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# Phenol content and antioxidant activity in seaweed fermented with lactic acid bacteria

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**Abstract**

Antioxidants and antimicrobials are rich in seaweed. Seaweed has been used as food for a long time and the potency to be used as a functional food. One of the most versatile foods is fermented beverages. Fermentation can increase the amount of antioxidants. This study aims to determine seaweed (*Gelidium* sp. and *Eucheuma cottonii*) extract fermentation with lactic acid bacteria (LAB), namely *Lactobacillus plantarum* and *Lactobacillus acidophilus,* as a starter on phenol content and antioxidant activity. The method used was an experimental laboratory. The seaweed was obtained from Yogyakarta. Seaweed extract was given a LAB starter and then fermented for 24 hrs. The samples were analyzed before and after fermentation in parameter reducing sugar, TPC, LAB viability, pH, phenol content, and antioxidant activity $IC_{50}$ tests. The result showed that both in *Gelidium* sp. and *Eucheuma* cottonii, reducing sugar does not change before and after fermentation with the LAB starter. Fermentation could increase the TPC, LAB viability, phenol content, antioxidant activity $IC_{50}$, and lower the pH sample. *Eucheuma cottonii* fermented with *L. acidophilus* gives the best characteristic of antioxidant.

## 1. Introduction

Algae or seaweed is commonly consumed in Asia (Aparicio *et al.,* 2018). Algae are an important natural source of bioactive like polyphenols, carotenoids, vitamins, proteins, lipids, and polysaccharides (Rodrigues *et al.,* 2016; Suleria *et al.,* 2016; Mittal *et al.,* 2017; Zhao *et al.,* 2018; Cui *et al.,* 2019; Ozturk *et al.,* 2020) and have acted as an antioxidant as well as antibacterial (Roohinejad *et al.,* 2017).

There are numerous seaweed species and have not been used optimally. Seaweed growth does not compete with agricultural land (Hou *et al.,* 2017). Parada *et al.* (2019) reported that polyphenol could be utilized as an ingredient to make novel low glycemic response food.

Seaweed consumption is currently showing an increasingly interesting trend because humans are more concerned with healthy lifestyles. Many products from marine products are made and marketed as functional foods (Roohinejad *et al.,* 2017). Seaweed stock is abundant, and the price is low. Microorganisms can ferment seaweed to increase the bioactive component (Suraiya *et al.,* 2018).

The fermented product of seaweed has not been widely studied. Fermentation using lactic acid bacteria was reported in 2004 on *Ulva* spp. Besides, the *Chlorophyta, Phaeophyta,* and *Rhodophyta* groups have also been studied. Fermentation of either lactic acid or ethanolic makes the possibility of the making new fermented product (food and beverage) from seaweed and seagrass (Uchida *et al.,* 2017). *Gelidium* sp. and *Eucheuma cottonii* are edible seaweed and abundant in Indonesia.

Antioxidants and antimicrobials are rich in seaweed. Seaweed has been used as food for a long time and has the potency to be used as a functional food. One of the functional foods is fermented drinks. Fermentation can increase antioxidants. This research focused on studying the development of the fermented product from seaweed using lactic acid bacteria. The purpose of this study was to determine the effect of lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus acidophilus*) as a starter on phenol content and antioxidant activity during fermentation of *Gelidium* and *Eucheuma cottonii* extract.

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## 2. Materials and methods

### *2.1 Material*

Dried *Gelidium* sp. and *Eucheuma cottonii* were purchased from Gunung Kidul Yogyakarta, Indonesia. Natrium Chloride, Man Rogosa Sharpe media, Plate Count Agar, Natrium Azide, $CaCO_3$, DPPH, Methanol, Alcohol, Follin Denish, $Na_2CO_3$, Arsenomolibdat, Folin reagent, Nelson reagent. Starter preparation: The bacterial starters used in this study were *L. acidophilus* FNCC-0051, and *L. plantarum* FNCC-0027 obtained from Pusat Studi Pangan dan Gizi UGM. The starter was subjected to subculture twice in sterile MRS broth media then incubated at 37°C for 24 hrs before use.

### *2.2 Seaweed extract fermentation*

This research was conducted as described by Rianingsih and Sumardianto (2020). Seaweed *Gelidium* sp. and *Eucheuma cottonii* was soaked in water for 6 hrs and then chopped into approximately 1 cm in length. Each seaweed extract was put into a soymilk maker and added with mineral water. The ratio of seaweed and water was 1:12. Each seaweed extract was added with glucose 5% w/v and put in a glass jar approximately 200 mL, then sterilized in autoclave 121°C 15 mins. The seaweed extract allowed to cool and, after the temperature below 40°C then added with a 5% bacterial starter (*L. acidophilus* or *L. plantarum*) incubated 24 hrs at 37°C.

### *2.3 Measurement of reducing sugar*

The reducing sugar was determined following Martin *et al.* (2000). Aquadest was used to add one gram of sample until the volume reached 100 mL. The solution was mixed with Reagent Nelson and heated in a water bath for 30 minutes at 100°C. Allow the solution to cool before adding arsenomolibdat. At 540 nm, absorbance was measured.

### *2.4 Measurement of pH*

The pH was determined directly using a pH meter.

### *2.5 Measurement of TPC and viability of lactic acid bacteria*

Determination of TPC and viability of lactic acid bacteria were determined as described by Dissarapong *et al.* (2005). Total plate counts were determined using a plate count agar, and the dilution was using 0.85% NaCl sterile solution. A diluted sample (0.1 mL) was spread on the media's surface and incubated at 37°C. While lactic acid bacteria count was determined using Man Rogosa Sharpe agar (MRSA), and the dilution was using 0.85% NaCl. A diluted sample (1 mL) was poured into a petri dish and followed by 10 to 15 mL of MRSA media. Incubation was carried out at 37°C for 48 hrs.

### *2.6 Measurement of phenol*

The total phenolic content was determined using the Folin-Ciocalteu method described by Saravanan and Parimelazhagan (2014). Gallic acid was used to prepare a standard calibration curve.

### *2.7 Measurement of antioxidant activity*

The samples' free radical activity was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by (Tanod *et al.*, 2019).

### *2.8 Statistical analysis*

A completely randomized design was used throughout this study, and the experiments were done in triplicate. Data were subjected to analysis of variance (ANOVA), and mean comparison was carried out using the honest significance difference (HSD) test. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for Windows; SPSS Inc.).

## 3. Results and discussion

### *3.1 Reducing Sugar*

Microorganism, including bacteria, need media or nutrition to grow. Bacteria use food and convert it to energy, grow to produce several metabolites. Like reducing sugar, the carbon source is the primary energy source (Singh *et al.,* 2017). Component carbon and nitrogen sources usually play a crucial role in the fermentation medium (Khani *et al.,* 2016). The increasing yield of the cell during fermentation is influenced by carbon sources (Wang *et al.*, 2018).

Reducing sugar on 0 hrs and 24 hrs in *Gelidium* sp. and *Eucheuma cottonii* extract was shown in Table 1 and Table 2. The Reducing sugar ranges from 0.14% to 0.18%. Reducing sugar value was low and did not show a difference value in 0 hrs and 24 hrs fermentation ($p < 0.05$). This phenomenon was because the fermentation condition was not suitable yet for the starter to grow and can not hydrolyze the complex carbon from seaweed into the simple carbon like reducing sugar.

Table 1. Reducing sugar (%) in *Gelidium* sp. extract during fermentation.

| Fermentation length (hrs) | Starter (log CFU/mL) | |
|---|---|---|
| | *L. plantarum* | *L. acidophilus* |
| 0 | 0.14±0.03[a] | 0.17±0.01[a] |
| 24 | 0.14±0.04[a] | 0.16±0.02[a] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

 

Table 2. Reducing sugar (%) in *Eucheuma cottonii* extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 0.17±0.05[a] | 0.17±0.03[a] |
| 24 | 0.18±0.06[a] | 0.17±0.03[a] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

During fermentation, the starter's enzymes are expected to produce enzymes and hydrolyze the complex molecule into a simple molecule. For example, cellulose into glucose. Every enzyme has the optimum condition to hydrolyze the molecule. The factor that usually influences the enzyme activity is pH, temperature, ion, and surfactant. When the conditions are not suitable, the enzyme activity will be low (Palavesam, 2015).

Table 1 and Table 2 show that the amount of reducing sugar was meager. The reducing sugar was only approximately 0.14% to 0.18%. Low sugar content was challenging to support bacterial growth. Simple sugar is the most accessible source of energy to use during bacterial growth. *L. plantarum* and *L. acidophilus* are including a group of lactic acid bacteria. Lactic acid bacteria are fastidious. They need carbohydrates as well as other compounds to grow well (Singh *et al.*, 2017). Total plate count (TPC) was lower than lactic acid bacteria count. It is showed that lactic acid bacteria growth was better than other bacteria in this fermentation. Lactic acid bacteria can suppress the development of different bacteria, although not optimal. During gain, lactic acid bacteria will produce acid and lower the pH. The lower pH will stop the growth of other bacteria that cannot tolerate low pH to reduce the TPC value.

### *3.2 Total plate count*

TPC is the enumeration of aerobic, mesophilic organisms included pathogens and non-pathogen, and is used to determine the hygienic status of food production. Bacterial count (TPC) on 0 hrs and 24 hrs in *Gelidium* sp. and *Eucheuma cottonii* extract were shown in Table 3 and Table 4. The TPC of the sample ranges from 2.62 log CFU/mL to 4.53 log CFU/mL. This data showed that the count of microorganisms other than the starter is still high. This is because not all ingredient used to produce the seaweed extract was sterilized, so the contamination was still very high.

In *Gelidium* sp. extract, TPC did not change after 24 hrs of fermentation in both starters. While in *Eucheuma cottonii* extract, TPC was increased 1 log cycle after 24 hrs fermentation time. *Eucheuma cottonii* extract has higher reducing sugar ($p < 0.05$) that can support microorganisms' growth. Simple sugar, like reducing sugar, is easy to metabolize by microorganisms, including lactic acid bacteria as a starter and other bacteria present in the sample (Ficoseco *et al.*, 2018). Table 1 and Table 2 show that the amount of reducing sugar was meager. The reducing sugar was only approximately 0.14% to 0.18%. Low sugar content was challenging to support bacterial growth. Simple sugar is the most accessible energy source to use during bacterial growth (Singh *et al.*, 2017).

Table 3. Total plate count in *Gelidium* sp. extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 3.29±0.06[a] | 3.86±0.25[a] |
| 24 | 4.10±0.06[a] | 4.31±0.54[a] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

Table 4. Total plate count in *Eucheuma cottonii* extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 2.62±0.06[a] | 2.71±0.22[a] |
| 24 | 4.52±0.05[b] | 4.53±0.06[b] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

### *3.3 Viability of lactic acid bacteria starter*

Lactic acid bacteria count of the starter on 0 hrs and 24 hrs in *Gelidium* sp. and *Eucheuma cottonii* extract was shown in Table 5 and Table 6. The data ranges from 6.7 log CFU/mL to 8.21 log CFU/mL. The bacterial count of starters increased after 24 hrs of fermentation time in both seaweed and both starter ($p < 0.05$). It is showed that the starter could grow in seaweed extract. The increasing microbial count was about 1 log cycle.

Table 5. Viability of lactic acid bacteria in *Gelidium* sp. extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 6.73±0.14[a] | 7.13±0.16[b] |
| 24 | 7.96±0.14[c] | 8.21±0.21[c] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

*Eucheuma cottonii* extract has higher reducing sugar ($p < 0.05$) that can support starter growth. Simple sugar is easy to metabolize by microorganisms, including lactic acid bacteria. Lactic acid bacteria are fastidious. It needs a carbon source like a simple sugar, a nitrogen source like amino acid or peptide, and some other compounds.

 

Lack of carbon sources makes the microorganism's growth not optimal (Ficoseco *et al.*, 2018). Estevam (2018) reported that the early fermentation time of fermented milk added with seaweed extract will increase the microbial count and titratable acidity.

Table 6. Viability of lactic acid bacteria in Eucheuma cottonii extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 6.85±0.15[a] | 6.70±0.23[a] |
| 24 | 7.86±0.34[b] | 8.04±0.24[b] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

*3.4 pH*

The pH sample on 0 hrs and 24 hrs in *Gelidium* sp. and *E. cottonii* extract were shown in Table 7 and Table 8. The pH was decreased after fermentation ($p < 0.05$). These phenomena happen because the lactic acid bacteria starter was grown and converted the fermentation medium into lactic acid to lower the pH.

The addition of aqueous seaweed extract to fermented milk will increase the total acid, thus will reduce the pH during the early fermentation period (Estevam *et al.,* 2018). It showed that the starter could metabolize the carbohydrates in seaweed to produce lactic acid. Wu *et al.* (2007) showed that some lactic acid bacteria could use the oligosaccharides from some seaweed as a fermentation medium.

Table 7. pH in *Gelidium* sp. extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 7.40±0.23[a] | 6.90±0.20[b] |
| 24 | 5.90±0.10[c] | 5.53±0.05[d] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

Table 8. pH in *Eucheuma cottonii* extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 7.10±0.20[a] | 6.97±0.31[a] |
| 24 | 5.63±0.23[b] | 5.50±0.36[b] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

Changes in pH value after 24 hrs fermentation were different between starter ($p < 0.05$). The changes in pH that caused by acid production during bacterial growth is strain-specific characteristic. In this research, *L. acidophilus* pH was lower than *L. plantarum.* This result was the opposite with Solval *et al.* (2019), in which the lactic acid bacteria count in *L. acidophilus* was lower than *L. plantarum*; thus, pH in *L. acidophilus* was higher than *L. plantarum.*

*3.5 Phenol content*

Phenol content on 0 hrs and 24 hrs in *Gelidium* sp. and *Eucheuma cottonii* extract were shown in Table 9 and Table 10. The phenol content showed various phenomena during the fermentation, and the value ranges are from 43.33 to 145.67. In *Gelidium* sp. extract, the phenol content did not change after 24 hrs of fermentation in both starters ($p < 0.05$). While in *Eucheuma cottonii* after 24 hrs of fermentation, the sample with *L. plantarum* showed a lower phenol content but in *L. acidophilus* showed a higher phenol content than before fermentation ($p < 0.05$). Fermentation can increase the biological functionalities include antioxidant activity (Hifney *et al.*, 2018).

Table 9 and Table 10 show phenol content in *Gelidium* sp. extract and *Eucheuma cottonii* extract before and after fermentation. In *Gelidium* sp. extract, the phenol content did not change after 24 hrs of fermentation in both starters ($p < 0.05$). While in *Eucheuma cottonii* after 24 hrs of fermentation, the sample with *L. plantarum* showed a lower phenol content but in *L. acidophilus* showed a higher phenol content than before fermentation ($p < 0.05$). Fermentation can increase the biological functionalities include phenol content and antioxidant activity (Hifney *et al.*, 2018; Norakma *et al.*, 2019). Microorganisms start to modify plant constituents during fermentation. Many biochemical changes occur during fermentation, leading to an altered ratio of healthy and anti-nutritive plants' components, which affect product properties such as bioactivity and digestibility (Katina *et al.*, 2007). The microorganism growth during fermentation may hydrolyze the seaweed tissue, contributing to phenol

Table 9. Phenol content in *Gelidium* sp. extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 74±30[a] | 116±75[a] |
| 24 | 116±8[a] | 137.5±6.5[a] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

Table 10. Phenol content in *Eucheuma cottonii* extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 70±19.29[a] | 91±5[b] |
| 24 | 43.33±5.03[c] | 145.67±4.04[d] |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

 

increasing (Hifney *et al.*, 2018). But it is also reported that not all substances are increased by fermentation. Several substances are also reduced after fermentation. The increment and reduction of phenolic content can be related to organic acids' metabolism, derivation of phenolics among themselves, and other metabolic pathways (Li *et al.*, 2020).

### *3.6 Antioxidant $IC_{50}$*

Antioxidant activity on 0 hrs and 24 hrs in *Gelidium* sp. and *Eucheuma cottonii* extract were shown in Table 11 and Table 12. The data showed that after fermentation, the sample has lower $IC_{50}$ when compared to before fermentation. The antioxidant activity showed slightly higher after fermentation. These phenomena happen because the lactic acid bacteria starter was grown and converted the fermentation medium into lactic acid and other products that probably function as antioxidants. During fermentation, several microbial enzymes could hydrolyze the raw material and increase the phenol content (Cheng *et al.,* 2015; Huang *et al.,* 2017) and flavonoid content in seaweed fermentation (Hur *et al.,* 2014). It will increase the antioxidant activity.

Table 11. Antioxidant $IC_{50}$ in *Gelidium* sp. extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 44667± 378.37$^{ab}$ | 45728.33±964.45$^{b}$ |
| 24 | 43842.67±1372.15$^{ab}$ | 42179.33±1941.45$^{a}$ |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

Table 12. Antioxidant $IC_{50}$ in *Eucheuma cottonii* extract during fermentation.

| Fermentation length | Starter (log CFU/mL) | |
|---|---|---|
| (hrs) | *L. plantarum* | *L. acidophilus* |
| 0 | 44024.33±222.37$^{a}$ | 42872.33±986.28$^{b}$ |
| 24 | 41504±855.89$^{c}$ | 38766.67±222.34$^{c}$ |

Values are presented as mean±SD. Values with different superscripts within the row are significantly different ($p < 0.05$).

Antioxidant activity of fermented seaweed extract on 0 hrs and 24 hrs in *Gelidium* sp. and *Eucheuma cottonii* extract were shown in Table 11 and Table 12. Data $IC_{50}$ is the concentration of a sample with the ability to scavenge 50% of DPPH radicals. The antioxidant activity of seaweed extract ranges from 44667% to 38766%. This activity is still deficient. The data showed that after fermentation, it showed that the sample has a lower $IC_{50}$. The antioxidant activity showed slightly higher after fermentation. These phenomena happen because the lactic acid bacteria starter was grown and converted the fermentation medium into lactic acid and other products that probably function as antioxidants. This data is also in line with the phenol content result (Table 9 and Table 10). After fermentation, the phenol content was slightly increased. During fermentation, several microbial enzymes could hydrolyze the raw material and increase the phenol content (Cheng *et al.,* 2015; Huang *et al.,* 2017) and flavonoid content in seaweed fermentation (Hur *et al.,* 2014) and increase the antioxidant activity.

## 4. Conclusion

Fermentation on a seaweed extract with *L. acidophilus* and *L. plantarum* increased lactic acid bacteria's viability, phenol content, antioxidant activity, and reduce pH. However, the antioxidant activity was still low. Therefore, it needs to optimize the fermentation condition to obtain higher antioxidant activities.

## Conflict of interest

The authors declare no conflict of interest.

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