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Hydrolysis of Polysaccharide *Caulerpa racemosa* Seaweed with Fermentation *Lactobacillus plantarum* SK (5)

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ABSTRACT Hydrolysis of polysaccharides can increase biological activity by changing the structure of polysaccharide functional groups and reducing molecular weight. Enzyme hydrolysis is an environmentally friendly hydrolysis method because it does not form toxic by-products. *Lactobacillus plantarum* can produce amylase and cellulase enzymes, which can be used to hydrolyze *C. racemosa* polysaccharides. This research aims to determine the best fermentation time and starter concentration in the *C. racemosa* polysaccharide hydrolysis process and the *C. racemosa* glucose concentration before and after fermentation. The research was divided into phases: phase I and II. The fermentation time in Phase I is six days, while in Phase II, it is 48 hours. The starter concentrations used are 0%, 5%, and 10%. The analysis showed that differences in starter concentration and fermentation time caused changes in the total values of LAB, TTA, pH, reducing sugar and glucose concentration. The best treatment was shown by adding 5% starter with a fermentation time of 24 hours.

Keywords: *C. racemosa*; fermentation; hydrolysis; *L. plantarum*

INTRODUCTION

Seaweed is high in polysaccharides, widely used in chemistry, food, pharmaceuticals, and cosmetics, among other industrial fields. Polysaccharides are complex molecules composed of several monosaccharide units connected via glycosidic bonds (Muthukumar *et al*., 2021). Polysaccharides can be composed of the same monosaccharide units (homopolysaccharides) or from different monosaccharide units (heteropolysaccharides) (Hentati *et al*., 2020). Seaweed polysaccharides have various bioactive compounds that can be used for human health. These compounds have broad biological activities such as antioxidant, antitumor, antimicrobial, anticoagulant, antiviral, anti-inflammatory, and immunomodulatory activities (Charoensiddhi *et al*., 2017).

Caulerpa racemosa is a seaweed classified as a group of green algae (Chlorophyta), commonly called sea grapes (Kusumawati et al., 2018). The nutritional content of C. racemosa seaweed is 21.7% protein, 8.6% fat, 20.9% ash, 48.6% carbohydrates, and 8.4% crude fiber. Carbohydrates have the highest nutritional value in C. racemosa, most of which are sulfated polysaccharides (Ma'ruf et al., 2013). Sulfated polysaccharide from *Caulerpa racemosa* (SPCr) has water-soluble properties (Wang *et al*., 2014). It is classified as a heteropolysaccharide because it is composed of several monosaccharide units such as glucose (52.42%), galactose (15.62%), mannose (12,61%), xylose (8.21%), and fucose (1,30%) (Magdugo *et al*., 2020). The sulfated polysaccharide of Caulerpa racemosa has been widely reported to have several biological activities such as antioxidant, antiobesity, anticancer, antitumor, and antiaging (Permatasari *et al*., 2021). Nurkolis *et al*. (2023) reported that the SPCr has strong antioxidant activity because it has a lower EC50 value (93.81 µg/ml) compared to the EC50 value of the positive control trolox (102.5 µg/ml).

Seaweed polysaccharides are known to have a complex structure and high molecular weight (10-1000 kDa), so a hydrolysis process is needed so that their biological activity can be increased. Hydrolysis can increase biological activity by modifying the structure of functional groups and reducing molecular weight (Charoensiddhi *et al*., 2017). According to (Li et al., 2018), ulvan fraction with a molecular weight of 83 kDa had stronger antioxidant activity than the ulvan with a larger molecular weight of 190 kDa. Hydrolysis of seaweed polysaccharides can be carried out physical, chemical, biological (enzymes), or in combination (Mutmainnah *et al*., 2023). Hydrolysis using enzymes is considered more environmentally friendly because it does not form toxic by-products but has a higher conversion efficiency (Offei *et al*., 2018). Agar waste from seaweed Gracilaria sp. hydrolyzed with cellulase enzymes had a glucose concentration of 28.8 g/L. This concentration is higher than acid hydrolysis, which produces glucose of 0.85 g/L (Pabriani, 2022).

Amylase and cellulase are the enzymes that are utilized in the hydrolysis of seaweed polysaccharides. These two enzymes can be obtained naturally by fermentation or commercially. Cellulase and amylase enzymes are two enzymes naturally secreted by cellulolytic and amylolytic bacteria, such as *Clostridium*, *Bacillus*, *RuL. plantarum* bacteria include lactic acid bacteria (LAB), which can produce cellulase enzymes with an enzyme activity of 0.054 U/ml at pH 7 and a temperature of 65°C (optimum). The resulting cellulase enzyme activity index ranges from 1.24 to 1.98, which is classified as moderate (Putri, 2016). *L. plantarum* bacteria can also produce amylase enzymes with enzyme activities ranging from 0.403–0.641 U/ml at pH 5–8 (Khusniati *et al*., 2020). *L. plantarum* SK (5) is a Gram-positive bacterium isolated from bekasam seluang fish (*Rasbora* sp.) originating from Kayu Agung Village, Ogan Komering Ilir Regency,

South Sumatra (Desniar *et al*., 2012).

Hydrolysis of seaweed polysaccharides by physical, chemical, and enzymes (commercially) has been widely researched and developed. However, using enzymes produced by microorganisms through the fermentation process has yet to be widely used. Therefore, this research examined the characteristics of glucose produced through hydrolysis of *C. racemosa* seaweed polysaccharides by fermentation by *L. plantarum* SK bacteria (5). This research was also carried out to obtain optimal fermentation time and concentration of *L. plantarum* SK (5) bacteria with the best characteristics.

MATERIALS AND METHODS

Materials

The material used in this research was green seaweed *C. racemosa* obtained from Jepara, Central Java. The starter used in fermentation was the bacterial isolate *L. plantarum* SK (5), which was isolated from bekasam seluang fish (*Rasbora* sp.) from Kayu Agung Village, Ogan Komering Ilir Regency, South Sumatra. The additional ingredients used are *bacteriological peptone* (Oxoid) and glucose monohydrate (Merck). The equipment used in this research consisted of a 100 ml Schott bottle, pH meter (Walklab TI9000), Thomas-Wiley Laboratory Mill, centrifuge (Beckman J2-21), autoclave (Yamato SM 52), incubator (Thermolyne type 42000 incubator), homogenizer (Nissei AM-3, HPLC instrument (LC-20AB, refractive index detector RID-10A), and spectrophotometer UV-Vis (Hitachi U-2800).

Methods

Research design

Two phases comprise this research. Determining the seaweed *C. racemosa* fermentation period is the goal of the phase I research. Total LAB, Total Titratable Acidity (TTA), pH, and reducing sugar values in fermented *C. racemosa* seaweed (fermentation period of six days) were analyzed to determine the duration of fermentation. Phase I research consisted of several processes: preparing *C. racemosa* seaweed raw materials, preparing *L. plantarum* SK (5) starter, and determining the fermentation time for *C. racemosa* seaweed. Phase II research aims to see the effect of differences in *L. plantarum* SK (5) starter concentration and selected fermentation time on microbiological and chemical characteristics. In phase II research, 48 hours was the fermentation period. Phase II research consisted of one process: fermentation of *C. racemosa* seaweed at selected fermentation times.

Sample preparation

Samples of green seaweed *C. racemosa* were washed to remove dirt attached to the seaweed and dried indoors for 7 days. The dried *C. racemosa* samples were ground using a Thomas-Wiley Laboratory Mill-type grinding machine.

Preparation of bacterial culture

Isolate *L. plantarum* SK (5) was grown on MRSA slant media. The growth isolates were characterized by performing Gram staining, motility tests, and catalase tests. The characterized isolate was then inoculated into 15 ml MRSB medium and incubated at 37 oC for 24 hours. 10% inoculum was then added to 135 ml of MRSB working culture. The working culture is then placed back into the incubator in a closed container.

Determination of selected times of C. racemosa fermentation

Fermentation of *C. racemosa* seaweed according to Rafiquzzaman *et al*. (2015) with modification. As much as 5 g *C. racemosa* powders were put into a Schott bottle. The sample was added with 5% (w/v) glucose, 0.8% (w/v) peptone, and 1:20 distilled water. Samples were sterilized using an autoclave for 15 minutes at 121°C. Starter bacteria *L. plantarum* SK (5) as much as 5% and 10% (v/v), were inoculated into different Schott bottles. In phase I of the research, a control treatment (without a starter) was added as a comparison. Samples were incubated at 37 °C for 6 days and analyzed on days 0, 3, and 6 at each *L. plantarum* SK (5) starter concentration.

Fermentation of C. racemosa seaweed with selected fermentation time

Fermentation of *C. racemosa* seaweed with the selected fermentation time was carried out based on research by Ambarsari *et al*. (2018) with modifications. In this phase II study, 5 g of powdered *C. racemosa* seaweed was added to a Schott bottle along with 5% (w/v) glucose, 0.8% (w/v) peptone, and 1:20 distilled water. The autoclave sterilized the samples for 15 minutes at 121°C. Different Schott bottles were inoculated with varying concentrations of the starter bacteria *L. plantarum* SK (5) at 0%, 5%, and 10% (v/v). Samples were incubated for 48 hours at 37 °C. For every starting concentration, analyses were done at 0, 12, 24, 36, and 48 hours. The total LAB, pH, TTA, and reducing sugar values were used to determine a subset of fermentation samples. The selected samples were then centrifuged at 15,000×g for 15 minutes to separate the supernatant and pellet. The supernatant was pipetted and collected into a vial, and the glucose profile was analyzed using HPLC.

Total Microbes and Lactic Acid Bacteria (LAB)

The calculation of total microbes and total LAB refers to SNI 2332.3:2015. The media used were PCA for total microbial testing and MRSA added with $CaCO_3$ 0.5% sterile for testing total LAB. A sample of 10 ml was dissolved in 90 ml of sterile Butterfield's buffered phosphate (BPB) to obtain a 10^{-1} dilution. 1 ml of the solution was pipetted and put into a test tube containing 9 ml of sterile BPB solution for a 10^{-2} dilution. This process was carried out until the dilution was 10^{-8}. A sample of 1 ml was pipetted at each dilution (10^{-6}, 10^{-7}, and 10^{-8}) and put into a petri dish, then 6-7 ml of PCA/MRSA media was poured into a cup containing the sample and then homogenized. Samples were incubated in an incubator at 37°C in an inverted position for 48 hours. The total number of bacteria was counted (25–250 colonies) and expressed in CFU/ml (BSN, 2015). The calculation formula used is as follows:

$$N = \frac{\Sigma C}{[(1 \times n1) + (0.1 \times n2)] \times (d)}$$

Note:
N = Number of colonies (colonies/ml)
$\sum C$ = Number of colonies on all plates was counted
n_1 = Number of plates in the first dilution is counted
n_2 = Number of plates in the second dilution is counted
d = First dilution is calculated

Total Titratable Acidity (TTA)

Analysis of total titratable acidity using the AOAC method. Five grams of sample were added with 45 ml of distilled water and then homogenized. The sample was dissolved using distilled water in a volumetric flask until the 50 ml reading mark. The sample was filtered using filter paper and pipetted 5 ml into a beaker. Indicator solvent phenolphthalein (PP) 1% was added in 2 drops, then titrated using 0.1 N NaOH until the colour of the solution changed to pink (AOAC, 2005). Calculation of total acid is carried out using the formula:

$$\text{Total Titratable Acidity}(\%) = \frac{V \times N \times 90 \times FP}{W} \times 100\%$$

Note:
W = Sample weight (mg)
V = Volume of NaOH used
N = Normality of NaOH
Fp = Dilution factor
90 = MW lactic acid

pH measurement

Measurement of pH using a pH meter following SNI 6989.11:2019. The measurement process begins with calibrating the electrode with a pH 7 solution and then drying the electrode using a soft tissue. The calibrated electrode is then dipped into the liquid sample as much as ±20 ml until the pH meter shows a stable number displayed on the pH meter screen (BSN, 2019).

Reducing sugars quantification using DNS (3,5-Dinitrosalicylic Acid) assay

Analysis of reducing sugars was accomplished according to Miller (1959) using DNS (3,5-dinitrosalicylate). The standard galactose solution was made in 1000 ppm and diluted to 500 ppm, then standard solution concentrations were made at 50, 100, 150, 200, 250, and 300 ppm in different test tubes. 1 ml of solution was pipetted from each standard solution and mixed with 3 ml of DNS reagent. In addition, take 1 ml of sample solution and mix it with 3 ml of DNS. The sample was heated at 100°C for 10 min until the colour changed to dark brown-black. The sample was measured at 512 nm absorbance using a UV-Vis spectrophotometer, and the reducing sugar content was calculated using the regression equation from the glucose standard curve that had been created.

Analysis of glucose and xylose using HPLC

Glucose and xylose were analyzed using the High-Performance Liquid Chromatography (HPLC) method according to AOAC (2006). The analysis begins by weighing 0.5 g of the sample and placing it in a 50 ml measuring flask. Aquabidest was added to the volumetric flask until the tera mark, then stirred until homogeneous. The sample solution was filtered using a 0.45 µm microfilter into a 2 ml vial, after which it was injected into the HPLC. Mixed sugar standards (glucose and xylose) have been injected first into the HPLC with a concentration series, namely, 0.01%, 0.025%, 0.05%, 0.10%, 0.25%, 0.50%, and 1.00%.

Statistical analysis

Quantitative data on the analysis of total LAB, TTA, pH and reducing sugars in phase I and II research were processed using Microsoft Excel 2019 to obtain average values and standard deviations. Data is presented in the form of tables and graphs and analyzed descriptively. Glucose concentration data were processed statistically using ANOVA analysis of variance. The results that showed the differences are continued with the Tukey Test. Analysis was conducted using SPSS ver.27.

RESULT AND DISCUSSION

Characteristics of Bacterial Starter L. plantarum SK (5)

A starter culture is a material containing large amounts of microorganisms that are deliberately added to the fermentation raw material to speed up the fermentation process and improve the characteristics of the fermentation raw material. The microorganisms contained in starter cultures are generally known for their metabolic activity in producing final fermentation products (Abubakar *et al*., 2019). The starter used in this research was *L. plantarum* SK (5), which comes from the seluang fish (*Rasbora* sp.). *L. plantarum* is a LAB commonly used as a starter in fermented products. The results of the Gram staining of *L. plantarum* SK (5) bacteria can be seen in Figure 1.

The Gram staining results showed that *L. plantarum* SK (5) bacteria are Gram-positive bacteria and have a rod cell shape. Catalase and motility tests on *L. plantarum* SK (5) bacteria showed negative results and were non-motile. These results follow research by Desniar *et al*. (2012), which states that the bacteria *L. plantarum* SK (5) has the characteristics of being Gram-positive, rod-shaped, does not produce spores, is non-motile, and is negative in the catalase test. *L. plantarum* SK (5) bacteria are classified as homofermentative because they can produce organic acids in formic acid, fumaric acid, acetic acid, oxalic acid, lactic acid, and ascorbic acid.

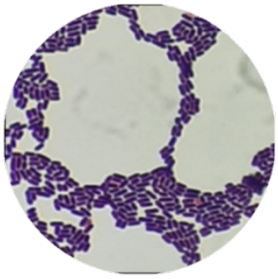

Figure 1. Gram stain of *L. plantarum* SK (5) bacteria (10x100 magnification).

The number of *L. plantarum* SK (5) bacterial colonies used as a starter in phase I research was 9.35 Log CFU/ml, while in phase II research was 9.24 Log CFU/ml. The quantity of colonies is in accordance with studies conducted by Oliveira *et al*. (2002), which indicate that 10^7 CFU/ml of bacterial colonies can be utilized as a starting.

Total Lactic Acid Bacteria (LAB)

L. plantarum SK (5) is a LAB that plays a role in the

fermented *C. racemosa*. The adaption, log, stationary, and death phases comprise *L. plantarum* SK (5) bacteria's growth phase. Each phase that occurs shows changes in the physiological activity of cells in the media during growth. Changes in total LAB of fermented *C. racemosa* can be seen in Figure 2.

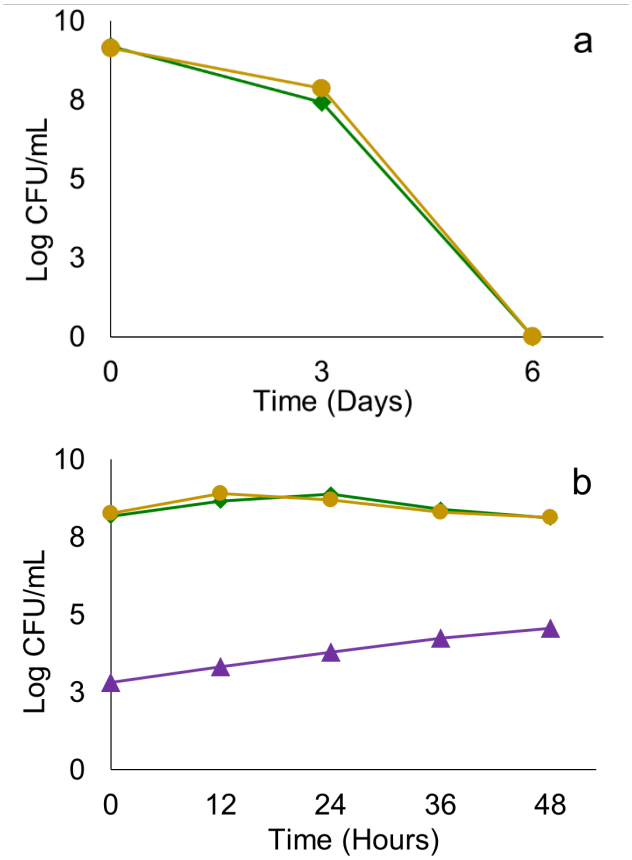

Figure 2. Changes in total LAB of fermented *C. racemosa* in research phase I (a) and phase II (b) with treatment without adding starter (0%) (▲), 5% starter (♦), and 10% starter (●).

The results of the total LAB analysis in phase I of the research showed that the treatments with the addition of 5% and 10% starter had relatively the same pattern of change, namely decreasing during the six-day fermentation. The decrease in lactic acid bacteria (LAB) is caused by the bacteria having passed the stationary phase and entered the death phase. These results showed that the logarithmic phase occurred before the third day of fermentation, so phase II research was carried out with a fermentation time of two days (48 hours). The results of total BAL analysis in phase II research showed different patterns of change in each treatment. The total amount of LAB with the addition of 5% starter increased up to a fermentation time of 24 hours to 8.89 log CFU/ml (up 8.9%), then decreased with a fermentation time of 48 hours to 8.12 log CFU/ml (down 6, 2%). The addition of 10% starter increased up to 12 hours of fermentation to 8.91 log CFU/ml (up 5.2%), then fell to 8.13 log CFU/ml (down 8.8%) at 48 hours of fermentation (Figure 2).

The increase in total LAB during the fermentation of *C. racemosa* grass was caused by bacteria in the logarithmic phase. Environmental conditions and the availability of sufficient amounts of nutrients influence the logarithmic phase of bacteria. Adding glucose and peptone as carbon and nitrogen sources also helps LAB growth. The higher the nutritional content in the fermentation substrate, the greater the number of lactic acid bacteria that grow (Agustine *et al*., 2018). The decrease in the number of LAB is due to the reduced availability of nutrients for bacterial growth, in addition to the accumulation of lactic acid formed during the fermentation process, causing the environmental pH to become too acidic. Environmental conditions that are too acidic can inhibit the growth of lactic acid bacteria (Silitonga *et al*., 2022).

In phase I of the research, total microbial analysis was carried out in the treatment without adding a starter (0%). This analysis aims to ensure that the bacteria that work in the fermentation of *C. racemosa* seaweed are *L. plantarum* SK (5) bacteria. The total number of microbes that grew in the control treatment during 6 days of fermentation was 2.5 x 10^5 CFU/ml. In phase II research, total LAB analysis was also carried out using MRSA selective media in the treatment without adding a starter (0%). The results of total LAB analysis in the treatment without adding 0% starter showed an increase during 48 hours of fermentation from 2.82 log CFU/ml at the start of fermentation to 4.57 log CFU/ml at 48 hours of fermentation. The results of this analysis show that microorganisms grow on the *C. racemosa* substrate after the sterilization process. The bacteria that grow are thought to be spore-producing bacteria or thermophilic bacteria. Spore-producing bacteria are bacteria that produce spores to protect themselves in extreme conditions. Bacterial spores are more resistant to heat and can develop into new individuals when they meet environmental requirements and the appropriate amount of nutrients (Mailia *et al*., 2015). *C. racemosa* seaweed has a carbon source in polysaccharides, which can encourage the growth of bacteria that can utilize it (Yap *et al*., 2019).

The results of bacterial isolation from *C. racemosa* seaweed originating from Jepara Waters, Central Java, show that there are bacterial isolates that are similar to *Caldalkalibacillus mannanilyticus* (Kartika *et al*., 2021). *C. mannanilyticus* bacteria are Gram variable, motile, aerobic, spore-forming, catalase positive, mesophilic, and form round yellow colonies. This bacterium was isolated from soil in Kunitachi City, Tokyo, Japan. *C. mannanilyticus* bacteria have a cell length of 3–6 µm with a width of 0.6–0.8 µm. The growth temperature for this bacterium ranges between 20-45 °C, with an optimal growth temperature of 37 °C, while the growth pH ranges from 8-10, with an optimal pH 9 (Nogi *et al*., 2005). The bacterium *C. mannanilyticus* was initially named Bacillus mannanilyticus (Gupta *et al*., 2020)

Total Titratable Acidity (TTA) and pH Value

The two main chemical parameters that indicate whether the fermentation process is successful are total titrated acid and pH levels. The amount of acid in a material is known as total titrated acid (TTA). The concentration of hydrogen ions (pH value) represents a solution's acidity level (Anwar *et al*., 2014). TTA content and pH value tend to change during fermentation, and the values are

inversely proportional. Changes in TTA and pH values of fermented *C. racemosa* can be seen in Figure 3.

TTA content in phase I research increased in all treatments from day 0 to day 6. TTA levels at the end of fermentation in each treatment were 1.2% (without a starter), 1.4% (5% starter), and 1.5 (10% starter). An increase in TTA content was followed by a decrease in pH values during six days of fermentation. The pH value in all treatments tended to decrease until the third day of fermentation and began to remain constant on the sixth day of fermentation. These results indicate that a decrease in the optimal pH value occurred before day three, so phase II research was carried out.

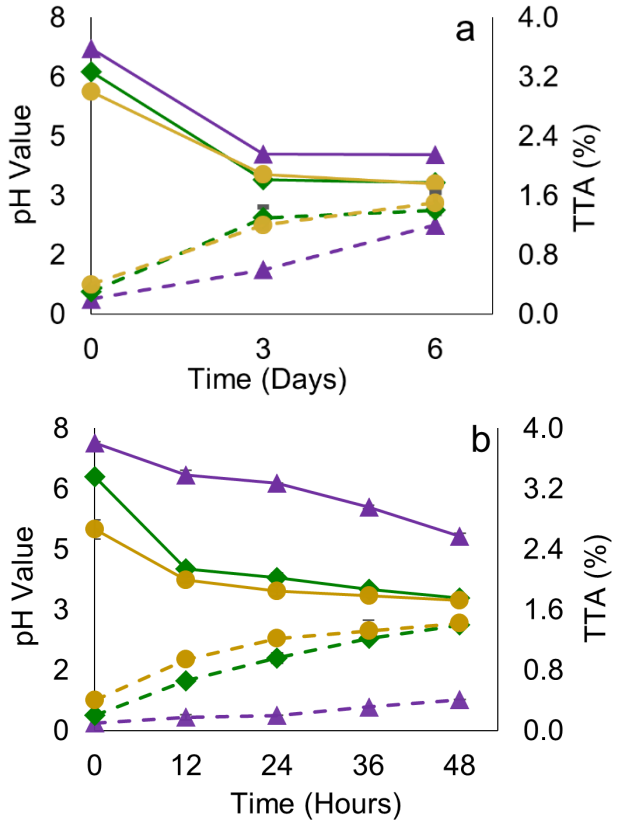

Figure 3. Changes in total titrated acid (- - -) and pH (——) of fermented *C. racemosa* in research phase I (a) and phase II (b) with treatment without adding starter (0%) (▲), 5% starter (♦), and 10% starter (●).

The results of TTA analysis in phase II research showed that TTA levels increased in each treatment, namely 0.41% (starter 0%), 1.40% (starter 5%), and 1.42% (10% starter) up to 48 hours of fermentation. The highest increase in TTA levels was shown by adding 5% and 10% starter, amounting to 1.2% and 1%, respectively. Increasing TTA levels during the fermentation process causes changes in environmental pH to become acidic and reduces the pH value during the fermentation process. The pH value of *C. racemosa* seaweed fermentation in phase II research with treatment without adding starter (0%) was 7.14–4.82 at the end of fermentation, while the pH value when adding 5% and 10% starter was 6, respectively. 3–3.29 and 4.99–3.24.

The increase in TTA levels was caused by bacterial activity utilizing *C. racemosa* seaweed carbohydrates as an energy source to produce lactic acid. The increase in TTA levels was followed by an increase in total LAB during 48 hours of fermentation (Figure 3b). Research by Susilowati *et al*. (2014) explained that bacteria will convert glucose into organic acids in large quantities, so an increase in the amount of LAB causes higher lactic acid production. The process of forming lactic acid by bacteria begins by converting glucose into two pyruvic acid molecules, 2 NADH and 2 ATP, through glycolysis. Two molecules of pyruvic acid are then converted into lactic acid by the enzyme lactate dehydrogenase.

The decrease in pH value was caused by the accumulation of lactic acid products produced by *L. plantarum* SK (5) bacteria during *C. racemosa* fermentation. *L. plantarum* bacteria can convert carbohydrates or sugar into lactic acid (primary metabolite) (Sumardianto *et al*., 2021). Desniar *et al*. (2012) explained that during fermentation, the bacteria *L. plantarum* SK (5) can produce lactic acid and acetic acid as the dominant organic acids, changing the environmental pH to acidic. The research results of Ambarsari *et al*. (2018) regarding the fermentation of Ulva lactuca seaweed with *L. plantarum* bacteria as a starter showed that the longer the fermentation time, the pH value decreases because bacteria produce lactic acid as a product of their metabolism.

The increase in TTA levels and decrease in pH values in the treatment without the addition of starter (0%) was caused by bacteria that grew in the *C. racemosa* substrate after the autoclave sterilization process, which is a bacteria that can utilize carbohydrates as a carbon source to produce several organic acids. Research by Kartika *et al*. (2021) explained that the results of bacterial isolation from *C. racemosa* seaweed showed bacterial isolates similar to the C. mannanilyticus bacteria. Research by Nogi *et al*. (2005) reported that the bacteria C. mannanilyticus could produce acid by fermentation of the monosaccharides glucose, mannose, galactose, and xylose.

Reducing Sugar Concentration

Reducing sugar is a sugar that can reduce compounds that accept electrons and have an aldehyde or α-hydroxy ketone group at their tip. All types of monosaccharides (glucose, xylose and galactose) and disaccharides (lactose and maltose), except sucrose as a reducing sugar (Afriza & Ismanilda, 2019). Fermentation of *C. racemosa* with *L. plantarum* SK (5) causes changes in reducing sugars. These changes result from the hydrolysis process of polysaccharides into monosaccharides and the use of monosaccharides as a source of nutrition for bacterial growth. Changes in reducing sugars concentration of fermented *C. racemosa* can be seen in Figure 4.

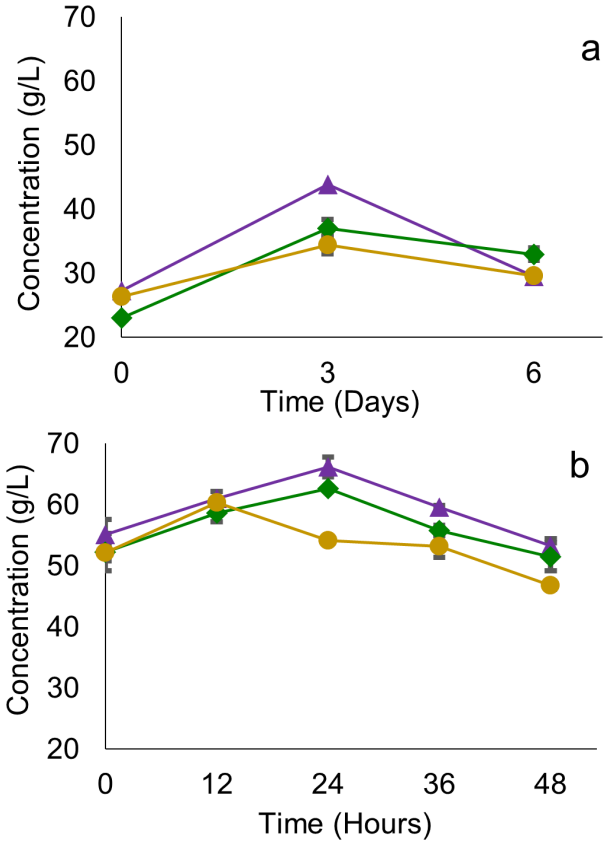

Figure 4. Changes in reducing sugar concentration of fermented *C. racemosa* in research phase I (a) and phase II (b) with treatment without adding starter (0%) (▲), 5% starter (♦), and 10% starter (●).

The results of the reducing sugar analysis in phase I research showed the same pattern of changes in all treatments. The concentration of reducing sugars in each treatment increased during three days of fermentation and then decreased during six days of fermentation. The highest increase in concentration (61%) occurred in the 5% starter addition treatment, from 23 g/L to 37.1 g/L in 3 days of fermentation. The treatment without adding starter (0%) saw the largest reduction in reducing sugar concentration (33%), from 43.9 g/L in 3-day fermentation to 29.5 g/L in 6-day fermentation. Phase II research was conducted by decreasing the fermentation time to 48 hours because these results demonstrated that the highest increase in concentration happened before the third day of fermentation.

Phase II research results from reducing sugar analysis showed different types of change in each treatment. After adding 10% starter, the reducing sugar content increased at 12 hours of fermentation and decreased until 48 hours. Different results were shown in the treatment without the addition of starter (0%) and the addition of 5% starter, which experienced an increase in reducing sugar concentration up to a fermentation time of 24 hours and then a decrease until fermentation took 48 hours. The highest increase in reducing sugar concentration (20.2%) was shown in the treatment without adding a starter (0%) from 55 g/L to 66.2 g/L at 24 hours of fermentation.

The increase in reducing sugar concentration is caused by the activity of enzymes produced by bacteria. Sulfated polysaccharides *Caulerpa racemosa* (SPCr) is composed of several monosaccharide units such as glucose, galactose, xylose, and mannose connected by glycosidic bonds (Magdugo *et al*., 2020). The concentration of reducing sugars will increase as the monosaccharides in SPCr are released when bacterial enzymes break down glycosidic bonds. According to research by Afoakwa *et al*. (2013), the increase in reducing sugar concentration is due to the activity of enzymes such as β-galactosidase, amylase, and cellulase, which hydrolyze polysaccharides into monosaccharides. Increasing the reducing sugar concentration during fermentation proves that *L. plantarum* SK (5) bacteria can produce enzymes that degrade seaweed polysaccharides.

The decrease in reducing sugar concentration was caused by utilizing monosaccharides resulting from polysaccharide hydrolysis by LAB as an energy source for growth. *L. plantarum* bacteria use monosaccharides like glucose as an energy source during fermentation (Nagarajan *et al*., 2022). The decrease in reducing sugar concentration is also caused by the activity of enzymes produced by microorganisms during fermentation. Each type of enzyme has ideal conditions to hydrolyze its molecules. Enzyme activity is usually affected by pH, temperature, and surfactants. Insufficient conditions lead to low levels of enzyme activity. The optimum conditions for the amylase enzyme activity produced by *L. plantarum* are 50 °C and pH 7 (Khusniati *et al*., 2020). Putri (2016) also reported that the optimum conditions for cellulase enzyme activity produced by *L. plantarum* occurred at pH 7 and a temperature of 65 °C.

The concentration of reducing sugars in the treatment without adding a starter (0%) changed during the research's phase I and II. These changes are thought to be caused by the activity of enzymes produced by symbiotic bacteria from seaweed. *C. mannanilyticus* which is thought to be a symbiotic bacterium of *C. racemosa* can hydrolyze starch and mannan polysaccharides (Nogi *et al*., 2005). The decrease in reducing sugar concentration also proves that the bacteria growing on the product can ferment carbohydrates. Research by Warsidah *et al*. (2021) explained that the carbohydrate fermentation test results on bacterial isolates isolated from *C. racemosa* seaweed showed positive results in the glucose, sucrose, and mannitol tests. The bacteria were identified as coming from the genera *Corynebacterium* and *Neisseria*.

Sugar concentration of C. racemosa hydrolyzate

Sulfated polysaccharides *Caulerpa racemosa* (SPCr) comprise several monosaccharides such as glucose, galactose, and xylose. The highest monosaccharide content in SPCr was glucose at 52.42%, galactose at 15.62%, and xylose at 8.21% (Magdugo *et al*., 2020). Fermentation can hydrolyze SPCr polysaccharides through enzyme activity produced by microbes. This process causes changes in the concentration of monosaccharides contained in *C. racemosa*. The glucose and xylose concentration in the physical hydrolysis and fer-

Table 1. Concentrations of glucose and xylose hydrolyzate *C. racemosa*.

Sample	Glucose (g/L)	Xylose (g/L)
Hydrolyzate *C. racemosa*	<2 (nd*)	<0,7 (nd*)
C. racemosa fermented with starter 0%	44,1±0,1^{c}	/
C. racemosa fermented with starter 5%	37±0,3^{b}	/
C. racemosa fermented with starter 10%	34,2±0,0^{a}	/

note: nd* : not detected.
/ : not do

mented (enzymatic) seaweed hydrolyzate is in Table 1.

The glucose concentration in the seaweed *C. racemosa*, which was physically hydrolyzed using an autoclave at 121°C for 15 minutes, showed a glucose concentration of less than 2 g/L and a xylose concentration of less than 0.7 g/L. The glucose concentration in *C. racemosa* seaweed, which was enzymatically hydrolyzed by fermentation for a fermentation period of 24 hours, decreased as the added starter concentration increased. The highest glucose concentration was shown in the treatment without adding a starter (0%) at 44.1 g/L, while the lowest glucose concentration was shown in the 10% starter treatment at 34.2 g/L (Table 1).

The small concentrations of glucose and xylose in the physically hydrolyzed *C. racemosa* samples indicate that the hydrothermal hydrolysis process by autoclaving at 121°C for 15 minutes was not able to break the glycosidic bonds entirely so that the monosaccharide units released were relatively small. Mutmainnah *et al*. (2023) reported that temperature and time influence the physical polysaccharide hydrolysis process. The higher the temperature and longer the hydrolysis time, the more influential the depolymerization process of *C. racemosa* seaweed polysaccharides will be. Research by Meillisa *et al*. (2015) reported that *S. japonica* seaweed, which was hydrolyzed using subcritical water at a temperature of 180°C with a pressure of 12.8 atm for 30-75 minutes, was able to produce a glucose concentration of 0.43 g/L, mannose 1.50 g/L, and gulose 9.80 g/L.

The difference in the addition of starter concentration in *C. racemosa* fermentation really influences the glucose concentration produced. Tukey's advanced test results showed that the three starter concentration treatments differed significantly. The treatment without adding a starter (0%) showed a higher glucose concentration than adding 5% and 10%. The high glucose concentration is thought to be glucose added to the fermentation medium. The high glucose concentration in the 0% starter treatment was also caused by differences in the number of bacterial colonies that grew. The number of bacterial colonies in the treatment without adding a starter (0%) with a fermentation time of 24 hours was 3.8 log CFU/mL. This value was smaller than in the treatment adding 5% and 10% starter, which had a total of bacterial colonies, each 8.9 log CFU/mL and 8.7 log CFU/mL. A smaller number of bacterial colonies causes less glucose to be used as a carbon source (Gupta *et al*., 2011).

L. plantarum SK (5) bacteria used as a starter are classified as homofermentative bacteria (Desniar *et al*., 2012). *Homofermentative bacteria* can convert more than 90% of glucose into lactic acid (Hutkins, 2006). Utami (2017) reported that the percentage of sugar consumption by *L. plantarum* SK (5) bacteria reached 92.35%. Based on these results, it can be concluded that in the treatment of adding 5% and 10% starter, more than 90% of the glucose (around 45 g/L) added to the fermentation medium was converted into lactic acid by the bacteria *L. plantarum* SK (5) so that the glucose that was detected Most of it was the result of hydrolysis of the SPCr polysaccharide. In contrast, in the treatment without adding a starter (0%), most of the glucose detected was glucose added to the fermentation medium.

CONCLUSION AND RECOMMENDATION

Conclusion

Different starter concentrations and fermentation times influence total LAB, TTA, pH value, reducing sugars, and glucose concentration in hydrolyzed *C. racemosa* by fermentation. The best treatment is shown with the addition of 5% starter with a fermentation time of 24 hours, which had a total BAL value of 8.9 log CFU/ml, TAT 0.96%, pH value 3.8, reducing sugar 62.6 g/L, and glucose 37 g/L. The glucose concentration of *C. racemosa* before fermentation was <2 g/L and xylose <0.7 g/L, while after fermentation, the glucose concentration was 44.1 g/L (without a starter), 37 g/L (5% starter) and 34.2 g/L (10% starter).

Recommendation

The studies that follow examine the profiles of various sugars, including mannitol, galactose, mannose, xylose, and xylitol. analyzing molecular weight, biological activity, bioactive chemicals, and functional groups in fermentation *C. racemosa*. bacterial isolation and identification from the seaweed *C. racemosa* in order to identify the microorganisms involved in spontaneous fermentation.

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REFERENCES

Abubakar, Y., M. Muzaifa, H.P. Widayat, M. Martunis & A. Maulina. 2019. Karakteristik starter kering dari isolat bakteri indigenous kakao Aceh. Gontor Agrotech Science Journal. 5 (2): 89-109. https://doi.org/10.21111/agrotech.v5i2.3278

Afoakwa, E.O., J.E. Kongor, J. Takrama & A.S. Budu. 2013. Changes in nib acidification and biochemical composition during fermentation of pulp pre-conditioned cocoa (Theobroma cacao) beans. International Food Research Journal. 20 (4): 1843-1852.

Afriza, R & I. Ismanilda. 2019. Analisis perbedaan kadar gula pereduksi dengan metode lane eynon dan luff schoorl pada buah naga merah (Hylocereus polyrhizus). Jurnal Temapela. 2 (2): 90-96. https://doi.org/10.25077/temapela.2.2.90-96.2019

Agustine, L., Y. Okfrianti & J. Jumiyati. 2018. Identifikasi total bakteri asam laktat (BAL) pada yoghurt dengan variasi sukrosa dan susu skim. Jurnal Dunia Gizi. 1 (2): 79-83. https://doi.org/10.33085/jdg.v1i2.2972

Ambarsari, N.D., I.R.P.A. Rushanti, A. Setyaji, T.R. Ningsih, N. Nurhana, I. Subekhi & E.N. Dewi. 2018. The influenced of Lactobacillus plantarum starter addition and the length time of fermentation process on the activity of seaweed antioxidant *Ulva lactuca* from Krakal Beach, Yogyakarta. IOP Conference Series: Earth and Environmental Science. 116: 012074. https://doi.org/10.1088/1755-1315/116/1/012074

Anwar, L.O., L. Hardjito & D. Desniar. 2015. Fermentasi tambelo dan karakteristik produknya. Jurnal Pengolahan Hasil Perikanan Indonesia. 17 (3): 254-262. https://doi.org/10.17844/jphpi.v17i3.8914

Association of Official Analytical Chemists (AOAC). 2005. The Association of Official Analytical Chemists, Inc.

Association of Official Analytical Chemists (AOAC). 2006. Separation of Sugar in Honey, Liquid Chromatography Method.

Badan Standardisasi Nasional. 2015. SNI 2332.3: 2015. Cara Uji Mikrobiologi-Bagian 3: Penentuan Angka Lempeng Total (ALT) pada Produk Perikanan. Jakarta.

Badan Standardisasi Nasional. 2019. SNI 6989.11:2019. Air dan Air Limbah – Bagian 11: Cara Uji Derajat Keasaman (pH) dengan menggunakan pH meter. Jakarta.

Charoensiddhi, S., M.A. Conlon, C.M.M. Franco & W. Zhang. 2017. The development of seaweed-derived bioactive compounds for use as prebiotics and nutraceuticals using enzyme technologies. Trends in Food Science & Technology. 70: 20-33. https://doi.org/10.1016/j.tifs.2017.10.002

Desniar, D., I. Rusmana, A. Suwanto & N.R. Mubarik. 2012. Senyawa antimikroba yang dihasilkan oleh bakteri asam laktat asal bekasam. Jurnal Akuatika. 3 (2): 135-145.

Gupta, A & J.P. Verma. 2015. Sustainable bio-ethanol production from agro-residues: A review. Renewable and Sustainable Energy Reviews. 41: 550-567. https://doi.org/10.1016/j.rser.2014.08.032

Gupta, R.S., S. Patel, N. Saini & S. Chen. 2020. Robust demarcation of 17 distinct *Bacillus* species clades, proposed as novel Bacillaceae genera, by phylogenomics and comparative genomic analyses: Description of *Robertmurraya kyonggiensis* sp. nov. and proposal for an emended genus *Bacillus* limiting it only to the members of the Subtilis and *Cereus clades* of species. International Journal of Systematic and Evolutionary Microbiology. 70 (11): 5753-5798. https://doi.org/10.1099/ijsem.0.004475

Gupta, S., N. Abu-Ghannam & A.G.M. Scannell. 2011. Growth and kinetics of *Lactobacillus plantarum* in the fermentation of edible Irish brown seaweeds. Food and Bioproducts Processing. 89 (4): 346-355. https://doi.org/10.1016/j.fbp.2010.10.001

Hentati, F., L. Tounsi, D. Djomdi,G. Pierre, C. Delattre, A.V. Ursu, I. Fendri, S. Abdelkafi & P. Michaud. 2020. Bioactive polysaccharides from seaweeds. Molecules. 25 (14): 1-29. https://doi.org/10.3390/molecules25143152

Hutkins, R.W. 2006. Microbiology and Technology of Fermented Foods. 15-66. Blackwell Publishing Ltd. United States. 15-66.

Kartika, A.I., M.S. Fitria & V. Oktaviola. 2021. Molecular identification of pathogenic bacteria causing foodborne disease in Caulerpa racemosa. Jurnal Teknologi Laboratorium. 10 (1): 31-39. https://doi.org/10.29238/teknolabjournal.v10i1.276

Khusniati, T., G.T. Dewi, A.P. Roswiem, S.A. Azhari, F. Ishfahani & S. Sulistiani. 2020. Carbohydrate degradation of tuber paste flour by the addition of α-amylase from two *Lactobacillus* species. Jurnal Teknologi dan Industri Pangan. 31(1): 60-65. https://doi.org/10.6066/jtip.2020.31.1.60

Kusumawati, I., F. Diana & L. Humaira. 2018. Studi kualitas air budidaya latoh (*Caulerpa racemosa*) di Perairan Lhok Bubon Kecamatan Samatiga Kabupaten Aceh Barat. Jurnal Akuakultura. 2 (1): 33-43. https://doi.org/10.35308/ja.v2i1.781

Li, W., N. Jiang, B. Li, M. Wan, X. Chang, H. Liu, L. Zhang, S. Yin, S. Liu & H. Qi. 2018. Antioxidant activity of purified ulvan in hyperlipidemic mice. International Journal of Biological Macromolecules. 113: 971-975. https://doi.org/10.1016/j.ijbiomac.2018.02.104

Magdugo, R.P., N. Terme, M. Lang, H. Pliego-Cortés, C. Marty, A.Q. Hurtado, G.Bedoux & N. Bourgougnon. 2020. An analysis of the nutritional and health values of *Caulerpa racemosa* (Forsskål) and *Ulva fasciata* (Delile)-two chlorophyta collected from the Philippines. Molecules. 25 (12): 1-23. https://doi.org/10.3390/molecules25122901

Mailia, R., B. Yudhistira, Y. Pranoto, S. Rochdyanto & E.S. Rahayu. 2015. Ketahanan panas cemaran *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* dan bakteri pembentuk spora yang diisolasi dari proses pembuatan tahu di Sudagaran Yogyakarta. Jurnal Agritech. 35 (3): 300-308. https://doi.org/10.22146/agritech.9341

Ma'ruf, W.F., R. Ibrahim, E.N. Dewi, E. Susanto & U. Amalia. 2013. Profil rumput laut *Caulerpa racemosa* dan *Gracilaria verrucosa* sebagai *edible food*. Jurnal Saintek Perikanan. 9 (1): 68-74. https://doi.org/10.14710/ijfst.9.1.68-74

Meillisa, A., H.C. Woo & B.S. Chun. 2015. Production of monosaccharides and bio-active compounds derived from marine polysaccharides using subcritical water hydrolysis. Food Chemistry. 171: 70-77. https://doi.org/10.1016/j.foodchem.2014.08.097

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 31 (3): 426-428.

Muthukumar, J., R. Chidambaram & S. Sukumaran. 2021. Sulfated polysaccharides and its commercial applications in food industries - A review. Journal of Food Science and Technology. 58 (7): 2453-2466. https://doi.org/10.1007/s13197-020-04837-0

Mutmainnah, M., D. Desniar & J. Santoso. 2023. Degradasi hidrotermal *Kappaphycus alvarezii*: Karakter hidrolisat dan kapabilitas sebagai prebiotik. Jurnal Pengolahan Hasil Perikanan Indonesia. 26 (1): 13-24. https://doi.org/10.17844/jphpi.v26i1.43568

Nagarajan, D., N. Oktarina, P.T. Chen, C.Y. Chen, D.J. Lee & J.S.

Chang. 2022. Fermentative lactic acid production from seaweed hydrolysate using *Lactobacillus* sp. and *Weissella* sp. Bioresource Technology. 344: 1-9. https://doi.org/10.1016/j.biortech.2021.126166

Nogi, Y., H. Takami & K. Horikoshi. 2005. Characterization of alkaliphilic Bacillus strains used in industry: proposal of five novel species. International Journal of Systematic and Evolutionary Microbiology. 55 (6): 2309-2315. https://doi.org/10.1099/ijs.0.63649-0

Nurkolis, F., R. Kurniawan, I. Kurniatanty, M.N. Park, M. Moon, S Fatimah, W.B. Gunawan, R. Surya, N.A. Taslim, H. Song & B. Kim. 2023. New insight on in vitro biological activities of sulfated polysaccharides from ulvophyte green algae. Molecules. 28 (11): 1-16. https://doi.org/10.3390/molecules28114531

Offei, F., M. Mensah, A. Thygesen & F. Kemausuor. 2018. Seaweed bioethanol production: A process selection review on hydrolysis and fermentation. Fermentation. 4 (4): 1-18. https://doi.org/10.3390/fermentation4040099

Oliveira, M.N., I. Sodini, R. Remeuf, J.P. Tissier & G. Corrieu. 2002. Manufacture of fermented lactic beverages containing probiotic cultures. Journal of Food Science. 67 (6): 2336-2341. https://doi.org/10.1111/j.1365-2621.2002.tb09550.x

Pabriani, R. 2022. Pengaruh Waktu Sakarifikasi Limbah Agar Menggunakan H_2SO_4 terhadap Gula Pereduksi yang Dihasilkan. Institut Pertanian Bogor.

Permatasari, H.K., F. Nurkolis, P.S. Augusta, N. Mayulu, M. Kuswari, N.A. Taslim, D.S. Wewengkang, S.C. Batubara & W.B. Gunawan. 2021. Kombucha tea from seagrapes (*Caulerpa racemosa*) potential as a functional anti-ageing food: In vitro and in vivo study. Heliyon. 7 (9): 1-9. https://doi.org/10.1016/j.heliyon.2021.e07944

Putri, S. 2016. Karakterisasi enzim selulase yang dihasilkan oleh *Lactobacillus plantarum* pada variasi suhu, pH dan Konsentrasi substrat. Universitas Islam Negeri Maulana Malik Ibrahim Malang.

Rafiquzzaman, S.M., I.S. Kong & J.M. Kim. 2015. Enhancement of antioxidant activity, total phenolic and flavonoid content of *Saccharina japonica* by submerged fermentation with *Aspergillus oryzae*. Korean Society for Biotechnology and Bioengineering Journal. 30(1): 27-32. https://doi.org/10.7841/ksbbj.2015.30.1.27

Silitonga, P.R.A., W.A. Setyati & M.T. Sibero. 2022. Pengaruh fermentasi *Gracilaria verrucosa* dengan penambahan starter *Lactobacillus plantarum* pada profil metabolit dan aktivitas biologisnya. Journal of Marine Research. 11 (2): 309-319. https://doi.org/10.14710/jmr.v11i2.33262

Sumardianto, S., P.H. Riyadi, A.D. Anggo, R. Romadhon & L. Rianingsih. 2021. Phenol content and antioxidant activity in seaweed fermented with lactic acid bacteria. Food Research. 5 (3): 7-13. https://doi.org/10.26656/fr.2017.5(S3).006

Susilowati, R., D. Koesoemawardani & S. Rizal. 2014. Profil proses fermentasi rusip dengan penambahan gula aren cair. Jurnal Teknologi Industri dan Hasil Pertanian. 19 (2): 137-148. http://dx.doi.org/10.23960/jtihp.v19i2.137%20-%20148

Utami, T.N. 2017. Penapisan Bakteri Asam Laktat Penghasil Bioetanol pada Substrat Cairan Hidrolisat Rumput Laut *Gracilaria* sp.. Institut Pertanian Bogor.

Wang, L., X. Wang, H. Wu & R. Liu. 2014. Overview on biological activities and molecular characteristics of sulfated polysaccharides from marine green algae in recent years. Marine Drugs. 12 (9): 4984-5020. https://doi.org/10.3390/md12094984

Warsidah, W., R. Rizky, M.S.J. Sofiana, I. Safitri, S. Minsas, M. Trianasta & S. Sumanti. 2021. Biodiversity of Symbiotic microorganisms of *Caulerpa racemosa* from Lemukutan Island, Indonesia and its antibacterial activity. International Conference on Science and Engineering. 211: 46-52. https://doi.org/10.2991/aer.k.211222.008

Yap, W.F., V. Tay, S.H. Tan, Y.Y. Yow & J. Chew. 2019. Decoding antioxidant and antibacterial potentials of Malaysian green seaweeds: *Caulerpa racemosa* and *Caulerpa lentillifera*. Antibiotics. 8 (3): 1-18. https://doi.org/10.3390/antibiotics8030152

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