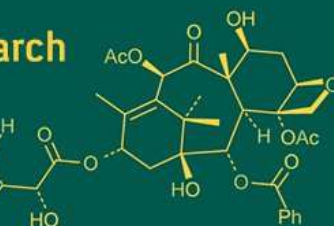
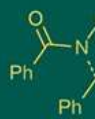
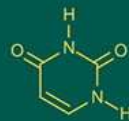
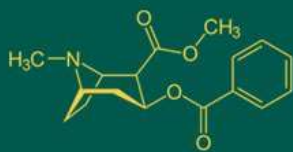


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Yield, proximate composition, and colour characteristics of sodium alginate extracted from the brown seaweed, *Sargassum tenerimum* (J. Agardh, 1848): A pathway towards sustainable biopolymer production

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Abstract

Marine macroalgae of the genus *Sargassum* are a relatively underexploited coastal bioresource with substantial industrial potential, notably as a renewable feedstock for sodium alginate. The present study was undertaken to evaluate the extraction, yield, proximate characterization, and colour attributes of sodium alginate obtained from the brown seaweed *Sargassum tenerimum* (J. Agardh, 1848) collected as drifted biomass from the rocky intertidal shores of Bhatkarawada (17°00'04.6" N; 73°17'15.2" E), Ratnagiri, Maharashtra, India. The fresh seaweed was shade-dried before extraction. Sodium alginate was extracted by alkaline digestion using sodium carbonate (Na₂CO₃) and acid precipitation with hydrochloric acid (HCl) and stepwise purification described by Jayasinghe *et al.* (2016). The stepwise processing yield was decreased from 100% (fresh biomass) to 15.50±0.11% (shade-dried). Sodium alginate yield was obtained at 25.30±0.18% on a dry weight basis, values within the range reported for tropical *Sargassum* species. The proximate analysis of the extracted sodium alginate supporting the low lipid, polysaccharide-dominant composition. The CIE L*a*b* colorimetric analysis of the alginate powder showed high lightness (L* = 76.42±0.38), near-neutral redness (a* = 0.84±0.12), and moderate yellowness (b* = 14.26±0.29), indicating effective pigment removal and a cream-white appearance in accordance with food-grade and pharmaceutical-grade alginate. The results confirmed that *S. tenerimum* is a suitable and locally available raw material for the synthesis of sodium alginate with obvious implications for biodegradable film formulations and food-grade biopolymer applications in the coastal fisheries industry of India.

Keywords: *Sargassum tenerimum*, sodium alginate, alkaline extraction, proximate composition, CIE L*a*b* colour analysis, yield, brown seaweed, biopolymer, biodegradable packaging

1. Introduction

Marine macroalgae, or seaweeds, are some of the most ecologically and economically important and prolific organisms in coastal habitats. Seaweeds are generally grouped under three categories, namely, Chlorophyta (green), Rhodophyta (red), and Phaeophyta (brown), which have been utilized for centuries in Asia for food, fertilizer, and medicine (Petchidurai *et al.*, 2023; Wells *et al.*, 2017) [12, 15]. Of these classes, brown seaweeds (Phaeophyceae) are of substantial industrial relevance mainly due to their high amount of alginate, a natural anionic polysaccharide, which accounts for 14-40% of their dry weight, depending on species and harvest season (Fertah *et al.*, 2017) [3]. Structurally, alginate serves in the cell wall as a hydrogel-forming matrix that enables the seaweed to survive hydrodynamic stresses in chaotic intertidal conditions (Lee & Mooney, 2012) [8].

Sodium alginate is the sodium salt form of alginic acid, which is commercially produced mostly from cold water brown seaweeds such as *Macrocystis pyrifera*, *Ascophyllum nodosum*, and *Laminaria* spp. However, increased worries about overharvesting of these temperate species and availability of tropical *Sargassum* species in plenty along the Indian coastline have generated interest in alternate tropical sources (Abka-Khajouei *et al.*, 2022; Mantri *et al.*, 2020) [1, 9].

The genus *Sargassum* (Family: Sargassaceae, Order: Fucales) has about 350 species worldwide, of which several are common along the west coast of India, especially in the rocky intertidal regions of Maharashtra, Goa and Tamil Nadu (Kamboj & Sharma, 2010) [7]. Despite this biological richness, the alginate production potential of regionally endemic species such as *Sargassum tenerimum* (J. Agardh, 1848) has been comparatively understudied, particularly in terms of proximate characterization and the colorimetric quality of the extracted alginate.

The global alginate market is estimated at more than USD 1.3 billion and is expected to develop at a steady pace because of the demand from the food, pharmaceutical, cosmetics, and packaging industries (Rhein-Knudsen *et al.*, 2015) [14]. Sodium alginate is widely utilized as a thickening, stabilizer, emulsifier, and film-forming agent in processed foods and as a matrix for wound dressing, medication encapsulation, and biomedical scaffolds (Lee & Mooney, 2012) [8]. More lately, alginate has received much interest as a base material for biodegradable packaging films. It provides a sustainable, compostable alternative to petroleum-derived plastics. Alginate-based bioplastic films generated from *Sargassum* species have shown promising tensile strength, water vapor permeability, and oxygen barrier qualities, a positive sign of the economic potential of tropical seaweed biomass for sustainable packaging (Mohammad *et al.*, 2023) [11].

One of the common approaches for seaweed biomass pretreatment before biopolymer extraction is in the form of shade drying to minimize the moisture content with minimum thermal degradation of labile bioactive chemicals (Phang *et al.*, 2015) [13]. It is known that drying concentrates the alginate content on a dry weight basis and increases the extraction efficiency by decreasing the water activity of polysaccharides in the cell wall matrix. The colour of the resulting alginate powder is an important quality indicator—high L* (lightness) values are associated with greater purity and acceptability for food-grade applications, as they reflect successful removal of phycobilin and carotenoid pigments during alkaline extraction and bleaching steps (Murata and Nakazoe, 2001) [10].

Maharashtra is endowed with extensive populations of drifted brown seaweeds, including *S. tenerimum* along its rocky coasts near the Konkan coast of India (17°00'N, 73°17'E). At present, these drifting biomasses are unused and discarded as marine garbage. The utilization of this biomass for alginate manufacture will not only offer economic value for coastal fishing communities but also add to the circular valorization of marine trash. It has been reported to occur in the region; however specific data on alginate extraction yields, proximate composition, and CIE L*a*b* colour quality of alginate from *S. tenerimum* collected from the Maharashtra coast are currently not available in the scientific literature.

In this context, the present investigation was undertaken with the following objectives: (i) to shade dry fresh *S. tenerimum* biomass and determine the stepwise processing yield; (ii) extract sodium alginate using an alkaline precipitation method; (iii) characterize the proximate composition of the extracted alginate; and (iv) evaluate the CIE L*a*b* color profile of the alginate powder at all processing stages. The results will provide a baseline data set for the sustainable exploitation of *S. tenerimum* as a

biodegradable biopolymer resource for food packaging and related applications.

2. Materials and Methods

2.1 Study Area and Seaweed Collection

Brown seaweed, *Sargassum tenerimum* (J. Agardh, 1848) was taken from rocky coasts of Bhatkarawada, Ratnagiri, Maharashtra, India (Latitude: 16°59'16.60"N; Longitude: 73°16'28.79"E) during morning low-tide intervals. Fresh, whole fronds without epiphytes and obvious contamination were hand-picked and transported to the laboratory in clean polythene bags containing seawater. The collected seaweeds were taxonomically identified using the accepted morphological keys of Indian *Sargassum* species.

2.2 Pre-processing and Shade Drying

The freshly acquired seaweed biomass was washed extensively in running tap water to remove clinging sand, salt crystals, epiphytes, and other marine detritus. The cleansed material was then drained of any surface water. The washed seaweed was uniformly spread in single layers on the clean drying racks with mesh surfaces and placed in an enclosure with good ventilation and shade, sheltered from direct sun and rain pollution. Shade drying was maintained at ambient temperature until a constant weight was achieved, as assessed by weighing the samples at 24-hour intervals. The dried material was then powdered finely using a laboratory mixer grinder (60-80 mesh) and stored in sealed air-tight containers at room temperature until further investigation. The yield at each step of processing, i.e., fresh biomass, cleaned and drained, shade-dried, and powdered dry seaweed, was expressed as a percentage of fresh weight.

2.3 Extraction of Sodium Alginate

Sodium alginate was obtained from powdered dried seaweed by the method of Jayasinghe *et al.* (2016) [6] with few changes (Flowchart 1). Briefly, powdered dried seaweed (100 g) was fixed for 30 min in 1% (v/v) aqueous formaldehyde solution to fix interfering colors and polyphenols. The pre-treated material was dispersed in a 2.5% (w/v) sodium carbonate (Na₂CO₃) solution with a ratio of seaweed to solvent of 1:20 (w/v) and heated at 60±2 °C for 3 hours under constant mechanical stirring in order to solubilize alginate. The viscous slurry so obtained was filtered through double layered muslin fabric and the filtrate was collected. Sodium alginate was recovered from the filtrate by acidifying with 10% (v/v) hydrochloric acid (HCl) to pH 2.0-2.5 to produce insoluble alginic acid. The precipitate was separated by centrifugation (3,000 rpm, 15 min), washed twice with distilled water to remove residual acid and then neutralized to pH 7.0±0.2 using 1 M sodium hydroxide (NaOH) to convert alginic acid into its sodium salt form. The re-dispersed sodium alginate solution was then bleached with 5% (v/v) sodium hypochlorite (NaOCl) for 30 minutes to improve colour and then re-precipitated with 70% (v/v) ethanol, filtered and dried at 50±2 °C in a hot air oven to constant weight. Sodium alginate powder was dried and stored in closed containers until analysed.

The yield of alginate was determined on the basis of dry weight, using the formula:

Yield (%) = (Weight of dried sodium alginate/Weight of dried seaweed powder) × 100

2.4 Proximate Composition Analysis

The proximate composition of the extracted sodium alginate powder was tested by conventional procedures of the Association of Official Analytical Chemists (AOAC, 2016) [2]. Moisture content was determined gravimetrically by drying at 105 ± 2 °C to constant weight. Crude protein was measured by the Kjeldahl method (conversion factor $N \times 6.25$). Crude fat was determined by Soxhlet extraction with petroleum ether (boiling range 60-80 °C). Ash content was determined by incineration at 550 ± 10 °C for 6 hours in a muffle furnace. Total carbohydrate was calculated by difference: Carbohydrate (%) = $100 - (\text{Moisture} + \text{Crude Protein} + \text{Crude Fat} + \text{Ash})$. All the analyses were performed in triplicate and the results are expressed as Mean $\pm$ SE.

2.5 Colour Analysis

The CIE 1976 $L^*a^*b^*$ colour parameters of fresh seaweed, dried seaweed, and sodium alginate powder were measured using a Lab Scan XE Spectrophotometer (Hunter Associates Lab., Inc., 2016). The instrument was calibrated against a standard white plate prior to measurements. L^* represents lightness (0 = black, 100 = white), a^* represents the green-red axis (negative = green, positive = red), and b^* represents the blue-yellow axis (negative = blue, positive = yellow). All measurements were performed in triplicate and reported as Mean $\pm$ SE.

2.6 Statistical Analysis

All data were shown as mean $\pm$ standard error (SE) of triple ($n = 3$) measurements. The one-way analysis of variance (ANOVA) was conducted to identify significant differences between processing stages, and Tukey's HSD post-hoc test was conducted for pairwise comparisons. For all analyses, a significance level of $p < 0.05$ was used. Statistical analyses were conducted using SPSS v.16 (IBM Corp., USA).

3. Results and Discussion

3.1 Colour Characteristics of Fresh and Dried Brown Seaweed

Table 1 shows the CIE $L^*a^*b^*$ colour values of fresh and shade-dried *Sargassum tenerimum*. Fresh seaweed had a low lightness value ($L^* = 32.50 \pm 0.42$) consistent with the dark olive-brown colouration characteristic of Phaeophyceae, mainly due to fucoxanthin and chlorophyll-c. The negative a^* value (-6.12 ± 0.18) is indicative of a green undertone, while the moderate b^* value (11.85 ± 0.33) corroborates the underlying yellow-brown color. After shade drying, L^* further decreased to 19.34 ± 0.38 , showing that prolonged exposure to ambient temperature conditions caused chlorophyll breakdown and production of phaeophytin, which may have caused higher surface browning. The a^* value was decreased from -6.12 to -2.12, indicating the partial loss of the green pigment component, while b^* greatly rose to 19.92 ± 0.41 , indicating the strong yellowing, which was consistent with the Maillard reaction products and oxidative pigment change. Earlier reports have indicated the occurrence of browning events during ambient drying of *Sargassum* species (Phang *et al.*, 2015) [13]. The observed decrease in lightness from fresh to dried seaweed indicates the necessity of shade drying over open sun exposure, since direct solar radiation would lead to a more rapid photo-degradation of chromophores.

Table 1: Colour characteristics of fresh and dried brown seaweed (*S. tenerimum*)

Sr. No.	Parameters (CIE $L^*a^*b^*$)	Colour values	
		Fresh Brown Seaweed	Dried Brown Seaweed
1.	L^* (Lightness)	32.50 ± 0.42	19.34 ± 0.38
2.	a^* (Redness/Greenness)	-6.12 ± 0.18	-2.12 ± 0.21
3.	b^* (Yellowness/Blueness)	11.85 ± 0.33	19.92 ± 0.41

Values are expressed as Mean $\pm$ SE ($n = 3$)

3.2 Yield of Brown Seaweed (*S. tenerimum*) at Different Processing Stages

The stepwise processing yield for *S. tenerimum* is given in Table 2. The 100% fresh biomass, after cleaning and draining, gave $74.00 \pm 0.21\%$ when surface water, epiphytes, and related debris were removed. In addition, the yield was reduced to $15.50 \pm 0.11\%$ with shade drying, which meant a loss of moisture of about 79%. This is in accordance with the normally high moisture content of fresh *Sargassum* biomass, which is in the range of 75-85% (wet weight basis). This decrease in moisture content was in agreement with the values reported for other tropical brown seaweeds (Phang *et al.*, 2015) [13]. Losses increased ($14.70 \pm 0.10\%$) due to the loss of small particles during milling and sieving with powder. A sodium alginate of $25.30 \pm 0.18\%$ (dry weight basis) was present in powdered dried seaweed, and *S. tenerimum* is within the competitive range of 15-31.7% stated for tropical *Sargassum* species. Jayasinghe *et al.* (2022) [15] showed that *S. wightii* yielded 31.7% of alginate (Sri Lanka) with 2.5% Na_2CO_3 , while Chee *et al.* (2011) obtained 49.9% of alginate from *S. siliquosum* at optimum hot extraction conditions. The variations in alginate yield of *Sargassum* species are due to the changes in cell wall architecture, season of harvest, and geographical factors (Abka-Khajouei *et al.*, 2022; Mantri *et al.*, 2020) [1, 9]. The increased yield of *S. tenerimum* suggests its potential as a feedstock for commercial alginate production on the coast of Maharashtra.

Table 2: Yield of brown seaweed (*S. tenerimum*) at different processing stages

Sr. No.	Specifications of Brown Seaweed (<i>Sargassum tenerimum</i> , J. Agardh) Processing	Yield (%)
1.	Fresh brown seaweed	100.00 ± 0.01
2.	Cleaned, branched, and drained brown seaweed	74.00 ± 0.21
3.	Dried brown seaweed	15.50 ± 0.11
4.	Powdered dried seaweed	14.70 ± 0.10
5.	Sodium alginate extracted from powdered dried brown seaweed	25.30 ± 0.18

Values are expressed as Mean $\pm$ SE ($n = 3$); yield was calculated as a percentage of fresh seaweed weight at each stage except alginate (dry weight basis of powdered seaweed).

3.3 Proximate Composition of Sodium Alginate from *S. tenerimum*

The proximate analysis of the extracted sodium alginate powder is shown in Table 3. The moisture level ($12.50 \pm 0.52\%$) is acceptable for polysaccharide powders and is indicative of the hygroscopic character of alginate under ambient storage conditions. AOAC (2016) [2] specifies that food-grade sodium alginate should not include more than 15% moisture, which is well within the range of the current extract. The low crude fat content ($0.52 \pm 0.03\%$) indicates a successful removal of lipids during the alkaline extraction phase. Crude protein ($2.12 \pm 0.18\%$) probably

corresponds to residual co-precipitated proteinaceous material from the seaweed cell matrix. The high ash content ($18.02 \pm 0.71\%$) suggested a large sodium ion loading of the sodium salt form of alginate, which was slightly higher than the 5.20% reported by Jayasinghe *et al.* (2022) [5] for *S. wightii*, probably because of the difference in the degree of sodium conversion during the neutralization step. Fertah *et al.* (2017) [3] reported ash values of 5-20% in alginate from *Laminaria digitata* as a function of purifying parameters, which indicated a strong process dependence of ash concentration. It contains a lot of polysaccharides, which is an important quality of economically relevant alginate. The primary carbohydrate type ($66.84 \pm 1.55\%$) The sum of all proximal fractions ($99.98 \pm 2.34\%$) was close to 100% confirming the completeness and accuracy of the analytical determination. Collectively these qualities make the *S. tenerimum* alginate a good quality biopolymer with potential application in food-grade and biodegradable packaging.

Table 3: Proximate composition of sodium alginate from brown seaweed (*S. tenerimum*)

Sr. No.	Parameters	Proximate Composition of Sodium Alginate from <i>S. tenerimum</i> (%)
1.	Moisture	12.50 ± 0.52
2.	Crude Protein	2.12 ± 0.18
3.	Crude Fat	0.52 ± 0.03
4.	Ash	18.02 ± 0.71
5.	Carbohydrates	66.84 ± 1.55

Values are expressed as Mean $\pm$ SE (n = 3)

3.4 Colour Characteristics of Sodium Alginate Powder

The CIE ($L^*a^*b^*$) color profile of the isolated sodium alginate powder from *S. tenerimum* is shown in Table 4. The alginate powder had a significantly higher lightness value ($L^* = 76.42 \pm 0.38$) than fresh seaweed (32.50 ± 0.42) and dried seaweed (19.34 ± 0.38), indicating the good removal of dark phycobilin and fucoxanthin pigments by alkaline solubilisation, acid precipitation, and hypochlorite bleaching during extraction. The a^* value changed from severely negative (-6.12 in fresh seaweed) to moderately positive (0.84 ± 0.12) in the alginate powder, suggesting the near-complete removal of chlorophyll-derived green chromophores. The b^* value of 14.26 ± 0.29 demonstrated

the lingering yellowness, which was likely caused by trace carotenoid residues and the inherent off-white to cream color of the sodium salt form of alginate. The L^* values ≥ 70 are usually accepted as an index of the commercial purity of alginate for culinary and medicinal uses (Murata and Nakazoe, 2001) [10], which is seen for the current extract. The present *S. tenerimum* alginate was in a similar colour range (b^* range: 13.4-18.2) as sodium alginate from *S. latifolium* (El-Sayed *et al.* 2021), indicating equal degrees of purifying effectiveness.

Table 4: CIE $L^*a^*b^*$ colour characteristics of sodium alginate powder extracted from brown seaweed (*S. tenerimum*, J. Agardh, 1848)

Sr. No.	Colour Parameters	CIE $L^*a^*b^*$ Colour Values of Sodium Alginate Powder from <i>S. tenerimum</i>
1.	L^* (Lightness)	76.42 ± 0.38
2.	a^* (Redness/Greenness)	0.84 ± 0.12
3.	b^* (Yellowness/Blueness)	14.26 ± 0.29

Values are expressed as Mean $\pm$ SE (n = 3).

3.5 Comparative Colour Profile across Processing Stages

Table 5 presents a comparative overview of CIE $L^*a^*b^*$ values of all three processing phases i.e. fresh seaweed, shade-dried seaweed, and extracted sodium alginate powder. The progressive increase of L^* from 32.50 (fresh) to 19.34 (dry) and to 76.42 (alginate) demonstrates a non-linear biphasic color change; The lightness decreased during the beginning of drying, due to the formation of phaeophytin and the Maillard browning and then increased significantly after the alkaline extraction and bleaching. The b^* (yellowness) value increased to a maximum at the drying stage and then decreased in the final alginate product, in agreement with the solubilization of carotenoids during the extraction. This stage-wise color evolution provides a realistic framework for the quality monitoring of *S. tenerimum* alginate extraction, which might be adopted by small-scale seaweed processors in coastal Maharashtra for rapid, non-destructive testing of product quality. Overall color trajectory supports the finding that the extraction process chosen is effective in obtaining a visually pure alginate powder that meets commercial criteria.

Table 5: Comparative CIE $L^*a^*b^*$ colour profile of brown seaweed (*S. tenerimum*) across processing stages

Sr. No.	Processing Stage	L^* (Lightness)	a^* (Redness)	b^* (Yellowness)
1.	Fresh Brown Seaweed	32.50 ± 0.42	-6.12 ± 0.18	11.85 ± 0.33
2.	Dried Brown Seaweed	19.34 ± 0.38	-2.12 ± 0.21	19.92 ± 0.41
3.	Sodium Alginate Powder	76.42 ± 0.38	0.84 ± 0.12	14.26 ± 0.29

Values are expressed as Mean $\pm$ SE (n = 3).

4. Conclusion

The present study indicates that the *Sargassum tenerimum* biomass drifted off the rocky coastlines of Ratnagiri, Maharashtra, India, is a potential and commercially feasible feedstock for the synthesis of sodium alginate. Moisture was effectively removed by shade drying to about 15.50%. The alkaline extraction process yielded $25.30 \pm 0.18\%$ alginate on a dry weight basis. This is in the range for top-performing *Sargassum* species. The proximate composition of the isolated alginate indicated a polysaccharide-dominant profile (carbohydrate: $66.84 \pm 1.55\%$). CIE $L^*a^*b^*$ analysis validated cream-white color quality ($L^* = 76.42 \pm 0.38$), commensurate with food-grade norms. The systematic color transition during processing stages provides a timely quality

monitor for coastal processors. Such outcomes establish *S. tenerimum* as a potential marine biopolymer source that is locally available and may be directly used for sustainable food packaging, edible film preparation, and marine entrepreneurship development in the coastal fisheries sector of India.

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7. Conflicts of Interest

The authors declare that there are no conflicts of interest.

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