60	I=90
Spirulina	3	3	15
Pea protein isolate	0	0	10
Whey protein isolate	0	0	17

Before digestion (T=0); after gastric phase (G60); after intestinal phase (I=90)

challenges of environmental contamination and algal toxins cannot be ignored. Therefore, there must be standards for step-by-step quality checks and adherence of Good Manufacturing Practices with stringent regulatory certifications. As there is rising interest in the food industry for alternative plant protein sources which are water positive and have less impact on environment, microalgal protein could be the solution for providing quality proteins, with added phytonutrients and associated minerals, for human consumption every day.

Financial support and sponsorship

Nil.

Conflicts of interest

S.K.V. and S.S. are employees of Parry Nutraceuticals, the producer of Parry Organic Spirulina and Parry Organic Chlorella protein powders, which were used in the study. E.J.C. and M.H.D. are employees of US Nutraceuticals, Inc.

d/b/a Valensa International, which is a subsidiary of Parry Nutraceuticals.

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