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DETERMINATION OF VITAMIN C AND VITAMIN B_6 CONTENT IN SELECTED SEAWEEDS FROM THE BAY OF BENGAL, BANGLADESH

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Abstract

Seaweeds contain a broad range of vitamins, particularly water-soluble vitamins. The objective of this study was to measure the levels of vitamin C and vitamin B_6 in seaweed samples by acidic and enzymatic hydrolysis, followed by reverse-phase high-performance liquid chromatography (RP-HPLC) analysis using a UV detector. Vitamin C and vitamin B6 contents ranged from 1.62 to 48.28 mg/100 g and 3.54 to 22.24 mg/100 g, respectively. *Sargassum* sp. collected from Teknaf showed the highest vitamin C content, while *Gracilaria* sp. contained the highest level of vitamin B_6. The vitamin levels varied depending on the seaweed species. However, this study revealed that seaweed can be a good source of vitamin C and vitamin B_6. Therefore, it is possible to conclude that this seaweed has the potential to serve as an alternative source of vitamins for food security in Bangladesh.

Introduction

Seaweeds are a type of macroscopic algae that are highly nutritious. They contain high levels of vitamins and secondary metabolites such as phenols, amino acids, alkaloids, saponins, tannins, flavonoids, glycosides, and terpenoids. They also include water-soluble vitamins like B_1, B_2, B_6, B_{12}, and fat-soluble vitamins such as A, D, E, and K (Kiran and Subbaiah 2020). These abundant vitamins, minerals, polysaccharides, and secondary metabolites contribute to their functional properties, making them valuable in the food, pharmaceutical, and nutraceutical industries (MacArtain *et al*. 2007, Holdt and Kraan 2011). Of these micronutrients, vitamin C (ascorbic acid) and vitamin B_6 (pyridoxine) are especially important, given their critical roles in physiology. Vitamin C is a powerful antioxidant involved in collagen synthesis, immune regulation, and iron metabolism (Padayatty *et al*. 2003). Vitamin B_6 plays a key role in amino acid metabolism, neurotransmitter synthesis, and cellular energy pathways, all essential for maintaining metabolic balance. The Bay of Bengal is renowned for its vibrant ecosystems and abundant marine life. Seaweeds are a natural treasure of the Bay, presenting in various colors, shapes, and sizes. These marine algae are not only visually stunning but also crucial to maritime ecosystems (Marinho-Soriano *et al*. 2006). They are considered the principal producers within the marine ecology (Wells *et al*. 2017).

Vitamin C is a water-soluble vitamin commonly referred to as ascorbic acid. It is a necessary vitamin for our body and has a significant nutritional value. It is involved in the tissue healing mechanism and enzyme creation, which is essential for the generation of certain neurotransmitters. It is also required for the functioning of many enzymes, all of which are critical for immune system functions (Barnes and Kodicek 1972). It also acts as an antioxidant (Meister 1992). Several clinical examples have been published demonstrating that mild vitamin C deficiency increases illness risk, including the common cold, cancer and COVID-19 (Hemilä and Chalker 2013).

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Vitamin B_6, also known as pyridoxine hydrochloride, is a water-soluble vitamin (Hanna *et al*. 2022). It is required for the proper development of the brain, neurological and immune system functions, and hormonal regulations. Vitamin B_6 produces antibodies and hemoglobin, degrades proteins, and maintains proper glucose levels. Vitamin B_6 deficiency has been related to microcytic anemia, electroencephalographic abnormalities, dermatitis with cheilosis (scaling on the lips and fissures at the corners of the mouth), glossitis (swollen tongue), sadness and anxiety, and impaired immune functions (Parra *et al.* 2018). Vitamin B_6 concentration can vary during the human life cycle. Adults require 1.2-1.9 milligrams of vitamin B_6 per day (Aktar *et al*. 2025).

The deficiency of vitamins is common in both low- and middle-income countries, where women are more affected than men. Vitamin C and B_6-deficient instances have recently been recorded in hospitals, medical institutions, and medical research facilities in Bangladesh (Parra *et al*. 2018). Vitamin B_6 is found in poultry meat, fish, potatoes, and starchy vegetables, but seaweeds may be the richest source of such vitamins and are widely accessible in Bangladesh, particularly in the coastal areas (Bay of Bengal) of Teknaf and Cox's Bazar. Seaweed is high in vitamin K, but it also contains vitamins A, D, C, B_1, B_2, and B_6.

To assess the vitamin content in the seaweed, it is necessary to develop some simple vitamin C and B_6 extraction and determination methods, and to enable the use of seaweed as a source of such vitamins (Ali *et al*. 2024, Mia *et al*. 2024).

In this study, seaweed samples were collected from the vicinity of Cox’s Bazar and the Teknaf area in the Bay of Bengal, Bangladesh, to evaluate the levels of ascorbic acid (vitamin C) and pyridoxine hydrochloride (vitamin B_6) for food security. Vitamin C and vitamin B_6 were extracted from seaweeds using a step-by-step process that included hydrolysis with HCl, treatment with the takadiastase enzyme, and analysis with HPLC (Kayesh and Sultan 2015, Begum *et al.* 2019, Islam *et al*. 2025). Using this method, we aim to provide a thorough understanding of the vitamin C and B_6 content in seaweeds.

Materials and Methods

Ascorbic acid (vitamin C) and pyridoxine hydrochloride (vitamin B_6) standards were purchased from Sigma Aldrich, Germany. All of the chemicals used in vitamin extraction and standard solution preparation, including hydrochloric acid, acetic acid, takadiastase enzyme, sodium acetate trihydrate, sodium dihydrogen phosphate, trimethylamine, and HPLC-grade methanol were also purchased from Sigma Aldrich (St. Louis), and double-distilled water was provided by the Drug Analysis Research Laboratory, Centre for Advanced Research in Sciences (CARS), University of Dhaka, Bangladesh.

Ten fresh seaweed samples, namely *Sargassum platycarpum, Ulva fasciata, Ipomoea pescaprea, Ulva reticulata, Eucheuma spinosum, Gracilaria lemineiformis, Enteromorpha clathrata, Caularpa* sp.*, Sargassum* sp., and *Glacilaria* sp.*,* were collected in zip-lock bags from the coastal areas (Bay of Bengal) of Cox-Bazar and Teknaf, Bangladesh and identified by a seaweed specialist. Although *Ipomoea pescaprae* is not a seaweed, it is a terrestrial creeping vine commonly found along the coast of Cox’s Bazar. Locally, it is known as Beach Morning Glory and is often mistaken for seaweed. Its leaves are rich in vitamin C and provide a good source of antioxidants (Manigauha *et al*. 2021); therefore, we included it as a seaweed sample in our study. Samples were dried at 30°C and stored at room temperature for further processing (Aktar *et al.* 2025).

One gram (1.0 g) of each dried homogeneous sample was taken in a 250 ml conical flask and mixed with 10 ml of (5% acetic + 3% metaphosphoric) acid solution to extract vitamin C. The 3% metaphosphoric acid solution is used as a stabilizing agent to prevent the oxidation of vitamin C.

Prepared solution was centrifuged at 6000 rpm for 20 min after being mixed. Centrifugation is used to clear the extract and remove solid debris, preventing damage to the HPLC machine and ensuring accurate results. Supernatant from each sample was filtered through a Whatman filter paper No. 1 and collected in a 5 ml volumetric flask, followed by further filtration with a membrane filter (0.22 µm). After filtration, the sample was taken into a 1.5 ml amber-colored HPLC vial (Sami *et al.* 2014). All the samples were analyzed immediately after extraction to minimize the oxygen exposure during vitamin C analysis, and each analysis was triplicated for statistical evaluation.

Two grams (2.0 g) of each dried sample was taken in a 250 ml individual conical flask. To extract vitamin B_6, 50 ml of 0.1 M hydrochloric acid was added to each flask and kept in a water bath at 100°C for 30 min. After cooling to room temperature, 100 mg/g of taka-diastase enzyme with 2.5 M sodium acetate buffer (pH 4.5) was added to each flask and incubated at 37°C for 24 hrs. After incubation, they were heated to 100°C to inactivate the enzyme. For effective extraction, starches are broken down and bound vitamin B_6 is released by using takadiastase. Samples were then filtered with Whatman filter paper No. 1 and kept in amber flasks. After membrane filtration (0.22 µm), samples were taken in 1.5 ml amber-colored HPLC vials and preserved at 4°C until analysis. Each sample was analyzed in triplicate to ensure statistical reliability.

2.5 mg of vitamin C and vitamin B_6 were taken in 100 ml (5% acetic acid + 3% metaphosphoric acid) solution and 0.1 M HCL solutions, respectively, to make 100 ppm concentration of each vitamin stock solution. Standard vitamin solutions were treated in the same way as samples to ensure accurate comparison, degradation, accounting for any losses, or changes that may occur throughout the extraction and analysis process. After that, the standard vitamins solution of 10, 20, 40 and 60 ppm was prepared in different Erlenmeyer flasks from the stock solution and used as working solutions. To prepare for HPLC analysis, an aliquot of each concentration was filtered through a 0.22 µm membrane filter and stored in a 1.5 ml amber glass vial (Ali *et al.* 2024).

The analyses were conducted on a reversed-phase C18 (4.6 mm × 250 mm, 5 µm, Kromasil) column with a mobile phase of methanol-water (15:85) and pH 2.5 with 0.1 N acetic acid solutions and the flow rate was maintained at 1.0 ml/min. The analyses were monitored by a UV detector at 254 nm and the system was equilibrated to get a stable baseline and consistent peak height and retention time (Ali *et al*. 2024).

The analyses were carried out on a reversed-phase C18 column (4.6 mm × 250 mm, 5 µm, Kromasil) using the mobile phase consisting of methanol-phosphate buffer (15:85) and 0.018 M trimethylamine (pH 3.55) at a flow rate of 1.0 ml/ min. The system was equilibrated and monitored with a UV detector analysis at 290 nm to get a steady baseline, as well as uniform peak height and retention times (Lebiedzińska *et al.* 2007).

The extracted standard of vitamin C and vitamin B_6 was prepared at four distinct concentrations: 10, 20, 40, and 60 ppm. Fifty microliter (50 µl) of each standard solution was injected. The UV-detector at 254 nm was used to monitor vitamin C analyses (Abdullah 2016), while the UV-detector at 290 nm was used to monitor vitamin B_6. Each study was repeated three times to get an accurate result, and a calibration curve was prepared by plotting peak area against concentration. Regression coefficient r^2 values were calculated to prove the linearity of the method (Islam *et al.* 2024, Mia *et al.* 2024). The r^2 value $\geq$0.995 indicates linearity and confirms that the method is suitable for accurate quantification.

Results and Discussion

The average areas of the peaks were plotted against concentration to get the calibration curve for ascorbic acid (vitamin C) and pyridoxine hydrochloride (vitamin B_6) (Mia *et al.* 2024). The correlation coefficients (r^2) for vitamin C and vitamin B_6 were found as 0.9934 and 0.9977, respectively (Fig. 1).

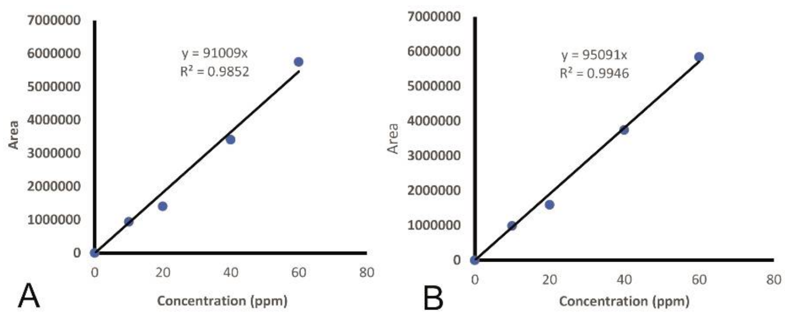

Fig. 1. Calibration curves of (A) ascorbic acid (vitamin C), and (B) pyridoxine hydrochloride (vitamin B_6).

At 254 nm, the peak for the vitamin C standard solution appeared at 2.87 ± 0.001 min, and for the extracted seaweed sample at 2.85 ± 0.012 min (Figs 2A and 2B). At 290 nm, the retention time (Rt) of vitamin B_6 was found at 3.51 ± 0.015 min for the reference standard, whereas 3.55 ± 0.017 min for samples (Figs 2C and 3D).

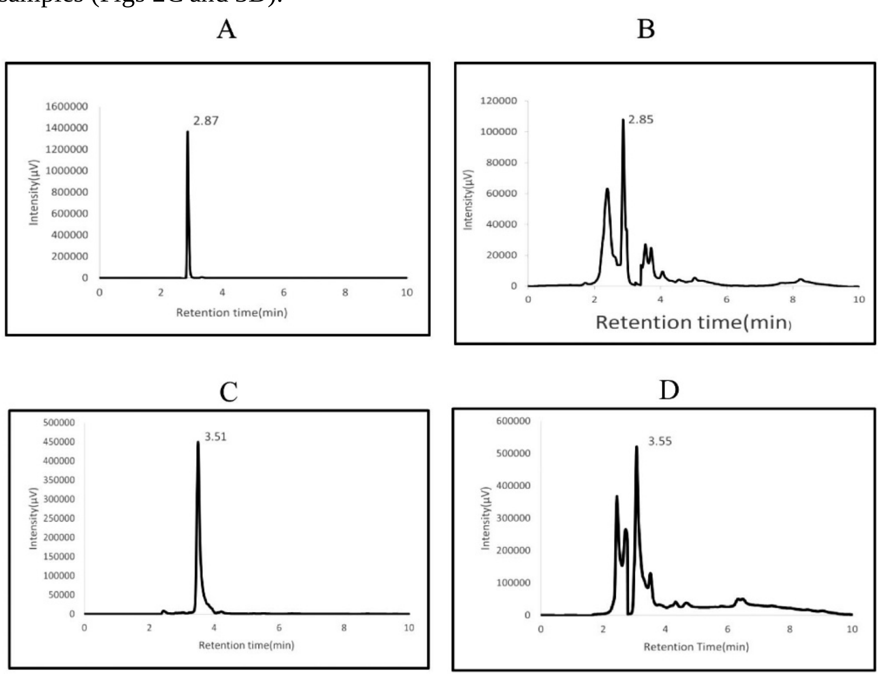

Fig. 2. Chromatograms for the standards and samples. (A) ascorbic acid (vitamin C, 60 ppm standard), (B) vitamin C in sample *S. platycarpum*, (C) pyridoxine hydrochloride (vitamin B_6) (60 ppm standard), and (D) vitamin B_6 in sample *G. lemineiformis.*

HPLC was used to determine the levels of vitamin C and vitamin B_6 in ten seaweed samples. The values (Table 1) derived from the calibration curve were used to calculate the amount of vitamin C and vitamin B_6. The Limit of Detection (LOD) and Limit of Quantification (LOQ) in this study were calculated from the standard curves using the equation $LOD = 3.3 \times (\sigma/S)$ and $LOQ=10 \times (\sigma/S)$, where σ is the standard deviation of the response, and S is the slope of the calibration curve. σ is typically determined from the standard deviation of the y-intercept or the residual standard deviation of the regression line. The values indicate the lowest concentrations of the analyte that can be reliably detected and accurately quantified, respectively. The vitamin content of seaweed samples is summarized in the Table 2.

Table 1. Analytical parameters employed to assess vitamin C and vitamin B_6 content in marine seaweed samples.

Parameters	Vitamin C	Vitamin B_6
Slope (m)	91009	95091
Y- Intercept	0.0	0.0
Regression coefficient (r^2)	0.9934	0.9977
LOD (ppm)	0.16	0.17
LOQ (ppm)	0.48	0.50

Table 2. Levels of ascorbic acid (vitamin C) and pyridoxine hydrochloride (vitamin B_6) found in the seaweed samples.

Voucher No.	Samples Name	Level of vitamin C and vitamin B_6 (mg/100 g) in seaweeds	
		Vitamin C	Vitamin B_6
DARL 63	*Sargassum platycarpum*	5.66 ± 0.71	6.95 ± 0.98
DARL 64	*Ulva fasciata*	3.75 ± 0.44	3.54 ± 0.01
DARL 65	*Ipomoea pes-caprea*	11.07 ± 1.32	3.79 ± 0.10
DARL 66	*Gracplania lemineiformis*	10.61 ± 4.08	7.51 ± 0.53
DARL 67	*Eucheuma spinosum*	9.37 ± 0.74	15.59 ± 1.63
DARL 68	*Enteromorpha clathrata*	21.34 ± 0.17	17.26 ± 0.41
DARL 69	*Caularpa sp.*	1.62 ± 0.06	3.62 ± 0.63
DARL 70	*Sargassum sp.*	48.28 ± 0.29	17.08 ± 0.42
DARL 71	*Ulva reticulata*	13.27 ± 2.42	8.44 ± 0.66
DARL 72	*Glacilaria sp.*	14.43 ± 1.22	22.24 ± 0.02

*Values expressed in Mean ± SD in mg/100 g of Seaweed.

The analyses revealed that the vitamin C content in seaweed samples ranged from 1.62 ± 0.06 mg/100 g to 48.28 ± 0.29 mg/100 g**,** while vitamin B_6 content varied between 3.54 and 22.24 ± 0.02 mg/100 g**.** Overall**,** the **v**itamin B_6 level was higher than the vitamin C level across the samples. The highest vitamin B_6 content (22.24 ± 0.02 mg/100 g) was found in the *Gracilaria* sp. seaweed, whereas the highest vitamin C concentration (48.28 ± 0.29 mg/100 g) was observed in the *Sargassum* sp. Interestingly**,** *Sargassum* sp. also contained a high level of vitamin B_6 (17.08 ± 0.42 mg/100 g) (Fig. 3), suggesting that it could be a rich source of both vitamins. These findings indicate that seaweed may serve as an alternative source of essential vitamins for human nutrition.

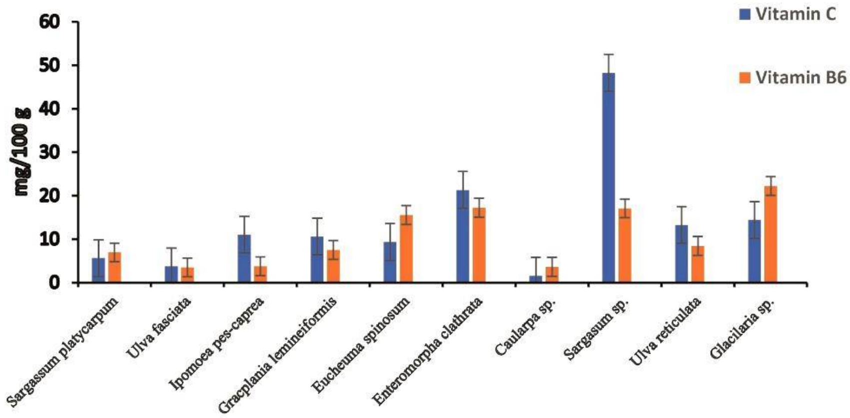

Fig. 3. Comparison of vitamin C and vitamin B_6 Content among seaweed samples.

This study evaluates vitamin C and vitamin B_6 levels in seaweed samples. The data show that seaweeds have the highest percentages of vitamin B_6 and vitamin C. However, most seaweed varieties contain higher amounts of vitamin B_6 than vitamin C. Both vitamins were present at comparable levels in *Sargassum platycarpum* and *Ulva fasciata* (Fig. 3). Advanced analytical methods were employed to accurately measure vitamin content. Based on the study, it can be concluded that seaweeds may offer a sustainable way to address vitamin deficiencies and provide nutritional benefits. We could develop oral supplements and vitamin pills from seaweeds to help reduce vitamin deficiencies.

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References

Abdullah R 2016. Determination Vit C in food samples using High Performance Liquid Chromatography. Chem. Mater. Res. **8**(6): 8-12.

Aktar F, Rahaman MN, Simol HA, Soma MA and Sultan MZ 2025. Quantification and evaluation of thiamine and riboflavin in marine resources of the Bay of Bengal. Biomed. Pharmacol. J. **18**(2).

Ali MA, Sultan MZ, Mia ML, Islam A, Ria UH and Salam MA 2024. Evaluation of thiamine and riboflavin in different varieties of rice grown in Kurigram district, Bangladesh. Heliyon **10**: e39868.

Barnes MJ and Kodicek E 1972. Biological hydroxylations and ascorbic acid with special regard to collagen metabolism. Vitam. Horm. **30**(C): 1-43.

Begum R, Sultan MZ, Chowdhury JA and Amran MS 2019. *In vitro* pharmaceutical equivalence study of three brands of atenolol tablets available in Bangladesh. Dhaka Univ. J. Pharm. Sci. **18**: 43-48.

Hanna M, Jaqua E, VanNguyen and Clay J 2022. B Vitamins: Functions and uses in medicine. Perm. J. **26**.

Hasan MMU, Ahmed S and Sultan MZ 2024. Assessment of residue levels and health risk of carbofuran, chlorpyriphos, abamectin, imidacloprid and pretilachlor in locally available fruits in Dhaka, Bangladesh. J. Food Tech. Res. **11**: 109-117.

Hemilä H and Chalker E 2013. Vitamin C for preventing and treating the common cold. Cochrane. Database Syst. Rev. Page No. 1-95 Issue 1. Art. No.: CD000980.

Holdt SL and Kraan S 2011. Bioactive compounds in seaweed: functional food applications and legislation. J. Appl. Phycol. **23**:543-597.

Islam A, Sultan MZ, Ali MA, Mia ML and Salam MA 2025. Assessment of thiamine and riboflavin levels in common green leafy vegetables grown in Shariatpur, Bangladesh. Heliyon **11**: e41419.

Kayesh R and Sultan MZ 2015. A novel ion-pair RP-HPLC method for simultaneous quantification of naproxen and esomeprazole in pharmaceutical formulations. J. Chromatogr. Sci. **53**: 687-693.

Kiran MV and Subbaiah M 2020. Phytochemical, amino acid, fatty acid and vitamin investigation of marine seaweeds *Colpomenia sinuosa* and *Halymenia porphyroides* collected along the southeast coast of Tamil Nadu, India. World J. Pharm. Res. **9**: 1088-1102.

Klimczak I and Gliszczynska-̄wiglo A 2015. Comparison of UPLC and HPLC methods for the determination of vitamin C. Food Chem. **175**: 100-105.

Lebiedzińska A, Marszałł ML, Kuta J and Szefer P 2007. Reversed-phase high-performance liquid chromatography method with coulometric electrochemical and ultraviolet detection for the quantification of vitamins B1 (thiamine), B6 (pyridoxamine, pyridoxal and pyridoxine) and B12 in animal and plant foods. J. Chromatogr. A **1173**: 71-80.

MacArtain P, Gill CIR, Brooks M, Campbell R and Rowland IR 2007. Nutritional value of edible seaweeds. Nutr. Rev. **65**(12): 535-543.

Manigauha A, Gupta V, Ganesh N and Kharya MD 2021. Antioxidant and Cytotoxic Activities of Beach Morning Glory (*Ipomoea pes-caprae*). Lett. App. Nano-biosci. **10**(4): 2898-2917.

Marinho-Soriano E, Fonseca PC, Carneiro MAA and Moreira WSC 2006. Seasonal variation in the chemical composition of two tropical seaweeds. Bioresour. Technol. **97**: 2402-2406.

Meister A 1992. On the antioxidant effects of ascorbic acid and glutathione. Biochem. Pharmacol. **44**: 1905-1915.

Mia ML, Sultan MZ, Ali MA, Simol HA and Salam MA 2024. Quantification of riboflavin and thiamine in GI (Geographical Indication) branded yogurts collected from Bogura, Bangladesh using HPLC equipped with a fluorescence detector. Bangladesh Pharm. J. **27**: 59-66.

Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O and Lee JH 2003. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J. Am. Coll. Nutr. **22**(1):18-35.

Parra M, Stahl S and Hellmann H 2018. Vitamin B6 and its role in cell metabolism and physiology. Cells **7**: 84.

Sami R, Li Y, Qi B, Wang S, Zhang Q, Han F, Ma Y, Jing J and Jiang L 2014. HPLC analysis of water-soluble vitamins (B2, B3, B6, B12, and C) and fat-soluble vitamins (E, K, D, A, and β-Carotene) of okra (*Abelmoschus esculentus*). J. Chem. **2014**(1): 831357.

Shah ST, Ria UH, Mamun AAl, Miah MI, Rahman SR and Rahman MM 2023. Characterization of indigenous *Saccharomyces cerevisiae* strains for their potential use as Baker's yeast. Dhaka Univ. J. Bio. Sci. **32**: 211-223.

Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Smith AG, Camire ME and Brawley SH 2017. Algae as nutritional and functional food sources: Revisiting our understanding. J. Appl. Phycol. **29**: 949-982.

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