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Effects of nitrogen fertilizer on the growth, chemical composition and antioxidant activity of *Chara corallina*

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Abstract The results showed nitrogen (N) fertilization can increase the growth performance, total chlorophyll, protein content, lysine, threonine, aspatatic acid, glutamic acid, arginine, phenolic content of *Chara corallina*. The alga in four treatments showed significant on chlorophyll, crude protein, amino acid profile. Most of the biochemical compositions in *C. corallina* increased significantly with the increased concentrations of nitrogen. The increasing nitrogen content in the culture system did not affect the phytochemical content of *C. corallina,* because nitrogen is not an essential element in the structure of phenolic, flavonoid and tannin compounds. Urea supplementation enhanced non-essential amino acids associated with flavor, particularly Glutamic acid and Aspartic acid, with the highest levels observed at 0.10 mg/L urea supplement, improving the nutritional and sensory quality of *C. corallina.* Additionally, nitrogen enrichment increased the antioxidant activity, likely due to elevated chlorophyll content. These findings suggested that optimizing nitrogen supply in algal cultivation can simultaneously enhance both the nutritional value and bioactive properties of *C. corallina*.

Keywords: Fertilizer, Phytochemical, Amino profile, Nitrogen, Freshwater algae

Introduction

Freshwater algae are an important source of food for humans, particularly in Thailand. Edible freshwater algae such as *Cladophora* and *Spirogyra* have long been consumed (Khuantrairong and Traichaiyaporn, 2012). In addition, *Chara corallina* (Cc) is a freshwater alga commonly consumed in Krabi Province, Thailand. Local communities collect *Chara corallina* (Cc) for

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household consumption, and it is also commercially available in local. Cc has attracted increasing attention as a promising nutritional and functional resource. Recent studies have demonstrated that Cc contains substantial levels of proteins, pigments, phenolic compounds, and flavonoids, along with notable antioxidant and enzyme-inhibitory activities. These properties highlight its potential applications as a nutraceutical or functional food ingredient (Chankaew *et al*., 2020; Khangsai *et al*., 2023; Chankaew *et al*., 2024). However, studies on its cultivation remain limited. Currently, Cc is primarily cultivated on a small household scale for local consumption, while most supplies are still harvested from natural water bodies. Therefore, the development of effective cultivation strategies is essential to ensure consistent production of high-quality biomass, particularly for enhancing bioactive compounds with potential health benefits.

Fertilizer is a material which contains a number of nutrient needed for algae to grow and survive. Nutrients especially nitrate and phosphate are the main limiting factors of algal growth in aquatic ecosystems, nitrogen (N) is a vital macronutrient regulating algal growth, protein biosynthesis, and the accumulation of secondary metabolites that contribute to antioxidant capacity. Nitrogen availability is widely recognized as a key factor regulating biomass production, nutrient allocation, and biochemical composition in aquatic macrophytes and macroalgae, particularly in relation to protein and pigment synthesis pathways (Lobban and Harrison, 1994; Markou *et al*., 2012). However, little is known about how nitrogen fertilization specifically influences Cc. Addressing, the present study investigates the effects of nitrogen fertilizer on its growth, nutritional value, phytochemical contents, and antioxidant activity, with the aim of identifying optimal conditions for producing biomass with both high yield and beneficial bioactive properties.

Nitrogen is one of the most essential macronutrients that plays a pivotal role in regulating plant and algal growth, metabolism, and productivity. As a major component of amino acids, nucleic acids, chlorophyll, and various secondary metabolites, nitrogen availability directly influences biomass accumulation and biochemical composition (Markou *et al*., 2014). In aquatic ecosystems and cultivation systems, the supplementation of nitrogen fertilizers has been widely applied to enhance the growth rate, improve nutritional quality, and stimulate the production of bioactive compounds. The present study was conducted and analyzed the effects of different concentrations of nitrogen simultaneously on growth and quality of *C. corallina*.

Materials and methods

Algal collection and culture experiment

Algal samples were collected from natural water sources and local markets in Krabi Province and were maintained in clean water under greenhouse conditions. Thalli of Cc obtained from natural habitats were thoroughly cleaned and acclimated in clean water in the greenhouse for two weeks. After the acclimation period, the thalli were cleaned again, and only healthy and intact thalli were selected for cultivation.

The experiment was conducted using a Completely Randomized Design (CRD) with four treatments and three replications. Bio-fermented (BF) solution was applied at a concentration of 0.026 mg/L, combined with three levels of urea supplementation (0.050, 0.075, and 0.100 mg/L, respectively). The cultivation was carried out using the plant seeding method, with an initial algal biomass of 0.25 g per liter of water. Plastic tanks with a capacity of 600 L were prepared and filled with a 5.0 cm layer of cleaned sand to serve as a substrate for algal attachment. The cultivation period was maintained for 35 days under these experimental conditions. At the end of the Cc cultivation, growth performance and quality were evaluated.

Measurement of biomass production

The fresh weight of Cc thallus was measured at the beginning and end of the experiment. The average daily gain ($ADG = (W_1\text{-}W_0)\ t^{-1}$), and specific growth rate ($SGR = [100 \ln (W_1/W_0)]\ t^{-1}$) were calculated using the following formula of Lobban and Harrison (1994).

Where, W_0 is the initial values of fresh weight; W_1 is the final values of fresh weight and t is the number of experimental days.

Algal quality analysis

Pigment analysis: Pigment composition was quantified by spectrophotometric methods. Chlorophyll *a*, chlorophyll *b*, and total carotenoids were determined following Costache *et al*. (2012), while β-carotene content was analyzed according to Nagata and Yamashita (1992).

Color measurement; Algal color was analyzed using Adobe Photoshop. Digital images were calibrated prior to analysis, and color values were obtained in the CIE L*a*b* color system.

Protein and amino acids; crude protein content was determined following the AOAC (1990) procedure. Amino acid profiles were analyzed using an in-house method adapted from the Journal of the Association of Official Analytical Chemists (1989).

Phytochemical analysis; ethanolic extracts of Cc were analyzed for phytochemical constituents. Total phenolic content was determined using the Folin-Ciocalteu method (Wolfe *et al*., 2003), total flavonoid content was assessed following Jia *et al*. (1999), and tannin content was quantified according to Tembe and Bhamba (2014).

Antioxidant assays; antioxidant activity of the ethanolic extracts was evaluated using three complementary assays. DPPH radical scavenging activity was determined following Fenglin *et al*. (2004), ABTS radical scavenging activity according of Re *et al*. (1999), and ferric reducing antioxidant power (FRAP) following Benzie and Strain (1996).

Water samples from experiment ponds were collected and analyzed (APHA, AWW and WPCF, 1985).

Statistical analysis

The data were presented as mean value ± standard deviation, comparision of mean values was made by one way analysis of variance followed by Tukey multiple comparision at a significance level of $p<0.05$.

Results

Growth performance and color contents

After 35 days of cultivation, growth performance of Cc, including average daily gain (ADG) and specific growth rate (SGR), differed significantly among treatments ($p<0.05$). The highest growth was observed in the fourth treatment, which received the highest level of urea fertilizer, whereas the control treatment showed the lowest growth performance, with ADG and SGR values of 0.57± 0.41 g/day and 1.77±0.61%/day, respectively (Table 1).

No significant differences were observed in the L* (lightness), a* (green - red), and b* (blue-yellow) color values of the Cc thallus among the experimental treatments (Table 1). The fourth treatment tended to exhibit a greener coloration than the other treatments.

Table 1. Growth performance and color values of *Chara corallina* cultivated at different nitrogen concentrations

Treatments	growth performance		color		
	ADG (gd^{-1})	SGR ($\%d^{-1}$)	*L**	*a**	*b**
1 (BF+U.0)	$0.57{\pm}0.14^{d}$	$1.77{\pm}0.61^{c}$	$38.33{\pm}4.72^{a}$	$-19.66{\pm}1.15^{a}$	$50.33{\pm}3.78^{a}$
2 (BF+0.050U)	$1.91{\pm}0.28^{c}$	$2.95{\pm}0.29^{b}$	$51.00{\pm}4.35^{a}$	$-23.33{\pm}1.52^{a}$	$35.33{\pm}4.16^{a}$
3 (BF+0.075U)	$2.21{\pm}0.29^{b}$	$3.25{\pm}0.27^{b}$	$45.77{\pm}1.15^{b}$	$-23.00{\pm}2.65^{a}$	$35.00{\pm}1.00^{a}$
4 (BF+0.100U)	$2.98{\pm}0.41^{a}$	$3.91{\pm}0.32^{a}$	$45.00{\pm}3.00^{a}$	$-24.33{\pm}0.58^{a}$	$36.33{\pm}4.51^{a}$
p-value	0.010	0.010	0.020	0.060	0.065

Note: values are presented as mean ± SD (n = 3), different superscript letters within a column indicate significant differences at p<0.05

Chemical composition

Pigment contents

The pigment content of Cc varied significantly among treatments. Chlorophyll *a* and chlorophyll *b* levels differed significantly ($p<0.05$), with treatment 4 showing the highest concentrations of both pigments. In the case of β-carotene, significant differences ($p<0.05$) were also observed; treatments 2, 3, and 4 contained similarly high levels, whereas treatment 1 had the lowest content. In contrast, no significant differences ($p>0.05$) were detected in the total carotenoid content among treatments. The crude protein content of the cultivated Cc was found to differ significantly among treatments ($p<0.05$) (Table 2). A clear trend was observed in which protein levels increased with increasing nitrogen concentrations.

Phytochemical content and antioxidant activities

Phytochemical content

The total phenolic content of the Cc differed significantly among treatments ($p<0.05$). However, no significant differences were observed among the nitrogen-fertilized groups ($p>0.05$), although a decreasing trend was evident with increasing nitrogen levels (Table 3). In contrast, total flavonoid content varied significantly among treatments ($p<0.05$), with the treatment 2 exhibiting the highest value was 1,497±29.33 mg catechin/g extract.

Table 2. Pigment contents of *Chara corallina* cultivated at different nitrogen concentrations

Treatments	Chlorophyll *a* (mg/g dw)	Chlorophyll *b* (mg/g dw)	β-carotene (mg/g dw)	Total carotenoids (mg/g dw)	crude protein (g/100g dw)
1 (BF+U.0)	1.30±0.01^{b}	0.56±0.06^{b}	0.028±0.00^{b}	0.225±0.00^{a}	16.87±1.22^{b}
2 **(BF+0.050U)**	1.35±0.00^{b}	0.73±0.05^{ab}	0.042±0.00^{ab}	0.203±0.00^{a}	18.70±2.20^{ab}
3 (BF+0.075U)	1.45±0.00^{ab}	0.79±0.00^{ab}	0.041±0.00^{a}	0.227±0.00^{a}	20.11±1.38^{a}
4 (BF+0.100U)	1.69±0.02^{a}	0.92±0.00^{a}	0.047±0.00^{a}	0.251±0.00^{a}	21.31±2.44^{a}
p-value	0.010	0.010	0.020	0.061	0.040

Note: Values are presented as mean ± SD (n = 3), different superscript letters within a column indicate significant differences at p<0.05

Table 3. Phytochemical composition of *Chara corallina* cultivated under different nitrogen concentrations

Treatments	Total phenolic content (mg GAE/g extract)	Total flavonoid (mgCAT/g extract)	Total tannin (mgTAE/g extract)
1 (BF+ U.0)	**149.82±9.35^{b}**	**830.67±26.52^{c}**	**682.81±132.72^{b}**
2 (BF+0.050U)	**370.11±24.05^{a}**	**1,497.00±29.33^{a}**	**1,656.60±207.45^{a}**
3 (BF+0.075U)	**356.00±35.83^{a}**	**1,215.96±55.02^{b}**	**1,308.73±229.79^{a}**
4 (BF+ 0.100U)	**345.09±5.52^{a}**	**1,160.23±28.34^{b}**	**1,429.88±85.41^{a}**
p-value	0.010	0.024	0.022

Note: Values are presented as mean ± SD (n = 3), different superscript letters within a column indicate significant differences at p<0.05

Antioxidant activities

The crude extracts of Cc showed significant differences (p<0.05) in DPPH radical scavenging activity (IC_{50}). Among the treatments, the extract from the fourth treatment exhibited the highest activity, with an IC_{50} value of 0.066±0.005 mg/mL. However, all crude extracts demonstrated lower DPPH scavenging capacity compared to the standard antioxidants, BHT and ascorbic acid. Similarly, the Cc extracts showed significant differences (p<0.05) in ABTS radical scavenging activity. The fourth treatment also exhibited the highest activity, with an IC_{50} value of 0.001±0.00 mg/mL. Nevertheless, all crude extracts showed lower ABTS scavenging efficiency compared to Trolox, a standard antioxidant (Table 4).

Ethanolic extracts of Cc cultured exhibited significant differences ($p< 0.05$) in Ferric reducing power assay. The crude extract from treatment 4 showed the strongest radical scavenging activity (DPPH, IC_{50}=0.066±0.005 mg/mL; ABTS IC_{50}=0.001± 0.00 mg/mL) (Table 4).

Table 4. Antioxidant activities of *Chara corallina* cultivated under different nitrogen concentrations

Treatments	Antioxidant activities (IC_{50}:mg/ml)		
	DPPH	**ABTS**	**FRAP**
1 (BF+ U.0)	0.069±0.005[c]	0.061±0.00[c]	7.25±0.26[b]
2 (BF+0.050U)	0.056±0.000[c]	0.008±0.00[b]	18.44±1.19[c]
3 (BF+0.075U)	0.068±0.003[c]	0.009±0.00[b]	16.19±1.23[c]
4 (BF+ 0.100U)	0.066±0.005[b]	0.001±0.00[a]	16.67±1.03[c]
ascorbic acid	0.009±0.000[a]	-	-
BHT	0.007±0.000[a]	-	-
trolox	-	0.001±0.00[a]	-
ascorbic acid	-	-	0.001±0.00[a]
p-value	0.01	0.01	0.032

Note: Values are presented as mean±SD (n = 3), different superscript letters within a column indicate significant differences at $p<0.05$

Amino acid profile

The amino acid profile of Cc obtained from cultivation showed the presence of 10 essential amino acids and 8 non-essential amino acids. Among the essential amino acids, Phenylalanine was found in the highest concentration, followed by Valine. For the non-essential amino acids, Glutamic acid was the most concentration of 2,877.12 mg/100 g dw, followed by Aspartic acid at 2,499.66 mg/100 g dw (Table 5).

Discussion

Growth performance, amount of pigments and color

The growth rate of Cc increased with increasing nitrogen concentrations. This pattern indicates that nitrogen availability plays a decisive role in promoting algal growth. Nitrogen is a fundamental element required for the synthesis of proteins, nucleic acids, and chlorophyll, all of which are essential for cellular metabolism and photosynthesis. The enhanced growth performance observed in this study is consistent with previous reports indicating that increased nitrogen availability promotes biomass production and metabolic activity in macroalgae

(Markou and Georgakakis, 2012). Similar findings have been reported in *Ulva* species, where increased nitrogen supply significantly enhanced growth and pigment accumulation (Zheng *et al*., 2019). These findings underscore the critical role of nitrogen supply in optimizing algal cultivation systems and enhancing biomass productivity

Table 5. Amino acid profile of *Chara corallina* cultivated under different nitrogen concentrations

Amino acid (mg/100 g dw)	**Treatments**			
	1 (BF + 0 U)	**2 (BF + 0.050 U)**	**3 (BF + 0.075 U)**	**4 (BF + 0.100 U)**
Essential amino acid				
Arginine	486.11	836.10	641.19	651.75
Histidine	332.37	629.80	504.03	476.21
Isoleucine	492.85	704.12	626.37	585.97
Leucine	641.06	1,085.65	788.73	722.55
Lysine	797.40	733.09	808.73	1,141.63
Methionine	232.77	380.25	314.63	295.81
Phenylalanine	713.07	1,059.21	934.16	791.99
Threonine	1,255.67	1,046.13	1,025.23	1,403.72
Tryptophan	231.20	251.93	373.63	379.50
Valine	1,008.73	1,416.69	1,259.96	1,183.80
Nonessential amino acid				
Alanine	1,590.95	1,915.00	1,709.29	1,765.42
Aspartic acid	1,791.70	1,908.68	1,746.19	2,588.35
Cystine	1,168.79	1,337.56	1,478.46	1,756.99
Glutamic acid	2,873.69	3,908.96	3,776.16	4,489.86
Glycine	1,117.78	1,261.38	1,550.46	2,018.91
Proline	858.36	974.96	1,101.52	1,000.59
Serine	743.08	901.10	902.82	1,061.37
Tyrosine	349.04	488.54	372.83	355.79
Other amino acid				
Asparagine	430.43	630.01	556.88	518.78
Hydroxylysine	nd	nd	nd	nd
Hydroxyproline	nd	nd	nd	nd
Cysteine	nd	nd	nd	nd

Note: nd = not detected

The color of Cc thallus tends to greener hues with increasing nitrogen levels, consistent with the increases in chlorophyll *a* and *b* contents. Enhanced nitrogen availability likely stimulated chlorophyll biosynthesis, improving photosynthetic efficiency and energy capture. This, in turn, may have supported higher growth rates, as reported in green macroalgae where nitrogen enrichment enhanced both pigment accumulation and biomass production (Kim *et al*., 2015; Gao *et al*., 2017). The positive effect of urea supplementation may be further attributed to its rapid conversion to ammonium, which can be readily assimilated for chlorophyll and amino acid synthesis, promoting overall algal productivity.

Phytochemical contents

The increasing nitrogen content in the culture system did not significantly affect the phytochemical content of Cc as nitrogen is not a structural component of phenolic, flavonoid, and tannin compounds (Crozier *et al*., 2006; Taiz and Zeiger, 2010). The trend was consistent with that of total phenolic content and total tannin content, suggesting that different levels of nitrogen fertilization did not markedly influence these two groups of phytochemicals. Nevertheless, the tannin content in ethanolic extracts of Cc cultured with urea fertilizer was higher than that reported for extracts obtained using other organic solvents such as ethyl acetate, ethanol, and methanol (Chankaew *et al*., 2020). Under high nitrogen conditions, algae preferentially allocate nitrogen to the synthesis of proteins, nucleic acids, and other primary growth processes, which consequently downregulates the production of secondary metabolites such as flavonoids (Stewart *et al*., 2001; Goiris *et al*., 2012). This metabolic allocation indicates that flavonoid biosynthesis is tightly regulated by nutrient availability and the trade-off between primary and secondary metabolism. Conversely, nitrogen limitation can stimulate the accumulation of flavonoids and other phenolic compounds as part of a stress-induced response, enhancing antioxidant capacity (Goiris *et al*., 2012). Therefore, the observed reduction in flavonoid content under elevated nitrogen levels reflects a reallocation of cellular resources from secondary metabolite synthesis toward growth-related processes and protein production.

Antioxidant activities

The crude extracts obtained in this study exhibited stronger radical scavenging activity than those derived from organic solvents such as ethyl acetate, ethanol, and methanol, as reported by Chankaew *et al*. (2020). These antioxidant effects can be attributed to phenolic compounds and flavonoids, which act as hydrogen or electron donors to neutralize free radicals (Rice-Evans *et al*., 1997; Prior *et al*., 2005). The FRAP results indicated that urea supplementation did not significantly influence ferric reducing capacity, consistent with the comparable total phenolic and flavonoid contents across treatments. This finding suggests that nitrogen availability selectively modulates radical scavenging activity rather than overall reducing power.

The strong ABTS radical scavenging activity observed in the ethanolic extract of Cc, particularly in treatment 4, can be attributed to its high flavonoid content (Table 4). Flavonoids act as effective hydrogen or electron donors, thereby stabilizing free radicals and interrupting oxidative chain reactions (Rice-Evans *et al*., 1997). This mechanism is reflected in the low IC_{50} values obtained in this study. Similar findings have been reported in other algae, including *Ulva* spp. and *Sargassum* spp., where flavonoid-rich extracts exhibited strong ABTS scavenging activity (Yangthong *et al*., 2009; Ganesan *et al*., 2019). Moreover, studies in terrestrial plants have demonstrated a positive correlation between total flavonoid content and antioxidant capacity, reinforcing the role of these compounds as major contributors to radical scavenging activity. Therefore, the superior ABTS scavenging ability of *Caulerpa* ethanolic extract highlights the importance of flavonoids as key bioactive constituents in marine algae with potential nutraceutical.

The ferric reducing power observed in the crude ethanolic extracts of Cc is generally associated with the presence of phenolic compounds, particularly flavonoids, which are capable of donating electrons to reduce Fe^{3+} to Fe^{2+}. This mechanism underlies the strong correlation frequently reported between total phenolic content and FRAP values in both algae and terrestrial plants (Prior *et al*., 2005). However, in the present study, no significant differences were detected among the urea-supplemented treatments (2-4), which is consistent with the comparable levels of total phenolics, flavonoids, and tannins measured in these groups. This finding suggests that nitrogen fertilization did not markedly alter the biosynthesis of these secondary metabolites in Cc, thereby resulting in similar ferric reducing capacities. Such results highlight that, while phenolic compounds are key contributors to FRAP activity, their antioxidant effects may reach a plateau when their concentrations are maintained within a narrow range across treatments.

The FRAP antioxidant activities did not differ significantly with increasing nitrogen content. It can be concluded from the results that increasing nitrogen content in the culture system did not affect the antioxidant content or antioxidant activity of Cc. This may be because nitrogen is not an essential structural component of phenolic, flavonoid and tannin. That was because nitrogen is not an essential element in the structure of phenolic, flavonoid and tannin compounds. These findings are consistent with those reported by Tanchaiha *et* *al.* (2021), who found that differences in nitrogen fertilizer concentrations did not significantly affect phenolic content or antioxidant activity.

The antioxidant activity observed in Cc may be attributed to the presence of photosynthetic pigments, such as chlorophyll *a*, chlorophyll *b*, β-carotene, and total carotenoids. These pigments play a crucial role in quenching singlet oxygen and neutralizing free radicals through electron or hydrogen atom donation, thereby stabilizing radical species. This mechanism may partly explain the higher scavenging capacity of the crude extract in treatment 4 compared with other treatments. However, the IC_{50} values obtained were higher than those reported for freshwater algae such as *Spirogyra* spp., which are known to contain various bioactive compounds with antioxidant properties, suggesting that although pigments contribute to radical scavenging in *Caulerpa* sp., other bioactive compounds present in freshwater algae may exert stronger antioxidant effects.

Amino acid profile

The experimental results indicated that the amino acid composition of cultured Cc varied significantly in response to different levels of urea combined with biofertilizer. Among the essential amino acids, low urea supplementation (supplement 0.05 mg/L) resulted in higher concentrations of several amino acids, including Leucine, Phenylalanine, and Valine, suggesting an effective nitrogen utilization for protein synthesis. However, when the urea supplement was increased to 0.10 mg/L, the levels of certain amino acids, such as Histidine and Leucine, decreased, whereas Threonine and Lysine reached their highest concentrations. These findings indicate that Cc can modulate amino acid biosynthesis in response to nitrogen availability, reflecting an adaptive balance in protein metabolism according to the nutrient environment.

For non-essential amino acids, the results demonstrated that urea supplementation stimulated higher levels of amino acids associated with flavor, including Glutamic acid, Aspartic acid, Alanine, and Glycine. This effect was particularly pronounced at the higher urea level (0.10 mg/L), where Glutamic acid reached 4,489.86 mg/100 g dw and Aspartic acid 2,588.35 mg/100 g dw. These findings are significant, as these amino acids contribute to the umami and sweet taste of Cc, enhancing both its nutritional value and sensory quality.The observed enhancement of essential amino acid (EAA) synthesis in *Caulerpa* sp. in response to urea supplementation can be attributed to the direct provision of inorganic nitrogen in the form of ammonium ions (NH_4^+), which serve as key precursors for amino acid biosynthesis. Biofertilizer likely contributed additional growth-promoting compounds, such as vitamins, phytohormones, and trace minerals, which may have synergistically enhanced nitrogen assimilation and metabolic activity.

These findings are consistent with previous studies in marine and freshwater algae, where nitrogen supplementation was shown to increase the total EAAs content and alter the amino acid profile. For instance, Phan *et al*. (2017) reported that urea addition to *Ulva lactuca* cultures enhanced Lysine and Threonine synthesis, while Zhao *et al*. (2020) found that combined organic and inorganic nitrogen sources improved both protein content and EAAs composition in *Chlorella vulgaris*. Similarly, the use of biofertilizers or organic amendments has been reported to improve nitrogen assimilation efficiency and overall protein quality in edible algae (Hassan *et al*., 2019). Overall, these results suggest that the combined supplementation of urea and biofertilizer not only provides readily available nitrogen for amino acid synthesis but also supplies growth-promoting factors that optimize metabolic flux through EAAs biosynthetic pathways. This integrated approach results in a more complete and balanced EAA profile compared to biofertilizer when used individually, highlighting its potential for enhancing both the nutritional and functional quality of Cc as a protein-rich food source.

In conclusion, the combined supplementation of urea and biofertilizer improved radical scavenging activity and enhanced amino acid composition, yielding a nutritionally superior and functionally enriched Cc biomass. This integrated approach demonstrates the potential of strategic nitrogen and biofertilizer application to enhance both antioxidant capacity and protein quality, thereby increasing the value of cultured edible algae as sustainable sources of high-quality protein and functional ingredients for food and nutraceutical applications.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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