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Characteristic of *Ulva lactuca* Freshness Under Different Temperatures at Short-term Storage

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Abstract

Ulva sp. is green macroalgae with a high potential for nutrient-dense food production. This species is found on a coastline in the intertidal zone of Indonesia's waters. There has been research on this species starting from post-harvest handling, nutrition, probiotics, and its ulvaran, however, information on the effect of temperature storage on *Ulva lactuca* freshness is still lacking. This study was undergone to evaluate the freshness quality of *U. lactuca* by sensory changes in different temperatures short-term storage. In order to evaluate the effect of the different storage circumstances, the fresh *U. lactuca* was collected from its natural habitat (intertidal zone of Sepanjang Coast, Yogyakarta Indonesia). It was rinsed from debris and epiphyte, stored in a transparent polyethylene bag, and stored at 4 °C, 15-20 °C, and room temperature for five days. Color, pH, ash, moisture, crude protein, chlorophyll, Total Plate Count, and sensory analysis was undergone during the storage period. The sensory evaluation score of *U. lactuca* in 4 °C was more than 6, and better than other storages. The crude protein (*U. lactuca* stored in 4 °C) decreased significantly on day 4 by 5.53%, it was lower than others. The TPC of all samples varied from 147x10^{3}in 0 days of storage to 2,462.5x10^3 CFU/ml on the last day of storage. In summary, the sensory scores of *U. lactuca* are more consistent and higher at 4 °C than in other storage conditions, despite a slight decline in nutrient content.

INTRODUCTION

The potential of macroalgae resources in Indonesia is about 6.42% of the world's total macroalgae biodiversity (Lalopua, 2018), including *Ulva* sp., which is easily found in Indonesian waters. The habitat distribution of *Ulva* sp. is in the coastal area intertidal zone (Rao *et al*., 2018). They are grown by attaching the holdfast to dead coral, sand, or coral fragments mixed with sand (Isham *et al*., 2018). Green macroalgae *Ulva* that lives in Indonesian waters has several species: *Ulva lactuca, Ulva reticulata, Ulva rigida,* and *Ulva fasciata* (Ferawati *et al*., 2014;

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Handayani, 2016; Kepel *et al*., 2016; Tarigan *et al*., 2020). *Ulva* sp. belongs to the green macroalgae group (Chlorophyta) because it contains a green pigment derived from chlorophyll (Tarigan *et al*., 2020). Fresh *U. lactuca* has the shape of a fresh green thallus (dark green to bright green), the thallus is a thin sheet, smooth, widened to light, the edges are wavy, and the base is thickened (Handayani, 2016; Kepel *et al*., 2016) with a length between 30-50 cm (can reach about at 100 cm) (da Costa *et al*., 2018), because it has a broad thallus also known as sea lettuce. As a food, *Ulva* is consumed as a salad or soup in European countries, as aonori in Japan, and as a snack (chips/ crackers) in Gunungkidul, Yogyakarta, Indonesia coastal area (da Costa *et al*., 2018; Poeloengasih *et al*., 2019). In Indonesia's coastal area, *Ulva* grows during the dry season or when it is the rainfall is low near the intertidal zone.

Ulva is very potential for high nutrient content. Study results of *Ulva*'s nutrition by Abirami and Kowsalya (2011) showed that nutritional value per 100 g, namely 12.9 g of protein; 64.2 g of carbohydrates; 1.2 g of lipid; 10.5 g of ash; 1094 mg of calcium; 86 mg of phosphorus; 2.3 mg of Fe; 3080 mg of magnesium; 4.7 mg of niacin and study result from Rasyid (2017) showed nutrition of dried *U. lactuca* which contained 13.6% of protein; 0.19% of lipid; 11.2% of ash; 58.1% of carbohydrates; 28.4% of fiber; <0.5 IU/100 g of vitamin A; 4.89 mg/kg of vitamin B; 0.86 mg/kg of vitamin B2; 364 mg/100 g of calcium; 1828 mg/100g of potassium; 14.0mg/100g of Fe; 467 mg/100g of potassium; 0.05% of phosphorus. The study results by Kurniawan *et al*. (2019) showed that *U. lactuca* contained bioactive alkaloids, flavonoids, phenols, steroids, and triterpenoids. *U. lactuca* also has the potential for large ulvan polysaccharides, which can be used in the food industry as an additional ingredient in the manufacture of prebiotics (Shalaby and Amin, 2019).

Since the great potential of *Ulva* was known, research related to this green macroalgae study was done in late several years. Postharvest techniques for *U. lactuca* have been carried out by Poeloengasih *et al*. (2019), studies on *U. lactuca* nutrition by Abirami and Kowsalya (2011) and Rasyid (2017), studies on *Ulva* as ulvaran potential for prebiotics were carried out by Shalaby and Amin (2019), Nayyar and Skonberg (2019) were known to study about the quality freshness of red macroalgae and (Sánchez-Garcia *et al*., 2021) studied about freshness quality and shelf life of *U. rigida*, but there is still lack of information about the storage of freshness *U. lactuca*. A study of *U. lactuca* freshly-postharvest quality is needed because of its potential as fresh food for diet healthy consumed (such as salad, soup, or garnish) and is widespread in Indonesian waters. Nowadays, the change in lifestyle has made a healthy diet a popular lifestyle by consuming fresh ingredients, and it has also made an opportunity for open-market in Western countries to sell fresh macroalgae products as a vegetable (Nayyar and Skonberg, 2019). Therefore, maintaining the optimal condition of fresh macroalgae needs further study.

After post-harvesting, fresh macroalgae can be stored only in the short term for several days, because it is a perishable food that can be damaged by microbial growth activity. Low- temperature storage in a refrigerator is a simple, easy, effective, and low-cost method to keep fresh products (Zhao *et al.,* 2022). Previous studies about the maintenance of fresh products in low temperatures have been done by Gouble *et al*. (2022) and the study of fresh macroalgae maintenance has been done by Nayyar and Skonberg (2019) for *Palmaria palmata* and *Gracilaria tikvahiae* from the cultivation of Maine Fresh Sea, Bristol, ME, USA, and by Sánchez-Garcia *et al*. (2021) for *U. rigida* from the waters of San Fernando, Spain. Different species and habitat locations of macroalgae will show characteristic differences to define

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optimal conditions and quality of fresh macroalgae. Evaluate quality changes in fresh macroalgae could be done by sensory evaluation, microbiological analysis, chemical analysis (soluble protein), physical analysis (drip loss, colorimetric, and instrumental texture (Nayyar and Skonberg, 2019; Sánchez-Garcia *et al*., 2021), but the sensory evaluation is faster from others to define product quality changes. According to Sánchez-Garcia *et al*. (2021), sensory analysis has been considered to evaluate freshness and determine quality macroalgae changes.

This study purposed to evaluate the freshness quality of *U. lactuca* by sensory changes in different temperatures short-term storage. Chemical, physical, and microbiology analyses were measured to define the quality of freshness of *U. lactuca* from the Sepanjang coast area, Gunungkidul Yogyakarta-Indonesia.

METHODOLOGY

Place and Time

This research was conducted from January to March of 2021. The sample was obtained at the coast of Sepanjang, Daerah Istimewa Yogyakarta, Indonesia. The experiment was performed in the laboratory of the Research Unit for Natural Product Technology, Indonesian Institute of Sciences (BPTBA LIPI), Gunungkidul Daerah Istimewa Yogyakarta.

Research Materials

The materials applied in this study were *U. lactuca* hand harvested from a local area that was in intertidal Sepanjang Coast habitat, Daerah Istimewa Yogyakarta, transparent polyethylene bags (± 30x20 cm), acetone, NaOH, HCl, PP indicator, NaCl, Plate Count Agar (PCA) medium. While some equipment were Refrigerators, chromameter (Konica Minolta Color Reader CR-20, Japan), digital pH-meter (Eutech Instruments PC 700, Singapore), equipped with a probe (Eutech Thermo Scientific, Singapore), oven (Memmert, Germany), UV-Visible Spectrophotometer, blender (Philips HR 2116, Indonesia), erlenmeyer, incubator (Binder, Jerman), porcelain crucible, furnace (Thermolyne, Thermo scientific, F 48010-33, USA).

Research Design

The experiment featured three distinct storage conditions: room temperature, 15-20 °C, and 4 °C for 0-4 days. Each day, the seaweed's Total Plate Count (TPC), total chlorophyll, color, and proximate were evaluated and recorded. A sensory test involving trained panelists was also done to evaluate the appearance, texture, color, and odor of the seaweed.

Work Procedure

Preparation and Collection of Sample

U. lactuca was collected in a plastic/ styrofoam container, which is then added clean seawater to keep it in fresh circumstance. *U. lactuca* was then cleaned and rinsed with drained seawater to make sure that there was very minimum debris (sand, gravel, biota) right after the sample arrived at a laboratory. The seaweed was divided into more and less 500 g batches in transparent polyethylene bags and closed loosely. The sample in the plastic bag was stored in three different conditions, refrigerator at 4 °C, refrigerator at 15-20 °C, and room temperature circumstance. The sample was collected from the three conditions for analysis every day for 5 days (0-4 days), such as pH, protein, ash, moisture, chlorophyll, TPC, and color. A similar procedure of providing fresh *U. lactuca* was also conducted for sensory tests. Fresh seaweed obtained from the same location keeps in the transparent polyethylene, ± 400 g each. There were four bags for every refrigerator at 4 °C, refrigerator at 15-20 °C, and room temperature for sensory tests on days 1-4, and one bag for fresh seaweed sensory test (day 0).

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Physical Analysis

The color of *U. lactuca* was determined using a chroma-meter with a white calibration cap (Konica Minolta, inc, Japan). The color parameter was L* value (representing brightness/lightness), a* value (representing for redness-greenness), b* value (representing yellowness-blueness), hue angle/color purity (h*), Polar coordinates of color Chroma (C*), Total color difference (ΔE) (Yasir and Qin, 2010; Hoang *et al*., 2016; Kaemba *et al*., 2017; Uribe *et al*., 2018; Charles *et al*., 2019). The Chromameter is calibrated first by placing the instrument on the cap calibration. The total measurement of degrees of color was used as a white color base as a standard. Hue angle (h*), Polar coordinates of color Chroma (C*), and Total color difference (ΔE) of *U. lactuca* were calculated using the following Equations (Uribe *et al*., 2018; Charles *et al*., 2019).

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

$$C^* = (a^{*2} + b^{*2})$$

$$\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$$

Where:

L and L_0 = difference in lightness
a and a_0 = difference in intensity (red color)
b and b_0 = difference in intensity (yellow color)

Chemical and Physical Analysis

Moisture content was determined gravimetrically according to the SNI 01-2891 (BSN, 1992) by drying 1-2 g sample (W). The sample was dried in the oven at 105 °C for 24 hours and stored in the desiccator for 30 minutes before weighing the sample. The sample was then dried again in the oven until it reaches constant weight (W2). The percent moisture content was calculated as follows:

$$\text{Moisture content} = \frac{(W1 \times 100\%)}{W}$$

Where:

W = sample weight before drying (g)
W_1 = lost weight after drying (g)
$W_1 = W - W_2$

The total chlorophyll of the sample was measured according to Sanger *et al*. (2018). Around 1-2 g sample was pulverized using mortar and pastel. For extracting the chlorophyll, 50 mL acetone was added to the sample. The sample was put into an aluminum foil-covered flask and stored at 4°C for 24 hours. The extracted sample was filtered through 100 mesh filter paper. The filtrate was measured using UV-Visible Spectrophotometer at 663 and 645 nm wavelengths. The total chlorophyll concentration (TC) was calculated by the followed formula:

$$TC\left(^{mg}/_{g}\right) = \frac{[(20.2\ (A_{.645}) + (8.02\ (A_{.663})V]}{1000xW}$$

Where:

W =weight of sample (g)
V = total volume of liquid extract
$A_{.645}$ = absorbance value of 645 wavelength
$A_{.663}$ = absorbance value of 663 wavelength

Crude protein content was analyzed using the Kjeldahl method. Samples (1-2 g) were mixed with a catalyst (1 g Kjeldahl tablet and 10 ml sulphuric acid) in a Kjeldahl flask and heated in a hot plate until got a clear solution. 30% NaOH was used to distill the solution and 10 mL of boric acid 2% with PP indicator caught the distillate. Titrated with HCl 0.02 N until red color (BSN, 1992) Volume of HCl equivalent with nitrogen in the samples then multiple with 5 (conversion factor) to determine the crude protein content (Angell *et al*., 2015). Protein content calculated by:

$$\%N = \frac{(ts - tb)\text{ml x N HCl x } 14.007 \text{ x } 100}{\text{sample x } 1000}$$

CP (%) = %N x conversion factor

Where:

ts = titrated sample
tb = titrated blank
CP = crude protein

For ash content, samples (1-2g) were placed into a porcelain crucible (Wo), in five replicates. Before the burning process in a furnace, the porcelain crucibles are placed on a hot plate electric stove for preheating. The samples were then burnt in a furnace for 5-6 hours at 550 °C. As the samples returned to room temperature, their weight stabilized in the oven.

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110 °C overnight. The samples were placed in a desiccator for 1-2 hours before being weighed as Wt. The ash is calculated by using the following formula:

$$\text{Ash (\%)} = \left(\frac{\text{Wt} - \text{Wo}}{\text{Wo}}\right) \text{x } 100$$

pH values were determined using a digital pH meter. Before each use, the pH meter was calibrated with buffers at pH 4.0 and 7.0. Around 20 g of *U. lactuca* was pulverized in a 30 ml beaker glass, then the pH probe was dipped into the sample to get the pH level. The pH values were determined by averaging the duplicates.

Microbial Analysis

Approximately 10 g of *U. lactuca* and 90 ml of sterile 0.85% NaCl were put into a sterile blender (Philips HR 2116, Indonesia) and pulverized to obtain a smaller size sample. The crushed sample was then put into a sterile Erlenmeyer and sealed. The sample was diluted to a concentration of 10^{-1} to 10^{-5} and then each 1 mL diluted sample was plated in a sterile petri dish (duplo) and added Plate Count Agar (PCA) medium (22.5 g PCA in 1 L distilled water) until the agar hardened, followed by 48 hours at 35°C in an incubator (Binder, German). After incubation, the colony was enumerated in CFU/mL.

Sensory Analysis

Physical alterations (texture, appearance, odor, and color, Table 1) of stored *U. lactuca* were determined by a sensory test that involved 19 trained panelists. Panelists (researchers and researcher assistants from the Research Division for natural products technology, (Indonesian Institute of Sciences) had been trained in examine of *Ulva* evaluation characteristics (appearance, color, odor, texture) analysis before the sensory test was conducted. Approximately 10 g of sample was served on a small white plastic plate (Ø 12 cm), which was wrapped in clear plastic wrap and labeled with a 3-digit unique code. The panelist then evaluated the sample in the sensory test room. Sensory evaluations were undertaken from day 0 to day 4 of storage.

Table 1. Descriptors *Ulva* sensory evaluation.

No	Descriptors	Scale	Code sample tested XXX
1	Appearance		
	fresh, bright, shiny	9	
	fresh, bright, less-shiny	7	
	less fresh, less bright, not shiny	5	
	not fresh, not bright, a little dull	3	
	complete loss of freshness, not bright, dull	1	
2	Color		
	dark green	9	
	dark green, less transparent	7	
	a slightly faded green, less transparent	5	
	faded green, less transparent	3	
	faded green, transparent	1	
3	Odor		
	fresh *Ulva* odor	9	
	less *Ulva* odor	7	
	faded *Ulva* odor, coast aroma	5	
	less *Ulva* odor, a little bit strong coast aroma, a little bit fishy	3	
	Ulva odor disappears, strong coast aroma, fishy, and strong rotten odor	1	
4	Texture		
	Rigid, slippery, difficult to rip	9	
	Rigid, slippery, a bit difficult to rip	7	

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Less rigid, less slippery, a little difficult to rip)	5
a little bit mushy, not slippery, easy to rip)	3
mushy, not slippery, very easy to rip)	1

Data Analysis

One-way ANOVA was used to evaluate the effect of time on seaweed freshness parameters, followed by Duncan to identify significant differences among sampling points using R program series 4.0.4. (Austria) at a 5% level of significance, different alphabetic letters in the column are statistically different.

RESULTS AND DISCUSSION

Proximate and pH of *Ulva lactuca*

Moisture Content and Ash

Figure 1 shows the value of fresh *U. lactuca* moisture content at different temperatures was about 80 g (100 g)$^{-1}$. This result is similar to Uribe *et al*. (2018), which showed that these results were consistent with marine product moisture content. Gupta *et al*. (2011) also mentioned that while fresh, nature-macroalgae contain a considerable amount of water (75-85%) and 15-25% of organic components and minerals. The moisture content is still normal, except for room temperature storage. *Ulva* stored at room temperature (day 4) showed signs of damage/rottenness, which looked slight of mucus on the thallus surface and the amount of free-water content. Lower temperature (4 °C) treatment was better for temperature storage of fresh *Ulva* since it maintained a low moisture content until the fourth day (82.11 g (100 g)$^{-1}$).

Meanwhile, the sensory evaluation also showed that 4 °C temperature storage treatment made *Ulva* relatively stable, there was no significant quality deterioration until the 4th day of storage. According to Paull and Chen (2008), the postharvest life of all species (including macroalgae) is approximately four days. Low/cold storage temperatures for fresh/perishable products are the best treatment to maintain product freshness and quality. Ahmad *et al*. (2020) state that cold storage is one way for preserving perishable food in its fresh state for a longer period, meanwhile Mercier *et al*. (2017) said that refrigeration is one of a method to enhancing of perishable food to maximize shelf life.

On the other hand, the ash component of *U. lactuca* increased gradually until day 4, and the percentage changed between days 0 and 4. The fresh seaweed (day 0) contained 31.9±0.94% of ash and at the end of the experiment increased to 43.59 ± 1.23%, 36.23 ± 1.43%, and 35.46 ± 1.07% for the seaweed stored at room temperature, 15-20 °C, and 4 °C respectively.

Crude Protein Content

The crude protein content of *U. lactuca* varied from 9-25% (Shuuluka *et al*., 2013; Pangestuti and Kim, 2015; Jatmiko *et al*., 2019). In this study, the initial crude protein content of fresh *U. lactuca* was 10.34 % (db). The crude protein content of *U. lactuca* at room temperature reached maximum concentration on day 2. However, crude protein content from day 0 until day 4 was not significantly different. Microbial metabolic rates were nearly double with every 10 °C increase in temperature (Dutta and Dutta, 2016), it was possible that microbial activity at room temperature (25-30 °C) increased. The microbial activity could cause early deterioration of *U. lactuca* that affected to the crude protein content behavior during storage time.

The maximum crude protein content of *U. lactuca* at room temperature was reached on day 2 while the maximum crude protein content at chiller and refrigerator was reached on day 3. Deterioration affected the crude protein content of *U. lactuca*. At room temperature, *U. lactuca* had a maximum crude protein concentration, of 11.36 ±_0.64 % (db) while at chiller and refrigerator temperature were

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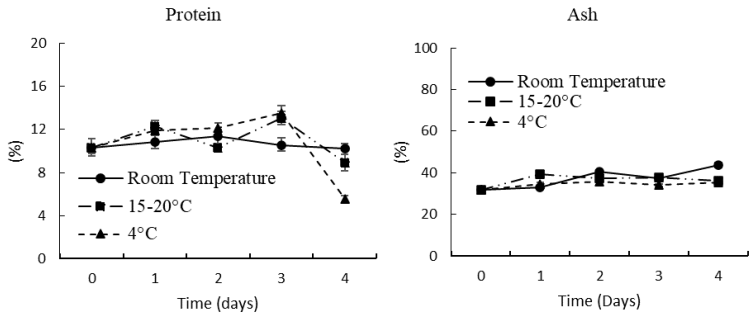
13.09 ± 0.63 % (db) and 13.51 ± 0.68 % (db).

Crude protein content behavior at chiller temperature is unique because the content reduced on day 2 but reached the highest crude protein content on day 3. Nevertheless, the decrease in crude protein content on day 2 was not significantly different from day 1 and day 3. Crude protein content increased during storage time at the refrigerator and decreased on day 4. Similar phenomena with crude protein content at the chiller during storage. In this study, the maximum duration allowed for the retention of crude protein content in *U. lactuca* was three days.

pH Analysis

As illustrated in Figure 1, the pH of fresh *U. lactuca* held at room temperature for four days ranged between 6.36-7.55; at 15-20 °C, the pH varied between 6.36-6.86; and at 4 °C, the pH varied between 6.36-7.4. This pH is similar to Sánchez-Garcia *et al*. (2021), where the pH value of fresh *U. rigida* stored for 12 days starting from 6.67 - 7.24 (4 °C) and 6.67 -7.67 (16 °C). There was a slight increase in pH following 0-4 days of room temperature storage, but it was not significant (6.36 - 7.55). Sensory evaluation of room temperature treatment likewise revealed a decline in quality for all criteria examined on the second day of storage. At 15-20 °C temperature, there was no significant pH value alteration, however, it displayed a pH value that was below the neutral pH condition range. Sensory assessment of the 15-20 °C temperature treatment also revealed a decrease in the values of the parameters assessed on each day of storage. While the lowest storage temperature treatment, there were no significant changes from 0 to 2nd days storage, but there was a significant increase in pH value on the last day. However, the rise in pH value for 4 °C temperature treatment at last day storage was still around 7. This was like the study conducted by Sánchez-Garcia *et al*. (2021) at 4 °C temperature on 4 day storage treatments showed a pH of around 7 for *U. rigida*.

Those values are close to the neutral pH condition. Each day of storage at a temperature of 4 °C was accompanied by a small drop in the evaluated value parameters. Maintaining perishable/fresh products in neutral pH conditions is the best way to keep proteolytic activity down (Rustad, 2003). Changes in pH value during storage time for fresh products probably took place by forming and accumulating acids, such as lactic acid, as occurs in other marine species (Sánchez-Garcia *et al*., 2021).



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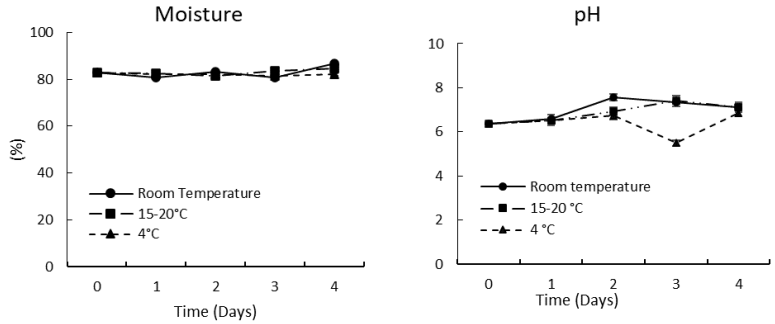

Figure 1. The proximate composition (protein, ash, moisture and pH of *Ulva lactuca* under different conditions.

Total Plate Count (TPC) and Total Chlorophyl of *Ulva lactuca*

During the storage period, a total of microorganisms were discovered on all treatments. The initial microbial population varied from 147 x 10^3 in 0 days of storage to 2,462.5 x 10^3 CFU/ml on the last day of storage. The microbial count increased rapidly from thousands to millions in 15-20 °C temperature storage on the last day of storage. The very large increase in microbial growth was due to the storage system of samples placed in an open plastic bag and it was possible to support rapid microbial growth from the surrounding. Paull and Chen (2008) also have the same phenomenon that red seaweed stored at 17 °C increased rapidly from thousands to millions in a few days. Only 4 °C storage temperature resulted in the smallest rise in microbial growth of all treatments. This result similar to Sánchez-Garcia *et al*. (2021) showed that 4 °C could maintain the quality of *U. rigida* by suppressing both the enzymatic and microbiological activity responsible for the deterioration stage.

Table 2. Total Plate Count (TPC) of *Ulva lactuca* stored at different temperature and day.

Storage time (days)	TPC (CFU/ml)		
	Room Temperature	15-20 °C	4 °C
0	147x10^3 ± 8,485	147x10^3 ± 8,485	147x10^3 ± 8,485
1	104.5x10^3 ± 7,778	195.25x10^3 ± 35,709	299x10^3± 31,113
2	21.092x10^3 ± 5,942	11.798x10^3 ± 2,430	3.313x10^3 ± 1,156
3	224.25x10^3 ± 22,274	30.575x10^3 ± 2,227	96.5x10^3 ± 19,092
4	290.75x10^3 ± 80,257	2,462.5x10^3 ± 1,771,302	21.75x10^3 ± 1,131

Colors of *Ulva lactuca*

Color is one parameter to define a fresh product quality by measuring the value of L* (showing light/brightness), a* (showing redness-greenness), and b*(showing yellowness-blueness). Table 3 showed that the L* value was affected by storage time and room temperature and 15-20 °C temperature treatments, except for the 4 °C temperature treatment. But all treatments showed L* value was dark green on each day of storage. The a* value increased and the b* value decreased in each day of storage for treatments. This indicates that more green and less yellow in each day of storage. Changing color through the storage period in different temperatures was because of pigment changes, possibly because of enzymatic browning reactions (Tello-Ireland *et al*., 2011).

The color parameter for hue angle, chroma, and color difference are shown in Table 3. The statistical analysis indicated that the color parameter for hue angle was not affected significantly by the storage time, except for the 15-20 °C treatment.

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The average hue angle values for room temperature treatment were found to be the highest on 2nd day of storage (0.68 ± 0.73) and the lowest on the 3rd day of storage (-0.76 ± 2.35), for 15-20 °C treatment were found to be the highest in the early of storage (0.76 ± 0.38) and significantly decreased until the last day of storage. In 4 °C treatment was found to be the highest on the 2nd day of storage (0.67 ± 0.58) and the lowest in ohe 4th day of storage (0.28 ± 0.36). The hue angle shown in Table 3 has a higher value compared with Uribe *et al*. (2018) were showed the hue angle for fresh *Ulva* spp. (-1.20+0.02).

The storage duration and temperature treatments had a substantial effect on the chroma values. The highest chroma value was found at room temperature and 4 °C storage (19.4 ± 4). In the early day of storage and the lowest was found at room temperature in 3rd-days of storage (4.22 ± 2.65). Uribe *et al*. (2018) show that the chroma value of fresh *Ulva spp*. higher (27.38 ± 0.98) than shown in Table 3. The statistical analysis indicated that the color difference was not affected significantly ($p<0.05$) by the storage time and 15-20 °C and 4 °C temperature treatments, except for the room temperature treatment. The highest were found at 15-20 °C temperature treatment (2.93 ± 1.24) and the lowest were found at 4 °C temperature treatment (1.29 ± 1.54).

Table 3. Color (L* (lightness) a*, (redness/greenness), b* (yellowness/blueness), the hue angle (h*), polar coordinates of chroma (C*), total color difference (ΔE)) of *Ulva lactuca* through time and temperature storage.

Storage time (days)	Lightness (L*)			Redness/greenness (a*)			Yellowness/blueness (b*)		
	Room Temperature	15-20 °C	4 °C	Room Temperature	15-20 °C	4 °C	Room Temperature	15-20 °C	4 °C
0	5.28±1.21^{a}	5.28±1.21^{a}	5.28±1.21^{a}	-1.95±0.29^{c}	-1.95±0.29^{b}	-1.95±0.29^{c}	3.93±0.41^{a}	3.93±0.41^{a}	3.93±0.41^{a}
1	4.2±0.32^{ab}	3.4±0.8^{b}	5.23±1.15^{a}	-1.35±0.25^{b}	-1.100±0.43^{ab}	-1.68±0.13^{c}	1.950±0.37^{b}	1.85±0.87^{b}	3.28±0.94^{a}
2	5.28±0.94^{a}	3.75±0.41^{b}	5.075±0.93^{a}	-1.53±0.21^{bc}	-1.28±0.39^{ab}	-1.53±0.15b^{c}	3.15±0.83^{ab}	2.15±0.82^{b}	3.2±0.73^{a}
3	3.58±0.85^{b}	4±1.06^{ab}	3.78±0.79^{a}	-0.8±0.08^{a}	-0.48±1.57^{a}	-0.925±0.28^{a}	1.8±0.67^{b}	2.1±0.8^{b}	2.1±0.77^{b}
4	4.43±0.94^{ab}	3.68±0.49^{b}	3.93±0.81^{a}	-1.23±0.1^{ab}	-1.23±0.17^{ab}	-1.15±0.39^{ab}	2.35±0.24^{b}	1.93±0.46^{b}	2±0.24^{b}
Storage time (days)	Hue angle (h*)			Polar coordinates of color Chroma (C*)			Total color difference (ΔE)		
	Room Temperature	15-20 °C	4 °C	Room Temperature	15-20 °C	4 °C	Room Temperature	15-20 °C	4 °C
0	0.51±0.26^{a}	0.76±0.38^{a}	0.51±0.26^{a}	19.4±4^{a}	17.65±3.46^{a}	19.40±4^{a}	2.34±0.49^{ab}	2.93±1.24^{a}	1.32±0.92^{a}
1	-0.12±0.22^{a}	0.09±0.32^{b}	0.46±0.57^{a}	5.78±1.94^{b}	5.34±4.56^{b}	14.21±6.64^{a}	1.31±0.6^{b}	2.85±0.96^{a}	1.29±1.54^{a}
2	0.68±0.73^{a}	0.09±0.2^{b}	0.67±0.58^{a}	12.80±5.56^{ab}	9.39±3.16^{b}	13.00±4.47^{a}	3.00±0.9^{a}	2.68±1.72^{a}	2.91±1.38^{a}
3	-0.76±2.35^{a}	0.37±0.29^{ab}	0.36±0.58^{a}	4.22±2.65^{b}	7.00±4.9^{b}	5.78±3.22^{b}	2.08±0.37^{ab}	2.45±0.62^{a}	2.60±0.76^{a}
4	0.43±0.4^{a}	0.03±0.5^{b}	0.28±0.36^{a}	7.07±0.94^{b}	5.39±1.77^{b}	5.48±1.95^{b}	2.34±0.49^{ab}	2.93±1.24^{a}	1.32±0.92^{a}

Note: Values expressed as means ± standard deviation followed by different letters within a column are significantly different by Anova analysis and Duncan test ($P < 0.05$, $n = 4$).

Sensory Evaluation of *Ulva lactuca*

The storage time and temperature significantly ($p<0.05$) influenced the sensory score. In general, the color, odor, and texture of *U. lactuca* markedly decreased ($p<0.05$) at room temperature and 15-20 °C during storage conditions (Figure 2). However, the sensory score was relatively greater when the sample was stored at 4°C compared to others.

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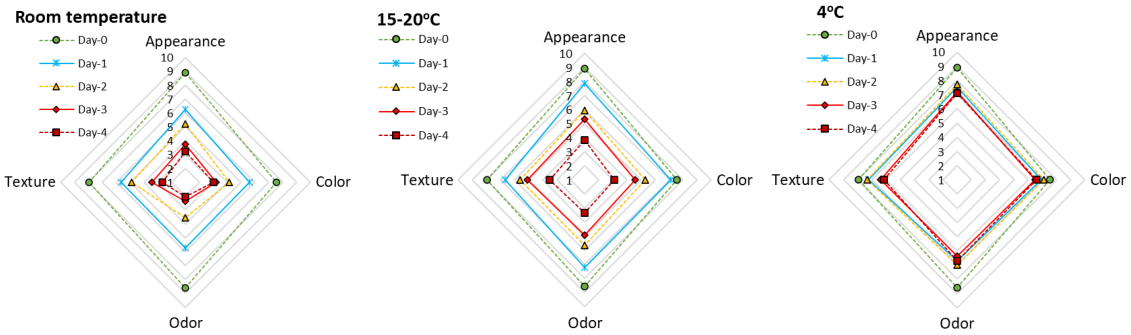

Figure 2. Sensory evaluation of *Ulva lactuca* through time and different storage situations.

On days 1 to 4, the appearance of *U. lactuca* stored in 4 °C was relatively stable, there was only a little alteration from day 0 to 1. According to the sensory test, the appearance score was still greater than 6 and the sample seemed fresher than the samples that were stored at the higher temperature. This is in agreement with Sánchez-García *et al*. (2021), where lower temperatures can keep the *U. rigida* chemical compound alterations slower. On the other hand, there was a significant physical shift of *U. lactuca* ($p<0.05$) when stored at 15-20 °C and room temperature. The appearance score drastically decreased from day 1 to the end of storage and the sample seemed dull and withered.

The color score of *U. lactuca* stored at 4 °C decreased but did not significantly ($p>0.05$) with still in the dark green. A previous study has also reported that the color degradation of *U. rigida* at 4 °C condition running slower (Sánchez-García *et al*., 2021). Nevertheless, the color changes appeared in the sample stored at 15-20 °C and room temperature. The color alteration of the seaweed sample stored at room temperature appeared started on day 1 during the storage and continued until the end of the experiment, meanwhile, the color shift seemed on day 2 for the sample in 15-20 °C storage. The color alteration started from dark green to yellowish green. The odor score of *U. lactuca* stored at room temperature, on the other hand, markedly decreased ($p<0.05$) from day 0 to 1, the score was lower than 6. The score continued declining and reached to be the lowest compared to the score of other parameters in the same sample. Moreover, the aroma of the seaweed altered from a fresh condition with a little fishy to be decayed smell and the particular fresh aroma of seaweed faded away. As noted by Sánchez-García *et al*. (2021), compound denaturation in *Ulva* is running faster at the higher temperature due to the higher microbial activities.

Despite the score of texture decreasing until the end of the experiment, the texture of the sample stored at 4 °C was still compact and not fragile. Different from the sample in room temperature storage, its texture score extremely declined on day one up to a lower of 6. On the other hand, the texture score of the seaweed in 15-20 °C storage also decrease significantly but it was still above 6. The compound degradation process of the sample was stored at 15-20 °C and room temperature probably due to the chemistry and enzymatic reaction running faster compare to the sample at 4 °C. Cell structure destruction by chemistry and enzymatic reactions and microbial activity cause a tissue more fragile and damaged easily (Blikra *et al*., 2019; Sánchez-García *et al*., 2021).

CONCLUSION

The current data demonstrate that temperatures (4 °C to room temperature) affect *Ulva lactuca*'s nutritional and sensory score alterations. Despite a minor loss in nutritional content, the sensory scores of *U. lactuca* are more consistent and higher at 4 °C than in other storage conditions.

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