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Morphology and Nutritional Composition of *Gracilaria changii* at Different Maturation Stages

Nurulain Najwa Suhaimai[1], Faten Nur Aisyah Zainuldin[1], Jalaludin Kassim[2] and Shahidah Md. Nor[1]*

[1]Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn, UTHM- Campus (Pagoh Branch), Pagoh Education Hub, KM 1, Jalan Panchor, 84600 Muar, Johor, Malaysia

[2]Laman Alam Jaya Sdn Bhd, Benteng Parit Bulat Laut, Parit Jawa 84150 Muar, Johor, Malaysia

ABSTRACT

Gracilaria changii is a red seaweed macroalgae that holds economic potential for the Malaysian economy, as it has now started to be cultivated commercially. Establishing the harvesting standards for seaweed is important for its marketing purposes. Thus, this study evaluated the effect of different growth stages on its postharvest quality. The seaweed was studied at three different maturation stages, which were Stage I (30–40 days), Stage II (40–50 days) and Stage III (60 days and above). Morphology, physicochemical properties, and bioactive compounds were evaluated to establish the postharvest quality. There was a significant difference in postharvest quality in terms of morphology and physicochemical properties at different maturation stages. Morphologically, the thallus of seaweed was straight and cylindrical. The secondary and tertiary branches were longer than the primary branches during stages II and III. During the whole development stage, *G. changii* was found in a pH range of 6.23–7.04, ash content (28.53–38.93%), (3.70–23.45 µg/g/100 ml) ascorbic acid, and (0.21–0.23 %) titratable acidity. Total phenolic content (TPC) was significantly highest at Stage III, whereas total flavonoid content (TFC) and the antioxidant activities measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays remained unaffected by the maturation stage. This study revealed that different maturation stages affect the postharvest quality of seaweed. This information is valuable to guide the establishment of the harvesting standard for local seaweed production.

Keywords: Antioxidant activity, *Gracilaria changii*, growth stages, morphology, nutritional composition

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E-mail addresses:
ainajwa.suhaimai@gmail.com (Nurulain Najwa Suhaimai)
fatennuraisyah99@gmail.com (Faten Nur Aisyah Zainuldin)
shahidahmn@uthm.edu.my (Shahidah Md. Nor)
jalalsulit@gmail.com (Jalaludin Kassim)
*Corresponding author

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INTRODUCTION

Gracilaria changii, also known as red seaweed, is an agarophyte that contains abundant nutrients. It can be consumed fresh or utilised in the phycocolloids industry. The succulent thallus contains a high amount of polysaccharides, minerals, vitamins, and some bioactive substances such as proteins, lipids, and polyphenols that demonstrate cytotoxic, antioxidant, anticancer, antibacterial, anti-viral, antifungal, antidiabetic, and anti-inflammatory activities (Bouzenad et al., 2024). These characteristics have enabled seaweeds to be employed as ingredients in functional food and nutraceutical applications (Chan & Matanjun, 2017). The chemical and nutritional profiles of seaweeds are subject to variation depending on species, habitats, growth stages, and environmental conditions. For instance, *Ulva lactuca* cultivated in the Israeli seashore has the maximum amount of lipid during summer compared to winter (Gnayem et al., 2024). The composition and nutritional content of *Sargassum fusiforme* vary according to different maturation stages, in which the seedling and early growth stages are recommended for preparing high-protein foods and health supplements. The chemical profile and antioxidant activity of *Caulerpa lentillifera* differ accordingly to different sites and environmental conditions in Thailand (Koodkaew et al., 2024).

Previously, the chemical composition of wild-grown *G. changii* at Santubong, Sarawak, Malaysia, was investigated by Chan & Matanjun (2017). This species was high in dietary fibre (64.74 ± 0.82%), low in fat (0.30 ± 0.02%) and a total amino acid of 91.90 ± 7.70%. Nevertheless, the composition at different maturation stages has not yet been investigated. The genus Gracilaria follows a triphasic life cycle known as the polysiphonia (Pereira & Yarish, 2008). Post fertilisation, a distinct cystocarp that evolved in a hemispherical shape will swell throughout the surface of the female gametophyte's thallus (Baweja et al., 2016). The cystocarp releases the diploid carpospores (2n), forming the tetrasporophyte (2n). The tetrasporophyte (2n) generates haploid tetraspores (n) through meiosis within cortical sporangia. These tetrasporangia eventually produce tetraspores, germinating into male and female gametophytes, forming into mature thallus, thus completing their whole life cycle (Baweja et al., 2016).

The growing thallus is harvested at different maturation stages. For the current cultivation practices, the farmers use the vegetative thalli to obtain rejuvenated juveniles that grow into a mature thallus (Pereira & Yarish, 2008). The cultivation date is counted once the process of putting thalli onto the ropes and lines is completed. Currently, there are three main stages used to refer to the maturity level of seaweed, involving: Stage I (30–40 days), Stage II (40–50 days) and Stage III (60 days above) (Laman Alam Jaya Sdn Bhd, 2024). The current agricultural practice of the maturation stage is the reference for the seaweed harvesting stage. It is believed that different maturity stages will produce different chemical and nutritional compositions that affect the final quality of seaweed used in the industry.

Seaweed was acknowledged as an important marine crop in Malaysia under the 10th Malaysia Development Plan through the National Key Economic Areas (NKEA) and Entry Point Project (EPP 3). The plan aimed to boost seaweed production from 13,500 metric tonnes in 2010 to a projected 150,000 metric tonnes by 2020 (Chan & Matanjun, 2017). In 2023, it was reported to increase to 225,048 metric tonnes valued at RM100 million. Recently, *G. changii* has gained popularity in Peninsular Malaysia, where it has been cultivated commercially in Muar, Johor. Since *G. changii* is a new commercialised species, knowledge of composition and nutritional contents at different maturation stages is still limited. This knowledge gap necessitates the current study to be undertaken. Knowing the composition difference between different maturity stages will lead to establishing a harvesting standard that can guide producers and marketers in deciding the market price by providing a common language for marketers and producers. This harvesting standard is expected to ensure that seaweed is harvested at the right time, providing optimal nutritional content and quality for industrial applications.

MATERIALS AND METHODS

Reagents and Apparatus

The chemicals used in this study were sodium hydroxide (QRec, Malaysia), phenolphthalein (Sigma Aldrich, Malaysia), malic acid (Sigma Aldrich, Malaysia), 2,6-dichloroindophenol (Sigma Aldrich, Malaysia), metaphosphoric acid (Sigma Aldrich, Malaysia), acetic acid (Sigma Aldrich, Malaysia), ascorbic acid (Sigma Aldrich, Malaysia), Folin-ciocalteu reagent (Merck, Germany), gallic acid (Merck, Germany), sodium carbonate (Merck, Germany), methanol (QRec, Malaysia), aluminium trichloride (QRec, Malaysia), ethanol (Sigma Aldrich, Malaysia), potassium acetate (Merck, Germany), quercetin (Sigma Aldrich, Malaysia), 2,2-diphenyl-1-picryhydrazyl (DPPH) (Merck, Germany), 2-2'-azino-bis (3-ethylbenzothiozoline-6-sulfonic acid) (ABTS) (Merck, Germany), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Merck, Germany), and potassium persulfate (Sigma Aldrich, Malaysia). They were analytical grades from procured sources.

The apparatus and instruments used in this study were a scanning electron microscope (SEM) (Coxem, South Korea), quorum sputter coater (QuorumTech, UK), high duty blender (XUETAO, China), muffle furnace (Carbolite, UK), calorimeter (PCE Instruments, Germany), texture analyser (Stable Micro System, UK), UV-VIS spectrophotometer (PG Instruments, UK), water bath (Memmert, Germany), pH meter (Trans instruments, Singapore), vortex mixer (Labmart, Malaysia), pipette (Eppendorf, Germany) and drying oven (Memmert, U40, Germany).

Collection of *G. changii* and Sample Preparation

The *G. changii* specimens were freshly collected from Laman Alam Jaya, a local Parit Bulat, Muar, Johor farm. The seaweed was raised using filtered seaweed water in a man-

made pond using standard seaweed cultivation practices. Seaweed at three harvesting stages: Stage I (30–40 days), Stage II (40–50 days), and Stage III (60 days above), was harvested randomly from nine different plots, with three biological replications representing one maturity level. Upon harvesting, the seaweed is thoroughly washed using filtered seawater to eliminate salt, debris, epiphytes, and foreign matter. The freshly cleansed seaweed samples were immediately subjected to morphological examination, including physical, colour assessment and texture analysis. The cellular structure of seaweed was also included in the morphological assessment. Post morphological assessment, the seaweed was subjected to dehydrator drying at 50 °C until constant weight was achieved. Small portions of dried thallus were subjected to cellular study using a scanning electron microscope, while the rest was processed into a powdered form. The powdered samples were subjected to physicochemical and antioxidant analysis. The dried thallus was pulverised into the powdered form using a Waring blender, passed through a sieve with a mesh size of 0.85 mm (pore size) and kept in an airtight bag in a freezer (-40 °C) until further analysis.

Morphology Studies

Physical Features

All individuals' thalli were photographed at the time of collection. The photographs were used to quantify the number of adventitious branch patterns.

Colour

The colour of the thallus was measured by using a handheld portable digital calorimeter (PCE-CSM 1, Germany). Three random spots at each sample were taken for the readings of L* (lightness), a* (green/red) and b* (blue/yellow) parameters. The C* (chroma) and h° (hue) parameters were calculated using a formula mentioned by Temocico et al. (2019).

Texture Analysis

A single-arm texture analyser (Stable Micro Systems Ltd., Godalming, UK) was used to evaluate the tensile strength (TS) and elongation at break (EAB) of the fresh thallus. The data were analysed using Texture Exponent software (Godalming, UK). Seaweed samples were uniformly cut to a length of 5 cm and loaded with a 30 kg load cell, following the ASTM D882-02 procedure. The tensile test was conducted with an initial grip separation (Lo) of 60 mm and a crosshead speed of 100 mm/min. The TS and EAB values were calculated from the tensile force and the length of the specimen after fracture, following a modified method of Hamdan et al. (2021).

Scanning Electron Microscope (SEM)

The surface morphology of the dried thallus was observed using an SEM. The sample was placed onto the SEM holder with double-sided electrically conductive carbon adhesive tape, and the specimens were then coated with a thin gold-palladium layer. The samples were observed using 5000 x magnifications (Khalil et al., 2016).

Physicochemical Properties Evaluation

Titratable Acidity (TA) and pH

TA was measured by acid-base titration, and pH using a pH electrode meter. Titration was performed using 10 mL of seaweed filtrate pre-added with two drops of phenolphthalein. Then, the solution was titrated against sodium hydroxide until a light pink solution appeared for 15 s. The result was expressed as a percentage of malic acid (Nor et al., 2023).

Ascorbic Acid (AA)

The ascorbic acid was determined using titration with 2,6-dichloroindophenol as an indicator. In a 50 mL Erlenmeyer flask, around 5 mL of metaphosphoric acid-acetic acid solution and 2 mL of the sample were carefully pipetted. The burette was loaded with 2,6-dichloroindophenol dye solution and titrated against each sample until a light rose-pink colour appeared within 5 s. Throughout the titration, the flask was swirled continuously. The initial and final readings of the burette were recorded, and the result was quantified as a µg/100 ml ascorbic acid (Nor et al., 2023).

Ash Content

The ash content was calculated using the muffle furnace method. The empty crucibles were dried at 110 °C for 3 h, followed by cooling in a desiccator before weighing. About 1 g of the sample was transferred to a silica crucible and heated in a muffle furnace at 550 °C for 3 h. Following incineration, the remaining content was cooled and weighed. The result was expressed as a percentage (Zakaria et al., 2018).

Antioxidant Activity Analysis

Sample Preparation and Extraction of Bioactive Compound

Approximately 1 g of powdered seaweed was dispersed in 5 mL of 95 % ethanol in a conical flask and heated in a water bath at 35 °C for 30 min. Centrifugation of the sample was done at 4200 × *g* for 10 min. Finally, the supernatant was filtered and collected for quantification (Nor et al., 2023).

Total Phenolic Content (TPC)

A 0.5 mL aliquot of the extracts was added to 2.5 mL of Folin-Ciocalteu reagent in a test tube. The mixture was then diluted tenfold with distilled water, and 2 mL of sodium carbonate was added. The sample was incubated for 30 min in the dark, with the test tube wrapped in aluminium foil. Following incubation, the absorbance was recorded at 765 nm using a UV-vis spectrophotometer. The total phenolic content (TPC) was quantified and reported as milligrams of gallic acid equivalents (GAE) per gram of sample (mg GAE/g) (Nor et al., 2023).

Total Flavonoid Content (TFC)

A 0.5 mL sample was combined with 2.8 mL of distilled water in a test tube wrapped with aluminium foil. Following that, 0.1 mL of 10 % aluminium trichloride and 0.1 mL of potassium acetate were added and thoroughly mixed. The mixture was incubated for 30 min and measured at 415 nm using a UV-vis spectrophotometer. The result was expressed as milligrams of quercetin equivalent (QE) per gram of sample (mg QE/g) (Nor et al., 2023).

2-2-diphenyl-1-picrylhydrazyl Radical Scavenging (DPPH) Assay

A 0.3 mM DPPH stock solution was freshly prepared on the analysis day. The analysis was conducted in the dark, with the test tubes wrapped in aluminium foil. Approximately 1 mL of the sample was added to each test tube and mixed with 3 mL of DPPH. The mixture was thoroughly vortexed before being incubated for 30 min. Absorbance was then measured at 515 nm using a UV-vis spectrophotometer. The DPPH stock solution was the negative control, while ethanol was the blank. Both the sample and the negative control were analysed in triplicate. Standard curve was done using a Trolox solution, and the result was expressed as µmol Trolox equivalent per 100 g of dried sample (µmol TE/100 g) (Nor et al., 2023).

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Assay

ABTS stock solution, consisting of 7 mM ABTS in water and 2.45 mM potassium persulfate, was prepared to perform the ABTS assay. This solution was allowed to stand in the dark at room temperature for 16 h. After the incubation period, the ABTS solution was diluted with ethanol to achieve an absorbance of 760 nm. Once the desired absorbance was reached, 150 µL of the extract was mixed with 3,850 µL of the ABTS solution. The mixture was immediately analysed by measuring absorbance at 734 nm. The results were compared to the Trolox standard curve and expressed as µmol of Trolox equivalent (TE) per 100 g of dried sample (µmol TE/100 g) (Nor et al., 2023).

Statistical Analysis

All analyses were conducted in triplicate, and the results were reported as mean ± standard deviation. One-way analysis of variance (ANOVA) with Minitab Software version 21, at a significance level of α=0.05, was used to evaluate significant differences between means. Tukey's test was employed to compare all treatments.

RESULTS AND DISCUSSION

Morphology Studies

Physical Features

The *G. changii*'s main body is known as the thallus. The thallus exhibited a cylindrical and straight shape, with constrictions at the base of the branches, swelling in the middle and tapering towards the ends (Figure 1). Branches formed sporadically, with the tips of

Figure 1. The branching pattern of freshly harvested seaweed from three random ponds at three different maturation stages. Stage followed by different letter and different number indicates different stages and different cultivation plots; (a1–a3) Stage I at 30–40 days; (b1-b3) Stage II at 40–50 days; and (c1–c3) Stage III at 60 days and above

secondary branches dividing into two short branchlets. The tertiary branches emerge along the secondary branch. During Stage I, it shows that the branching pattern of the thallus was shorter within a 5 cm length than secondary branches at 10–15 cm (Figure a1–a3). At stage 2 (Figure b1–b3) and stage 3 (Figure c1–c3), the primary thallus of the seaweed grew to 10 cm, while the secondary branches extended to 20–25 cm. The changes in seaweed morphology positively show that the seaweed is undergoing a growth and development process due to photosynthesis and nutrient accumulation responses (Roleda & Hurd, 2019).

Colour

Colour can be denoted as a prominent postharvest standard in rating the quality of fresh produce (Nor et al., 2023). This study shows that the harvesting stage impacts the colour of fresh *G. changii* (Table 1). The lightness (L*) values of stages 1 and II were similar. Then, it became significantly lighter ($p<0.05$) when the seaweed reached Stage III. For the a* value, stages 1 and II had negative values, indicating that the colour tends towards greenness. Later, when it reached Stage III, it changed significantly to 0.39, indicating the seaweed becoming redder in colour. The colour thallus was more likely to be brown-red as all stages possessed negative b*. The blueness (-b*) of *G. changii* decreased ($p>0.05$) along with the increase in the maturation stage. Rating on the colour intensity, the chroma (c*) value decreased progressively ($p>0.05$) from Stages I to III. The hue angle shifted towards brownness during Stage III. Rating on the colour quality, *G. changii* in Stage III, produced a more intense red brown colour during its late maturation stages. This finding aligns with studies of other seaweeds, such as Ulva fasciata, that shift towards red hues in a response to age development, which have been attributed to carotenoid accumulation and degeneration of chlorophyll (Beer et al., 2000). Research conducted by Chan and Matanjun (2017) has quantified that *G. changii* contains multiple tetrapyrrole pigments such as chlorophyll, carotenoid, xanthophylls, zeaxanthin, lutein and antheraxanthin. These pigments may change at various stages of maturation, as indicated by the corresponding shifts in colour.

Table 1
The effect of different maturation stages of G. changii *on colour (L*, a*, b*, c* and h°)*

Factors	L*	a*	b*	c*	h°
Stage I	24.68±0.28^{a}	-1.30±0.12^{a}	-5.30±0.49^{a}	5.46±0.50^{a}	256.13±0.32^{a}
Stage II	24.77±0.25^{a}	-1.07±0.21^{a}	-4.91±0.32^{ab}	5.03±0.35^{ab}	257.74±1.77^{ab}
Stage III	27.97±0.85^{b}	0.39±0.98^{b}	-3.56±0.88^{b}	3.76±0.70^{b}	278.12±14.64^{b}

Note. The result was expressed in means ± standard deviation ($n = 27$), followed by the same letter in the same column, which was not significantly different at ($p > 0.05$) according to Tukey's post-hoc tests

Texture Properties

Texture is a crucial factor influencing the acceptability of fresh and processed seaweeds in the industry. The tensile strength values of fresh *G. changii* were within the range of 10.00–33.85 MPa (Table 1). These values were lower than those of other Gracilaria species, such as *G. salicornia* (68.24 MPa) and *G. edulis* (85.05 MPa) (Phang, 2006). The difference in tensile strength could be due to the polysaccharide composition that builds up the main thallus structure (Rhein-Knudsen et al., 2017). For instance, *G. salicornia* contain 18%–30% agar (Buriyo & Kivaisi, 2004), *G. edulis* contain 23%–28% agar and *G. changii* contain 18%–22% agar (Lee et al., 2016). A thallus with a higher agar content, particularly rich in agarose, builds a stronger and more rigid thallus to resist mechanical stress from water currents and waves (Pica et al., 2024). Matured seaweed during stages II and III exhibited significantly higher tensile strength (29.79±2.85 MPa and 33.85±10.12 MPa) compared to Stage I (10.05±5.20 MPa). Additionally, the elongation (%) of the seaweed correlated with the tensile strength (MPa), showing that increased maturation time results in greater elasticity. This is likely due to the maturation process, during which the seaweed strengthens with age as its cell walls thicken by accumulating hydrocolloids such as agar, carrageenan, and alginates (Shao & Duan, 2022). Integrating these hydrocolloids into the cell walls of the thallus may form a composite material that enhances the seaweed's strength and elasticity (Norziah & Karim, 2006). In the global market, the fresh seaweed that is fibrous, sticky and tactile is highly appreciated by Western and Japanese consumers (Figueroa et al., 2022). From the rating on the consumer perspective, when the seaweeds are consumed fresh, consumers would appreciate Stage II and III more due to greater tensile strength and higher elongation (%), making the texture springier and chewier.

Scanning Electron Microscope (SEM)

The SEM micrograph in Figure 2 strengthens the texture analysis conducted in this study. Clearly, the thallus surface from all stages was dominated by tear ridges and irregular round pores known as dimples. This surface would cause the thallus to have a brittle and elastic in texture (Wang et al., 2022). In Stage I, I had a greater number of dimples on its surface than in the other stages, explaining its low ductility and low stretchability (Table 2). In addition, this study also displays that the dimple size of Stage I (Figure 2a) seaweed was smaller compared to the Stages II (Figure 2b) and III (Figure 2c). Wichard et al. (2015) explained that mitotic spots distributed across the thallus are responsible for active cell division, resulting in cell size and number variations during different developmental phases. During Stage I, the cells of the thallus could actively undergo mitosis. The small and high number of dimples in Stage I could be attributable to active cell division, the mitotic process (Figure 2a).

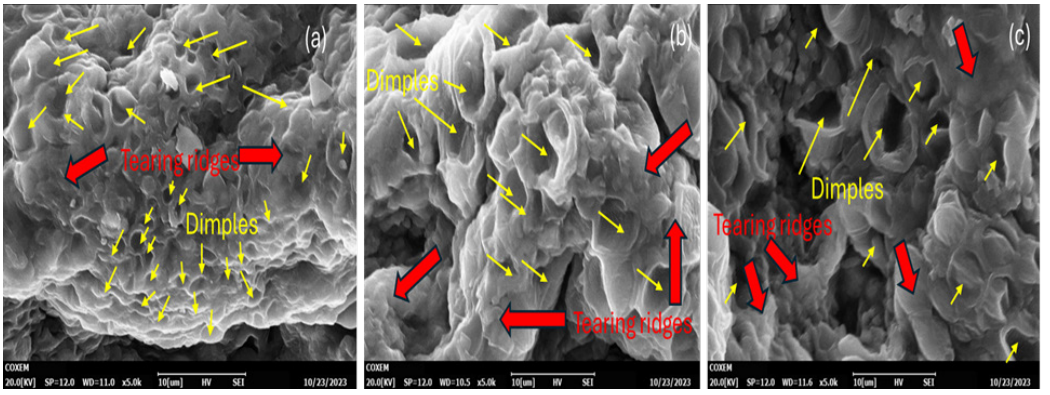

Figure 2. The surface of thallus G. changii at different stages of maturation: (a) Stage I at 30–40 days; (b) Stage II at 40–50 days; (c) Stage III at 60 days and above in 5000x magnification under SEM. The yellow arrows show the visible pores known as dimples, while the red arrows show tearing ridges on the surface of the thallus

However, cell division may cease upon reaching stages II and III, allowing the expansion process to occur. A few dimples characterise this, but their size increases (Figure 2b, 2c). Tear ridges is known as fold that running along thallus (Wang et al., 2022). Tear ridges are functional in adding mechanical strength to the thallus by preventing it from tearing under high water pressure (Pica et al., 2024). Tear ridges were more noticeable in stages II and III (Figure 2b, 2c). Tear ridges caused greater stretchability and higher tensile strength, as confirmed by the texture analysis, which showed increased tensile strength values in stages II and III (Table 2).

Table 2
The effect of different maturation stages of G. changii *on the texture profile*

Factors	Tensile strength (MPa)	Elongation (%)
Stage I	10.00±5.20[a]	39.11±8.57[a]
Stage II	29.79±2.85[b]	51.17±2.95[b]
Stage III	33.85±10.12[b]	50.79±2.32[b]

Note. The result was expressed in means ± standard deviation ($n = 27$), followed by the same letter in the same column, which was not significantly different at ($p > 0.05$) according to Tukey's post-hoc tests

Physicochemical Properties

Table 3 presents the physicochemical data of *G. changii* seaweed at different maturation stages, including pH, ascorbic acid (AA), titratable acidity (TA), and ash content. The pH of *G. changii* varied during the maturation stages, with the lowest pH (6.23) in Stage I and the highest pH (7.04) in Stage II, while the pH of Stage III was 6.94. Meanwhile, the titratable acidity (TA) showed no significant difference across different maturation stages.

Table 3
The content of ash, pH, ascorbic acid and titratable acidity of G. changii *at different maturation stages*

Factor	pH	Ascorbic acid (µg/100ml)	Titratable acidity (% malic acid)	Ash content (%)
Stage I	6.23 ±0.48[a]	23.45±5.31[a]	0.20±0.06[a]	38.93±3.10[a]
Stage II	7.04 ± 0.22[b]	13.69±10.25[ab]	0.23±0.08[a]	34.50±1.32[ab]
Stage III	6.94 ± 0.31[b]	3.70±3.90[b]	0.21±0.07[a]	28.53±4.15[b]

Note. The result was expressed in means ± standard deviation (n=27), followed by the same letter in the same column, which was not significantly different (p>0.05) according to Tukey's post-hoc tests

The ascorbic acid (AA) content of *G. changii* was found to be lower than that of wild *G. changii* grown in Santubong, Sarawak (2.51 mg/100 g) and higher than that of other brown and green seaweed species cultivated in Indonesia (Chan & Matanjun, 2017; Bocanegra et al., 2009). The current study reveals that the maturation stage influences antioxidant activity (AA), with values significantly decreasing from Stage I (23.45 µg/100 ml) to Stage II (13.69±10.25 µg/100 ml), and further to Stage III (3.70 µg/100 ml). Ascorbic acid is normally present in seaweed in all cell compartments, including the cell wall. It normally acts as an enzyme cofactor for controlling cell growth by aiding in the biosynthesis of hydrocolloid (Smirnoff et al., 2000). In Stage I, the ascorbic acid could be abundant because of the need to aid in the biosynthesis of hydrocolloid in the cell wall of the thallus (Smirnoff & Wheeler, 2024).

When the seaweed reached Stage II and III, the seaweed could focus more on structure development as more tear ridges were on the thallus surface (Figure 2b, 2c). Deposition of hydrocolloid could happen during this process, as evidenced by the increase in tensile strength and elongation percentage (Table 2). As a result, the demand for ascorbic acid in metabolic pathways reduced, leading to its gradual decline (Table 3). In industry, seaweed is normally added to food products to develop new products and nutraceuticals (Chandrasekhar et al., 2023). The pH, TA, and AA values indicated that seaweed had a natural pH and low acidity, making it suitable for incorporation into a wide range of food products. The characteristics would allow it to blend effortlessly with other ingredients without affecting the overall taste or stability of the final product.

The ash content is a key indicator of seaweed's nutritional quality, reflecting the presence of minerals and trace elements. The ash content of *G. domingensis* was 23.8%, while *G. birdiae* was 22.5% (Gressler et al., 2010). The lower amount of ash content was found in Gracilaria, ranging from 5% to 6% (Purwaningsih et al., 2024). This study observed that the ash content of *G. changii* decreased (p>0.05) progressively from Stage I to III, dropping from 38.93% in Stage I to 34.50% in Stage II and further to 28.53% in Stage III. Seaweed harvested during Stage I could have more mineral and trace elements due to a significant ash content (%). Chan and Matanjun (2017) reported that K and Na are

the most abundant minerals in *G. changii*, with K being eight times higher than Na. The presence of minerals in seaweed may result from the ability of its surface to absorb elements from the surrounding seawater (Kumar et al., 2011). The thallus surface can undergo ion exchange, facilitating the uptake of minerals from the marine environment into the cell cytoplasm (Roleda & Hurd, 2019). The decline in mineral content is likely due to a reduced ability of the thallus surface to absorb minerals from its surroundings, as reflected by the distinct changes in thallus morphology (Figure 1) and its surface characteristics observed from stages I to III (Figure 2).

Antioxidant Activity

Phenols and flavonoids are the most common bioactive compounds of land plants and seaweed, responsible for antioxidant activity (Sadeghi et al., 2024). The bioactive compounds present in seaweed can be exploited for functional ingredients to enhance the nutritional, textural, and sensory attributes of food products. TPC and TFC quantified phenolic and flavonoid compounds, respectively. The TPC measures the total amount of phenolic compounds in a sample, while TFC measures the total amount of flavonoids, a subclass of phenolic compounds (Nor et al., 2023). Parallelly, the antioxidant capacity of seaweed extract was assessed using DPPH radical scavenging activity and ABTS radical scavenging methods (Table 4). DPPH and ABTS assays are common techniques used to measure the antioxidant potential of a sample. The DPPH assay involves the generation of the DPPH• radical, a stable nitrogen-centred free radical characterised by its deep purple colour and maximum absorbance at 517 nm. In contrast, the ABTS assay produces the ABTS•+ radical cation, a blue-green chromophore with a strong absorbance peak at 734 nm. Flavonoids and phenols containing hydrogen-donating groups reduce DPPH and ABTS solutions by forming non-radical species, demonstrating their antioxidant potential (Ramli et al., 2023).

Table 4
Antioxidant properties of extracts of G. changii *quantified by TPC, TFC, ABTS and DPPH at different maturation stages*

Stages	TPC (mg GAE/g)	TFC (mg QCE/g)	ABTS (µmol TE/100g)	DPPH (µmol TE/100g)
Stage I	1.16±0.00016[a]	0.279±0.00417[a]	26.86±7.10[a]	11.84±0.086[a]
Stage II	1.139±0.000047[a]	0.264±0.00236[a]	24.53±5.11[a]	11.87±0.078[a]
Stage III	11.015±0.0000597[b]	0.296±0.00171[a]	24.63±3.83[a]	11.78±0.188[a]

Note. The result was expressed in means ± standard deviation (n=27), followed by the same letter in the same column, and was not significantly different (p>0.05) according to Tukey's post hoc tests. GAE=Gallic acid equivalent, QCE=Quercetin equivalent, DPPH =2,2-diphenyl-1-picryl-hydrazyl, ABTS = 2-2'-azino-bis (3-ethylbenzothiozoline-6-sulfonic acid)

The TPC values of the seaweed extracts ranged from 1.16 to 11.015 mg GAE/g, with Stage III exhibiting the highest TPC value. Meanwhile, the TFC contents were not significantly (p>0.005) different across different maturation stages, with values ranging from 0.264 to 0.279 mg QCE/g. The value of cultivated *G. changii* obtained from this study was higher than the TPC (0.1848–0.2365 mg GAE/g) and TFC (0.003–0.0032 mg CE/g) values of selected seaweed species grown in Sabah, including *Sargassum polycystum, Eucheuma denticulatum* and *Kappaphycus alvarezii* (Fu et al., 2016). Similarly, the ABTS (24.63–26.86 µmol TE/100g) and DPPH (11.84–11.87 µmol TE/100g) values for *G. changii* were higher than those of the species cultivated in Sabah, where DPPH and ABTS were reported to range from 0.3–3.0 µmol TEAC/100 g dried sample and 0.01–0.4 µmol TEAC/100 g, respectively (Fu et al., 2016). In the present study, TPC did not seem to significantly contribute to antioxidant activity, as TPC increased drastically (p<0.05) from Stage II to Stage III, while DPPH and ABTS activities remained stable (p>0.05). This trend parallels the antioxidant activity of the stem, peel and flesh of dragon fruit, which the TPC has not contributed to (Fidrianny et al., 2018; Nurliyana et al., 2010).

Flavonoids could contribute to antioxidant activity, as the similar trends observed in TFC, ABTS, and DPPH assays suggested. Hydrocolloids that build the seaweed thallus could potentially be the primary contributors to its antioxidant activity, which should be determined in the future. Several studies have demonstrated that hydrocolloids exhibit antioxidant properties due to their carbohydrate content, which contains hydroxyl groups capable of donating electrons or hydrogen to neutralise free radicals (Luo et al., 2024). Furthermore, sulphated polysaccharides enhance this antioxidant activity by increasing electron density and improving the molecule's capacity to donate electrons or hydrogen for free radical neutralisation (Hu et al., 2024). Since the antioxidant activity in all stages is not significantly different, Stage II could be pointed out as the optimal Stage for seaweed harvesting, considering other physicochemical and textural profiles.

CONCLUSION

The physical and chemical properties of *G. changii* have been shown to be influenced by the stages of maturation. The thallus of *G. changii* seaweed was cylindrical, straight and branched in all stages of development. The physical feature of young seaweed l (Stage I) produced light brown, possessed weak tensile strength, and was less elastic than stages II and III. This study suggests that Stage II is the optimal harvesting stage for fresh use and processing for various applications in the food and nutraceutical industries. In Stage II, the *G. changii* was red-brown as indicated by CIELAB colour. The texture of the seaweed stabilised during Stage II, resulting in strong mechanical properties that give it springy and tactile properties. The physicochemical properties of stages II *G. changii* can be described as having a natural pH, low acidity and an acceptable amount of ascorbic acid and ash

content. In addition, the contents of bioactive compounds were at a considerable amount as indicated by TPC, TFC, ABTS and DPPH, which could work as free radical neutralisers and reduce oxidative stress. In developing nutraceutical products, this antioxidant activity is a major criterion required for food ingredient incorporation. This study provides fundamental knowledge for establishing a postharvest standard for fresh quality seaweed to be used as food and functional ingredients in the industry.

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