Effect of storage temperature with vacuum packaging on physicochemical stability of *Ulva ohnoi.*

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Abstract. Indonesia's seaweed industry, despite its vast potential, predominantly exports raw materials, with value-added processing limited to certain red and brown seaweeds. Green seaweeds, such as *Ulva,* are largely untapped by the processing industry despite their abundance and potential natural pigment sources. *Ulva* undergoes rapid color deterioration in tropical climates, indicating pigment degradation and quality loss. This study investigated the impact of storage conditions at 4°C (chilling) and 28°C (room temperature) using minimal processing vacuum packaging on the stability of physicochemical properties, color profile, pigment concentration, and antioxidant activity over three months. Results showed that chilling with minimal processing significantly inhibits color degradation and maintains greenness *a** value of the start of -15,76±1,11 to -10.91±1,14 while the room temperature is -5.68±0.72. In the third month, chilled samples exhibited significantly higher concentrations of chlorophyll *a* at 5.71±0.50 µg/mL, in contrast to room temperature, which was 3.83±0.4 µg/mL. Antioxidant activity decreased in the third month. Furthermore, the moisture content in the chilled samples was lower and stable at 28.87±0.16%, compared to samples stored at room temperature, which measured 33.34±0.54%. Chilling temperature with vacuum packaging effectively preserves the quality of *Ulva ohnoi,* making it suitable for industrial applications. This study provides important insights for storage conditions and increasing the commercial potential of this underutilized seaweed.

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1 Introduction

Industrialization has profoundly impacted the economy, transforming it from an agricultural-based system to a mechanized manufacturing-focused one. This transition led to economic growth, as shown by The Industrial Revolution during the 19th century by developed countries such as Europe and North America towards other countries, notably Asia. A developing country, Indonesia shows strong evidence that industrialization and financial development are critical to long-term economic growth [1]. This country holds immense potential in seaweed industrialization as Indonesia contributed approximately 25% of the world's total seaweed production, which amounted to 36.5 million tonnes in 2022, making it the second largest producer after China [2]. At the same time, the market of commercial seaweed was valued at $6.5 billion in 2021 and is projected to reach $14.6 billion by 2031, growing at a Compound Annual Growth Rate (CAGR) of 8.7% from 2022 to 2031 [3]. This makes this commodity potential for the country's economic state.

Indonesia's seaweed industry was predominantly known by the global market for its red and brown seaweeds, which are well-established and economically significant. However, the green seaweed sector remains largely underutilized despite its potential. *Ulva* sp., a rapidly growing green seaweed, is particularly promising for biomass production and sustainable industrial utilization in Indonesia [4]. This seaweed has been traditionally used for food and feed [5]. Unlike other commercial seaweed, recent reports show *Ulva* species exhibit significantly higher growth rates than many other seaweeds, with fourfold daily growth rates of 0.37–0.89/day [6]. This gives *Ulva* high biomass, and we can tap into the potential of this abundant resource without depleting natural stocks. This seaweed has garnered considerable attention recently owing to its potential as a promising source of bioactive compounds [7-11]

However, there is limited information regarding the storage conditions. Traditionally, controlling the storage temperature is key to preserving seaweed quality. The physical properties of *Ulva lactuca* (sea lettuce) and other seaweeds like *Kappaphycus* and *Gracilaria* vary significantly due to their distinct biological characteristics. *Ulva* has a thin, flat thallus that is typically only two cells thick. This structure makes it more susceptible to storage conditions. *Ulva lactuca* is particularly sensitive to temperature changes. When stored at 16°C, it shows signs of deterioration within days, including increased microbial activity and color degradation, such as loss of greenness [12]. In tropical regions, where room temperatures are elevated, this sensitivity leads to a much shorter shelf life compared to other seaweeds that may have more robust structures or lower moisture content. Also, there are significant gaps in research, while this species is a new candidate for industrial applications, commonly using dried specimens, with most of the previous research focused on specimen storage conditions as fresh food [12-14].

This species has highly perishable characteristics, as it quickly soaks up water from the air. Thus, storing dried *Ulva* with packing that minimizes the moisture absorption to kept the quality and color is suggested. There has been a growing trend in using polyethylene vacuum (PEV) pouches to preserve the quality of raw materials for the pharmaceutical industry and food products [15]. Vacuum packaging has been known for decades to extend shelf life, preserve color, moisture protection, and oxygen exclusion [16]. Vacuum packaging was also shown to maintain a higher degree of greenness in fresh seaweed compared to the control group over a 14-day storage period [14]. However, from those reports, there are some limitations. The study was conducted over a short time frame, utilized only a single temperature, and does not align with industrial needs and capacities, as it focused on fresh *Ulva* rather than dried. Therefore, there is still a notable lack of research regarding the temperature and packaging conditions for storing dried *Ulva*, revealing a significant gap in the foundational knowledge of storage conditions for this seaweed.

2 Materials and methods

2.1 Materials and apparatus

The main material used in the research was dried *Ulva ohnoi*. Seaweed from Lombok, West Nusa Tenggara, extraction process using ethanol 100% (Merck), 1,1-diphenyl-2-picrylhydrazyl (TCI). The apparatuses used were an ultrasonic cleaner bath (DSA 100, 200W), tray dryer (Food dehydrator-30, 800 W), air jacket- incubator, refrigerator, analytical balance (Ohaus Adventurer), polyethylene vacuum packaging, vacuum chamber sealer, micropipette (Nichipet), thermometer, Whatman filter paper No. 41, and glassware (Pyrex). The tools used in the analysis are a UV-Vis spectrophotometer (BMG Labtech Omega), chromameter (CR 300 Minolta), a_w meter (Rotronic HP23-AW-A-SET-40), and rotary evaporator.

2.2 Preparations and Experimental Design Storage Conditions of Dried Seaweed

The present study aimed to investigate the effect of storage temperature on the stability of active compounds in fresh wild green seaweed *Ulva ohnoi*. The harvested seaweed is collected from Coastal Fisheries Cultivation Development Center (CFCDC) Sekotong, West Nusa Tenggara, Indonesia. The seaweed samples were collected, washed, and dried on bamboo racks. Dried samples were then shipped to the Aquatic Product Department, and samples were immediately stored at -18℃ to maintain the quality. The dried samples were packed using PEV packaging with a chamber vacuum sealer and selected by hand to evaluate the quality of dried seaweed. Each weighing 100 g samples were stored at two different temperatures (4℃ and 28℃ for chilling and room temperature, respectively) for three months. Samples were taken from each bag on the first week of every month from January to May 2024 and were analyzed immediately every month. The results were then compared for the room temperature (RT) and chilling temperature (CT) groups.

2.3 Analytical Procedure

2.3.1 Moisture content

The moisture content analysis was conducted according to AOAC standards [17].

2.3.2 Water activity

Water activity was measured using an a_w meter Rotronic HP23-AW-A-SET-40. The water activity was put into a particular container by leaving the part on the top open. Then, the tool section for measuring water activity was placed above the sample container to be measured. After that, the settings were made by pressing the power button to turn it on, then pressing enter twice to change the a_w reset display to a_w dwell for measuring water activities. The

warning that water activity measurements had been completed was marked by the appearance of a check mark on the tool.

2.3.3 Color profile

Color analysis used the Hunter method with the Chromameter CR tool 300 Minolta. The sample to be analyzed was placed on the measuring head, and then the MEASURE button was pressed. Measurements were carried out at three points, and the results were printed in Lab notation. L represented the brightness parameter (color achromatic, 0: black to 100: white). The mixed chromatic color of red and green was indicated by the value a (a+ = 0-100 for red, a- = 0 – (-80) for red and green). The chromatic color of a mixture of blue and yellow was indicated by the b value (b = 0-70 for yellow, b- = 0 – (-70) for blue). Additionally, the chroma value (C*) was calculated using the formula $C^* = \sqrt{(a^2 + b^2)}$, and the hue angle (h) was determined using the arctangent function h = arctan(b/a), with hue ranging from 0° to 360°, corresponding to different colors in the color wheel.

2.3.4 Pigment Concentration

To analyze the pigment in *Ulva* extract, 4 g of dry-weight *Ulva* was mixed with 100 mL of ethanol solvent (Merck, Germany). The mixture was then extracted in an ultrasonic bath at 40°C for 60 minutes. The suspensions were then filtered through a Whatman paper no. 41 [18]. Each extract was analyzed using a BMG Labtech Omega spectrophotometer, and the extract was placed 200 µl into a 96-well microplate (Biologix). The samples were analyzed for the 400–700 nm absorption spectrum. Measurements were done in duplicate. Based on the absorption spectra, chlorophyll *a*, *b*, total chlorophyll, and total carotenoid content were determined according to the method described by Lichtenhaler [19]. The content of pigments was expressed as µg/mL of extract and was calculated using the following equations:

$$Chlorophyll\ (mg/ml) = (13.36 \times Abs\ 664) - (5.19 \times Abs\ 648) \quad (1)$$

$$Chlorophyll\ b(mg/ml) = (27.43 \times Abs\ 645) - (8.12 \times Abs\ 664) \quad (2)$$

$$Total\ Chlorophyll\ (mg/ml) = (5.24 \times Abs\ 664) - (22.24 \times Abs\ 648) \quad (3)$$

$$Carotenoids(mg/ml) = \frac{((1000 \times Abs\ 470) - (2.13 \times Chl.a) - (97.64 \times Chl.b))}{227} \quad (4)$$

2.3.5 Antioxidant Activity DPPH

DPPH radical scavenging activity was determined with slight modifications [20]. The pigment extract was evaporated and diluted into various concentrations. Briefly, 100 µL of each extract was placed into a microplate at concentrations 1, 0.5, and 0.1 mg/mL dilutions. Sample dilutions were mixed with 100 µL of 0.16 mM DPPH solution. The mixture was inverted back and forth using a micropipette to mix and kept for 30 min in the dark. Then, the absorbance was measured at 517 nm in an automated microplate reader (BMG Labtech Omega). The antioxidant capacity was calculated using the following equation:

$$Percentage\ inhibition\ (\%) = \frac{Absorbance\ of\ sample - Absorbance\ of\ control}{Absobance\ of\ control} x100\% \quad (5)$$

The half-maximal inhibitory concentration (IC_{50}) was calculated by linear regression analysis and expressed as the mean of three determinations.

2.4 Data Analysis

Data analysis was conducted using SPSS version 25.0 and Microsoft Excel 2013. The study investigated various physicochemical properties (including moisture content and water activity), color profiles (CIE *L, a*, b**, C*, h* parameters), and pigment concentrations (chlorophyll *a*, chlorophyll *b*, and carotenoid), antioxidant capacity (IC_{50}) was assessed. These analyses were performed over a single month, comparing samples stored at temperatures of 4℃ and 28℃. Statistical significance was determined using an Independent T-test at a 95% confidence level (α=0.05), with p-values less than 0.05 indicating significant differences between groups. Before hypothesis testing, the data underwent rigorous testing for basic assumptions, including homogeneity of variances and normality. This preliminary testing was performed using the Shapiro-Wilk test for normality and the Bartlett test for homogeneity of variances. A p-value higher than 0.05 in both tests confirmed that the data was both homogeneous and normally distributed, addressing the prerequisites for further statistical analysis.

3 Results and Discussion

3.1 Moisture content and water activity

Moisture content is an essential criterion in determining the shelf-life and quality of processed seaweed, as high moisture may faster the growth of microorganisms. The moisture content of fresh *Ulva lactuca* is high, typically ranging from 75% to 85% of its total weight. This high moisture content makes it susceptible to rapid deterioration and microbial growth when improperly stored. The reseach findings showed shows that CT groups have significantly lower moisture content, which indicates better stability throughout the storage months. Also, the research findings revealed that the dried samples from Lombok had an initial moisture content of 30.27%, which is considerably high. This was linear with a previous study that showed lower temperature (4 °C) storage of fresh *Ulva* with a low moisture content until the fourth day [13]. The study also Previous studies on dried *Ulva* sp., which was only 16.9% [21]. Additionally, other studies have documented a range of moisture contents for dried *Ulva* from as low as 0.95% to 14.57% and 10.5% [22-23]. This variance highlights the critical drying method impacting moisture content in determining the shelf-life and quality of processed seaweed since high moisture can accelerate the growth of microorganisms [13].

Despite its abundance, the National Standardization Agency of Indonesia has not yet set a quality standard for *Ulva* seaweed. If compared with other commercial species that have been commonly commercialized in Indonesia, such as *Eucheuma* sp. (32%), *Gracilaria* sp. (25%), *Turbinaria* sp. (20%), and *Sargassum* sp. (20%). *Ulva lactuca* samples with higher moisture content (71.37% for brined samples) degrade chlorophylls more rapidly compared to those with lower moisture content (14.22% for air-dried samples) [24]. Higher relative humidity during storage accelerates the degradation of chlorophyll in thylakoid powders. For example, spray-dried powders stored at higher RH levels (49% and 61%) showed a significant decrease in chlorophyll content, from 68 mg/g to 42 mg/g (−38%) and 32 mg/g (−52%) respectively [25]. Therefore, storing dried *Ulva* at low temperatures can minimize chlorophyll degradation that leads to color alteration and ensure that the storage environment has low humidity to prevent rehydration and subsequent chlorophyll degradation. The results of physicochemical analysis throughout the study can be seen in Table 1.

Table 1 Moisture content and water activity of *Ulva ohnoi.*

Physicochemical	Storage temperature (°C)	Storage time (Months)			
		0	1	2	3
Moisture content (%)	4	30.27±0,14	30.55±0.08[a]	29.50±0.07[a]	28.87±0.16[a]
	28		29.48±0.26[b]	30.90±0.07[b]	33.34±0.54[b]
Water activity (a_w)	4	0.68±0,007	0.68±0.09[a]	0.68±0.02[a]	0.66±0.01[a]
	28		0.68±0.02[a]	0.68±0.01[a]	0.65±0.02[a]

Different letters following the numbers in the same column indicate differences at the 5% significance level between room temperature (RT) and chilling temperature (CT).

Water activity (a_w) is one of the main parameters for indicating stability, mainly microbiological, for food and preservation storage time. Table 1 shows that the dried *Ulva* in the initial month is 0.68±0,07 and stabilizes till the tendency to decrease in the last months. The RT and CT groups show no significant difference in water activity on every month. The dried *Ulva ohnoi.* on the initial month shows low aw and stabilizes throughout the storage month, showing the importance of using vacuum packaging for air exclusion. Fresh *Ulva* reported an aw value of 0.96±0.04, indicating a high level of moisture, and stabilized for 12 days stored in 4°C [20]. This was similar to other marine products (0.99–0.97), vegetables (0.97–0.98), and other seaweeds (0.978–0.989) [26].

Vacuum storage reduces the water activity of seaweed by removing water molecules from the surface through evaporation. This decrease in a_w inhibits the growth of microorganisms, ensuring food safety and extending shelf life [14]. Keeping the a_w below 0.85 prevents microbial growth and ensures compliance with FDA regulations [27]. Water activity plays a crucial role in the degradation of pigments in seaweed. Higher water activities can lead to faster degradation rates, particularly for chlorophyll *a*, which is sensitive to moisture and oxygen [28]. In another study on the effects of a_w and temperature on color and chlorophyll changes in yerba mate leaf, chlorophyll *a* was degraded at the highest rate at higher water activities [29]. Higher a_w also can lead to faster degradation rates of phenolic compounds, which are sensitive to moisture and oxygen [30].

3.2 Color analysis

3.2.1 Color photographic documentation

The Dried *Ulva* species were selected and secured within Polyethylene Vacuum (PEV) packaging. Photographic documentation was conducted in the initial week of each month. Prior to the initiation of storage, an examination was undertaken to see the differential impacts associated with RT and CT group storage conditions on the specimens. This photographic documentation shows the sample's conditions throughout the months. As illustrated in Figures 1B, D, and F, the findings predominantly reveal that specimens subject to RT exhibit noticeable discoloration, transitioning towards a paler hue from the first month. In contrast, specimens maintained under CT conditions, depicted in Figures 1C, E, and F, consistently exhibit a darker and richer green complexion across the observational period. Moreover, a more significant occurrence of natural bleaching or whitening has happened more in the RT groups. CT samples showed a better color from maintaining their initial color from the samples, showing a more likeliness to consumer preferability. The morphological changes of the sample are shown in Figure 1 below.

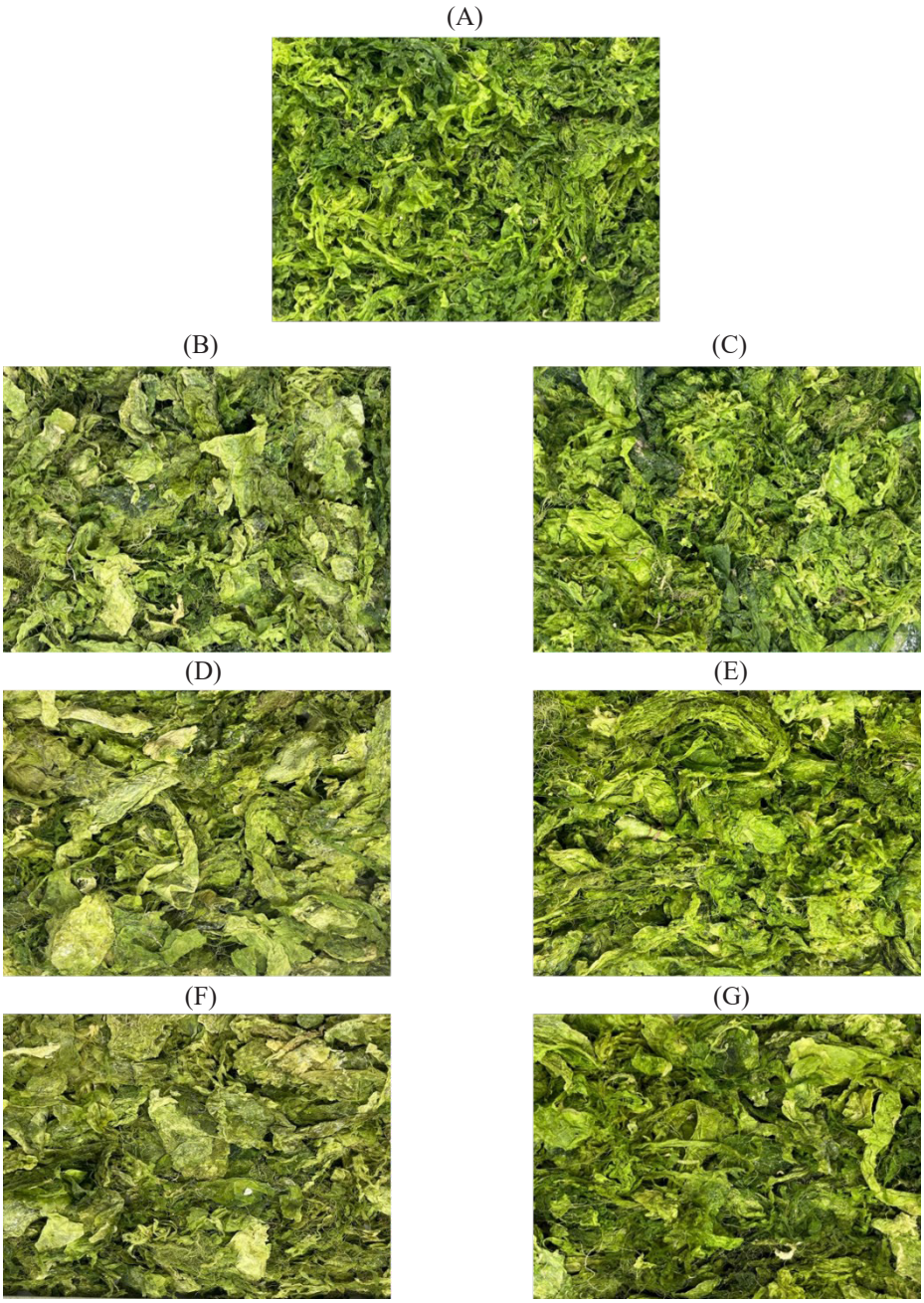

Fig. 1 *Ulva ohnoi*. Morphological change post room temperature and chilling storage using vacuum packaging. A). Before storage, B). One month after room temperature storage, C). One month after chilling temperature storage, D). Two months after room temperature storage. E). Two months after chilling temperature storage, F). Three months after room temperature storage, G). Three months after chilling temperature storage.

The conditions under which the specimens were stored presented marked differences, which were in line with the previous study's findings. Upon conducting sensory evaluations, it was determined that there was no significant discernible difference between the samples [13]. Supporting this observation, the pigment degradation process in *U. rigida* proceeds slower under 4℃ storage conditions [12]. Furthermore, contrasting methodologies involving variations in packing and rinsing procedures reported the development of an undesirable grey-brown hue in the specimens during the storage period [31]. This suggests that, apart from temperature, other storage handling practices are critical in maintaining the color stability of the specimens, indicating a complex relationship between storage conditions and the physiological reactions of *Ulva* leading to color alteration.

3.2.2 Color analysis

The green seaweed, notable for its distinct green hue, is crucial in determining the quality of its dried raw materials. Color significantly influences consumer preference and acceptability of products [32]. This is particularly relevant to species like *Ulva* because of its characteristic of being prone to color changes, transitioning towards a paler hue, indicating declining raw material quality. The evaluation of color is conducted using a colorimeter based on the CIE *L*a*b** color space, where '*L**' represents lightness, '*a**' the red to green spectrum, and '*b**' the yellow to blue spectrum. These parameters are vital in assessing discoloration in seaweeds such as *Ulva*, marking an essential criterion for quality analysis. Results show significant differences factored by the temperatures every month for every color indicator. Based on the data presented in Table 1, at 4℃, the CT groups initially exhibit lower lightness (*L**), followed by an increase over the months. Conversely, the RT groups demonstrate a decrease in lightness throughout the same period. The *a** value also indicates that the CT group shows a greener hue initially, which decreases over time. Similar trends are observed in the *b** value, with the CT group showing a more yellowish hue that decreases towards the end of the study period.

Table 2 Color Profile of *Ulva ohnoi* through time and temperature storage

Color	Storage Temperature ℃	Storage Time (Months)			
		0	1	2	3
*L**	4	44.72±1.61	38.19±0.89^{a}	47.71±2.36^{a}	56.73±1.99^{a}
	28		55.85±1.32^{b}	41.37±2.33^{b}	38.72±2.03^{b}
*a**	4	-15.76±1.11	-13.29±0.47^{a}	-11. 63±1.24^{a}	-10.91±1.14^{a}
	28		-8.00±0.68^{b}	-8.38±0.52^{b}	-5.68±0.72^{b}
*b**	4	28.09±1.04	24.45±0.38^{a}	26.22±0.92^{a}	29.68±0.83^{a}
	28		22.61±0.41^{b}	22.80±1.19^{b}	19.23±1.12^{b}
C*	4	32.21±1.41	27.61±0.55^{a}	28.69±1.18^{a}	31.63±1.05^{a}
	28		23.99±0.42^{b}	24.30±1.08^{b}	20.05±1.26^{b}
*h**	4	119.28±0.98	118.38±0.49^{a}	113.88±1.67^{a}	110.16±1.70^{a}
	28		109.48±1.59^{b}	110.22±1.67^{b}	106.42±1.10^{b}
Δ E	4	-	9.14±2.10^{a}	11.05±1.68^{a}	11.15±1.84^{a}
	28	-	18.78±3.40^{b}	13.39±5.12^{a}	8.18±1.19^{a}

Different letters following the numbers in the same column indicate differences at the 5% significance level between room temperature (RT) and chilling temperature (CT).

The CT groups show the greenest specimen, as seen on *a** value results, and preserved the best color. The observed phenomenon is linear, as previous findings in a temperature-dependent discoloration in specimens over 12 days, wherein lower temperatures (4℃) predominantly maintained greener and yellower hues [12]. Contrastingly, an elevation in temperature to 16℃ notably enhanced the *a** value, indicative of redness, and reduced the *b** value, suggesting a decrease in yellowness. In contrast, the *L** value, representing lightness, remained relatively stable throughout the storage period. Similarly, recent findings identified a gradual color transition from dark green to yellowish-green [13]. The presence of carboxyl and amino groups on the seaweed surface, which have an affinity for metal ions, is postulated as a contributing factor to the discoloration observed during storage [33]. This hypothesis is supported by the premise that elevated temperatures may expedite chemical reactions, thereby altering the pigments and structural integrity of the seaweed, manifesting as discoloration. The degradation of chlorophyll, the primary green pigment in seaweed, due to environmental stressors such as light, heat, or chemical exposure is a plausible mechanism for the observed color shift towards lighter shades.

The Δ E value Differences in perceivable color can be analytically classified as very distinct ($\Delta E^* > 3$), small differences ($1.5 < \Delta E^* < 3$), and undetectable differences ($1.5 < \Delta E^*$) by non-experimented observers. The results showed very distinct color alteration in every month of storage. The most significant color alteration happened in the first initial month by the RT groups, followed by a decrease, whereas chilled samples demonstrated more stable degradation rates over time. This indicates that storing *Ulva* in chilling temperatures showed slower color alteration and more stability in the color of the specimen. While storing at room temperature, there was significant color alteration starting from the initial month of storage.

3.3 Pigment Concentration

In tropical countries like Indonesia, using *Ulva* as food is not ideal because maintaining its freshness proves to be quite expensive, limiting its potential for a demanding local market. Therefore, exploring *Ulva* as a viable candidate for industrial applications presents a novel and promising direction [4]. This study is particularly relevant for industrial purposes, focusing on storing dried raw materials to ensure the effective preservation of the desired pigment compounds. The results consistently demonstrated higher pigment concentrations in the chilling groups for chlorophyll *a*. This was aligned with the color profile findings, where higher *a** values indicating greater greenness corresponded to higher chlorophyll content and more likely to consumer preferences. *Ulva's* carotenoid concentration indicates that storing at chilling temperatures preserves higher levels. However, carotenoid content is low compared to chlorophyll, the main pigment. The study also showed fluctuating results over the month, with an initial decrease in the first month and gradually increasing at the end of the study month. This suggests that storing the pigments at chilling temperatures significantly preserves their concentration. The results of the study are presented in Table 3.

Table 3 Pigment concentration of Chlorophyll *a,* Chlorophyll *b,* Total Chlorophyll and Carotenoid under different temperatures in *Ulva ohnoi.*

Pigment (μg/mL)	Storage Temperature (°C)	Storage Time (Months)			
		0	1	2	3
Chlorophyll *a*	4	5.01±0.15	2.25±0.38[a]	2.24±0.14[a]	3.83±0.44[a]
	28		4.22±0.30[b]	5.34±0.53[b]	5.71±0.50[b]
Chlorophyll *b*	4	5.67±0.15	3.15±0.80[a]	5.12±0.64[a]	7.22±1.31[a]
	28		6.72±0.93[a]	7.90±0.91[a]	6.06±0.09[a]
Total Chlorophyll	4	10.68±0.30	5.40±1.18[a]	7.84±0.13[a]	11.04±1.75[a]
	28		10.94±1.23[b]	13.24±1.44[a]	15.65±0.59[a]
Carotenoid	4	0.39±0.33	0.76±0.09[a]	0.99±0.07[a]	1.97±0.32[a]
	28		0.43±0.08[a]	0.44±0.02[b]	0.34±0.13[b]

Different letters following the numbers in the same column indicate differences at the 5% significance level between room temperature (RT) and chilling temperature (CT).

The findings were linear with the study on pitaya bark flour, which showed pigment degradation faster at room temperature than in refrigerated conditions [34]. The previous study indicates that temperature is the most relevant factor among many variables studied and may cause degradation in pigments [35]. Based on the findings, lower temperatures help maintain a more stable water content in the dried seaweed. High water content causes microbial growth and enzymatic activity, which degrade pigments [24]. The stable water content at 4°C helps preserve the pigments' integrity. The environmental conditions contributed to the initial decrease in chlorophyll. Slight increases after initial degradation may occur as the chlorophyll loss rate slows down, potentially due to reduced enzymatic activity or lower oxygen levels achieved through proper storage. In some cases, increases in chlorophyll can result from the breakdown of other pigments or the synthesis of new chlorophyll, typically under controlled atmospheres or low-temperature storage [36]. The timing of analyses (first week of each month) might also influence observed trends.

Chlorophyll degradation occurs through several mechanisms influenced by environmental factors and processing conditions. A primary pathway involves enzymatic degradation, where chlorophyllase removes the phytol chain from chlorophyll, producing chlorophyllide, which further degrades to pheophorbide by heat or acid [25]. Chemical degradation, particularly pheophytinization, involves replacing the central Mg^{2+} ion in chlorophyll with two H^+ ions to form pheophytin. This reaction is accelerated by acidic conditions and heat [37]. Additionally, saponification can break down the phytyl side chain of chlorophyll, leading to chlorophyllides or pheophorbides [38].

Additionally, oxygen can trigger oxidative stress, which contributes to chlorophyll degradation [39]. Vacuum packaging helps maintain chlorophyll levels, preventing discoloration from bright green to olive-green or olive-yellow, a process known as pheophytinization [40]. The low carotenoid content was partly due to extraction methods using polar solvents, like ethanol, while carotenoids are generally lipophilic (fat-soluble). Carotenoids demonstrate better stability at chilling temperatures due to their long chains of conjugated double bonds, which confer resistance to oxidative degradation via electron delocalization [41]. They can degrade through various mechanisms influenced by environmental factors and chemical reactions. Molecular oxygen can directly degrade carotenoids, forming 1,2- or 5,6-epoxy-carotenoids that separate chromatographically from their parent forms. Additionally, research indicates that increased temperatures accelerate carotenoid degradation, especially in the presence of oxygen, leading to greater losses of myxoxanthophyll and echinenone at higher temperatures [42].

Chlorophyll derivatives are recognized as benign additives that act as natural coloring agents and antioxidants in various food products, including juices, dairy, confections, gels, and soups. Global chlorophyll production is projected to reach approximately 1.2 billion units annually [40]. These derivatives and their copper complexes by their respective E140 and E141 are noted in the food industry for their safety as natural colorants. European regulations require chlorophyll extraction from consumable sources for food applications. Previous research showed that seaweed-derived chlorophyll enhances the aesthetic quality of jelly desserts, maintaining color stability for over a month at ambient conditions [43]. Additionally, a previous study reported the successful incorporation of seaweed C. racemosa powder in semi-sweet biscuits, reducing their yellowness, redness, and lightness due to chlorophyll content. [8]. These findings underscore the essential knowledge for storing *Ulva* dried raw materials for various applications.

3.4 Antioxidant activity

The antioxidant properties of algae are influenced by external environmental factors such as salinity, nutrient availability, light exposure, growth depth, and seasonality. Additionally, intrinsic factors, including algal species, age, length, and tissue type, play a significant role [44]. The primary antioxidant groups in seaweeds are pigments, polysaccharides, vitamins, and phenolics [45]. These antioxidants function as free radical scavengers, thereby protecting cellular and tissue integrity against oxidative damage. They are crucial in maintaining cellular health and offer various health benefits, including reducing the risk of chronic diseases [11]. Multiple species of *Ulva* have demonstrated potent antioxidant activity. This can be a big potential for the utilization of *Ulva* itself. The study's results show that storage temperature significantly affects antioxidant activity only in the third month, with chilling temperatures indicating more effectiveness in radical scavenging. Also showed, a slight increase in IC_{50} values was observed from month 2 to month 3, indicating a decrease in antioxidant activity. The results are presented shown in Table 4.

Table 4 Antioxidant activity pigment extract of *Ulva ohnoi.*

Antioxidant	Storage Temperature (℃)	Storage Time (Months)			
		0	1	2	3
IC_{50} (mg/mL)	4	0.82±0.06	0.91±0.22^{a}	1.32±0.36^{a}	7.56±0.18^{a}
	28		1.47±0.77^{a}	1.37±0.47^{a}	8.83±0.59^{b}

Different letters following the numbers in the same column indicate differences at the 5% significance level between room temperature (RT) and chilling temperature (CT).

The findings align with the research of storing matcha at higher temperatures, which resulted in a significant decrease in antioxidant activity by the seventh day, suggesting that lower temperatures are more effective for preserving antioxidant properties [46]. Complementing these findings, another study indicates that the antioxidant activities of *Ulva lactuca* extracts remained stable for five days when stored in cool and dark conditions [47]. Additionally, different species of *Ulva* from the Persian Gulf exhibited a range of IC_{50} values, from 0.881 ± 0.047 to 2.372 ± 0.22 mg/mL, highlighting the variability in antioxidant potential across species [20]. The slight increases in IC_{50} value in the third month for every samples can happen because the active antioxidant compounds like pigments and phenol have degraded or oxidized over time. Chlorophyll pigments in *Ulva* are not only essential for photosynthesis but also contribute to its antioxidant properties. The presence of magnesium in chlorophyll enhances its ability to scavenge free radicals and act as an antioxidant [48]. These compounds can scavenge free radicals and chelate metal ions, thereby reducing

oxidative stress. The antioxidant activity of ethanol extracts from *Ulva* is likely due to a combination of both chlorophyll pigments and phenolic compounds. The synergistic effects between these compounds can enhance the overall antioxidant capacity of the extract [20].

The antioxidant activity from the expected extracted compound, namely chlorophyll and carotenoid [49]. Chlorophyll and carotenoids exhibit antioxidant activity through several mechanisms, although their effectiveness can vary depending on the context and specific conditions. Chlorophylls can directly interact with free radicals, neutralizing them and preventing oxidative stress. This activity is particularly noted in chlorophyll *a* and chlorophyll *b*, which have been shown to scavenge peroxyl radicals and other reactive species [50]. In contrast, the chemical structures of carotenoids serve antioxidant activity. They can trap peroxyl radicals with the production of resonance-stabilized carotenyl radicals, thereby neutralizing oxidative stress [51]. Additionally, it reported pheophytins have higher antioxidant activity [50]. Recent studies of chlorophyll metabolites, their bioavailability, metabolic pathways, and precise oxidation mechanisms remain limited. In vitro studies have explored the stability and absorption of chlorophylls during digestion. Findings suggest that chlorophylls *a* and *b* are converted into their corresponding pheophorbides and pheophytins before intestinal absorption, with rates comparable to carotenoids [49]. These findings collectively underscore the importance of storage conditions in maximizing the antioxidant benefits of *Ulva* extracts.

4 Conclusions

The industrial potential of *Ulva* species, particularly as a natural pigment source, has been growing trends. With abundant *Ulva* resources in Indonesia, the base knowledge important to storage methods is essential to prevent rapid degradation and preserve the quality of this valuable raw material. This study provides key insights into the effects of different storage temperatures, specifically chilling (4°C) and room temperature (28°C), on the characteristics of dried *Ulva ohnoi* over a three-month period. The results indicate that chilling temperatures significantly preserve the moisture content, color, chlorophyll *a*, and antioxidant activity. These findings underscore the importance of chilling temperatures, in combination with vacuum packaging, to effectively minimize color changes and pigment degradation. This positions *Ulva ohnoi*. as a promising industrial resource, with proper storage being critical for maintaining its quality and extending its utility in novel applications.

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