Antioxidant activity in seaweed (Sargassum sp.) extract fermented with Lactobacillus plantarum and Lactobacillus acidophilus

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Abstract. Sargassum sp. has been known to contain rich of nutrients and bioactive compounds. In order to produce a non dairy probiotic beverage, this study tried to fermented the Sargassum sp. using Lactobacillus plantarum and L. acidophilus. The aim of this study was to understand the changes of pH, viability of lactic acid bacteria, aerobic plate count and antioxidant activity. Fermentation was done at 37 °C for 24 hours. Total aerobic plate count (APC), Total plate count lactic acid bacteria (LAB), sensory, pH, fenol concentration and antioxidant activity were measured. The data were analyzed by ANOVA and Tukey test in order to know which treatments are different. The result showed the fermentation reduced the pH, increased the reducing sugar, TPC, viability of LAB starter, and antioxidant activity.

1. Introduction

Modern lifestyle nowadays increases the variety of health problems in the society. People who live in big city have to deal with a busy and stressful life. Moreover, the environmental conditions also declining in quality. These problems are suggested to induce the occurrence of degenerative diseases [1-4].

Fortunately, the awareness of the society about the importance of living healthy is increasing by maintain their lifestyle. One of the methods carried out by them is eating healthy food, whole food and back to nature. Many people are already familiar with functional food and understand its function for health. One of the most popular functional foods nowadays is probiotic drinks. Most of the probiotic drinks in the market are made from a dairy product, nevertheless some other non-animal raw material has also begun to be developed into a probiotic drink, such as those are based on fruits [5].

In order to make a new probiotic product, marine environment offers various raw material that are potentially to be utilized. Seaweed is on of marine resources in Indonesia which has a cheap price and can be fermented to increase the levels of bioactive compound, such as polyphenols [6-8]. Several polyphenol derivatives are lignans, cinnamic acid, benzoic acid, quercetin, isoflavones, flavonoid and phenolic acid. These derivatives are reported as an antioxidant agent with potential free radical scavenging activity [3].

This study tried to formulate Sargassum sp. drink which was made from seaweed extract with the addition of probiotic bacteria (lactic acid bacteria) such as Lactobacillus plantarum FNCC 027 and L. acidophilus. Both of these bacteria are lactic acid bacteria that can reach the human digestive tract in a state of life and show inhibitory activity against pathogenic bacteria.
This study uses seaweed *Sargassum* sp. and different types of starter to produce antioxidant fermented drinks. The aim of this study was to know the changes of pH, viabilitas of lactic acid bacteri, aerobic plate count and antioxidant activity in *Sargassum* extract after 24 hours fermentation.

2. Methods
2.1. Material
MRSA, NaCl, Na-Azida, NaCl, CaCO₃, DPPH, Metanol, Na₂CO₃, Arsenomolibdat, Folin reagen, Nelson reagen. *Sargassum* sp. were collected from Gunung Kidul Yogyakarta Indonesia.

2.2. Starter preparation [15]
The bacterial starters (*L. acidophilus* FNCC-0051 and *L. plantarum* 0027) study were obtain from Pusat Studi Pangan dan Gizi UGM. Subculter was done 2 times before the starter used for fermentation. Starters were cultivated in sterile a MRS broth media then incubated at 37 °C for 24 hours.

2.3. Seaweed fermentation
The fermentation of *Sargassum* sp. was conducted by an addition of 5% of *L. plantarum* and *L. acidophilus*. Fermented seaweed extract made by soaked seaweed in water for 6 hours then cut into small pieces (1 cm). Sampel was put into soymilk maker with a ratio of seaweed and water 1:12 to obtain seaweed extract. Then, 200 ml seaweed extract was put in sterile glass jar and added with 5% bacterial starter and incubated 24 hours in 37°C.

2.4. Measurement of pH
The pH of fermented seaweed was measured as described by Benjakul et al. [9]

2.5. Measurement of TPC and viability of LAB [10]
A 10 g sampel was homogenized with 90 ml of physiological saline water for 60 s in stomacher. Aerobic and mesophilic counts were performed on Plate Count Agar, LAB on de Man, Rogosa and Sharpe (MRS) agar plates (Oxoid, CM361). Plates were incubated at 37°C for 3 days.

2.6. Measurement of total phenolic
Mesurement of total phenolic was performed according to Primurdia and Kusnadi [5].

2.7. Measurement of antioxidant activity
Measurement of antioxidant activity was performed according to Molyneux [11].

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 Honestly significanc difference test. Statistical analysis was performed using the statistical Package for Social Sciences (SPSS for Windows; SPSS Inc.) [12].

3. Results and Discussions
3.1 Reducing sugar
*Sargassum* is one of the famous brown seaweed. *Sargassum* is a good raw material that contain high amount of carbohydrates and could be convert into monosaccharides. Seaweeds have a specific carbohydrate such as agar, alginate, and carrageenan. Major carbohydrate in *Sargassum* are alginate 32.2%, holocellulose 41.6%, alpha-cellulose 22% and hemicellulose 19.6%. Tamayo and Rosario [13] reported that reducing sugar could be increased in *Sargassum* with enzymatic saccharification. The maximum amount of sugar was in 72 hours hydrolysis with the commercial cellulose enzyme. The
concentration of reducing sugar reach around 30 mg/mL. *L. plantarum* produced various extracellular enzyme such proteolytic, cellulolytic and hemicellulolytic. This microorganism could be selected as biotransformation agent in cellullosic material [14].

Table 1. Reducing Sugar Content of Fermented *Sargassum* sp.

| Fermentation time (Hours) | L. *plantarum* | L. *acidophilus* |
|---------------------------|----------------|------------------|
| 0                         | 0.12 ± 0.01<sup>a</sup> | 0.14 ± 0.06<sup>a</sup> |
| 24                        | 0.17 ± 0.01<sup>b</sup> | 0.22 ± 0.02<sup>b</sup> |

(Value represent mean ± SD from triplicate determination)

The data showed that sugar reduction content at the beginning fermentation was not significantly different between those given *L. plantarum* or *L. acidophilus* inoculum (Table 1). The sugar reduction in sample with *L. plantarum* was 0.12 ± 0.01 and sample with *L. acidophilus* was 0.14 ± 0.06. After fermentation the sugar reduction content rises to 0.17 ± 0.01 with *L. plantarum* and 0.22 ± 0.02 with *L. acidophilus*. This phenomena means that during the fermentation process there was a process of breaking down complex carbohydrate into simple sugar (reducing sugar). The starter was believed to produce an enzyme that able to hydrolyze complex compounds into simple compounds. In compared to hydrolysis with cellulose enzyme, sugar recution in this research was lower. It means that the starter only can hydrolize small amount of complex carbohydrate in the raw material. It is allegedly that starter was not grow optimally.

Carbohydrate is needed for living microorganisms as a source of energy. The simpler the carbohydrates available, the easier bacteria use it [15]. A longer fermentation period increased the sugar uptake by the lactic acid bacteria to grow. During the fermentation process, the lactic acid bacteria break down glucose into lactic acid or other sugars such as lactose, galactose, fructose, sucrose and maltose [5]. Viability of the starter can be seen in Table 2.

3.2 The viability of lactic acid bacteria in fermented *Sargassum* sp.

The data showed that the number of bacteria in the beginning of fermentation was not significantly different. After 24 hours of fermentation, the number of lactic acid bacteria in *Sargassum* extract were increased. The cell density was about 10<sup>8</sup> cfu/ml. The increasing means that the starter grew in seaweed extract juice media. The increasing number of lactic acid bacteria was only 1 log cycle. This might be due to the media merely contained *Sargassum* sp. without any addition.

Table 2. The viability of lactic acid bacteria in fermented *Sargassum* sp.

| Fermentation time (Hours) | L. *plantarum* | L. *acidophilus* |
|---------------------------|----------------|------------------|
| 0                         | 7.01 ± 0.04<sup>a</sup> | 6.91 ± 0.04<sup>a</sup> |
| 24                        | 8.11 ± 0.03<sup>b</sup> | 8.19 ± 0.06<sup>b</sup> |

(Value represent mean ± SD from triplicate determination)

Seaweed extract juice contains high amounts of carbohydrates but still in the form of complex carbohydrates which are relatively difficult to metabolize by the starter [15-17]. It means that the carbon source in the media was still need addition. In addition, nitrogen is an important element. In seaweed, there is not much protein so it is also need to be added. Addition of reducing sugars (sucrose, glucose) and N sources is thought to be able to increase the viability of the lactic acid bacteria.
3.3 Total Plate Count (TPC) of fermented Sargassum sp.

The data of TPC is shown in Table 3. An enhancement phenomenon was observed during the fermentation. The enhancement occurred from $10^3$ to $10^4$. It is expected that there will be an enhancement in LAB starter viability and a decrease in mesophilic TPC. But the data showed that TPC has increased one log cycle. An enhancement in TPC means that the LAB starter cannot dominate growth in seaweed extract. This might be due to the media is still need to be improved. The nutrient content was still not enough to support the growth of the starter thus still provides a chance for other microorganisms to grow. If starter grow well, it is expected that the pH will drop which later will inhibit the growth of other bacteria so that the TPC value will decreased [15].

Table 3. Total Plate Count (TPC) in fermented Sargassum sp.

| Fermentation time (Hours) | Starter (log cfu/ml) |
|--------------------------|----------------------|
|                          | L. plantarum        | L. acidophilus       |
| 0                        | 3.63 ± 0.02b         | 3.26 ± 0.09a         |
| 24                       | 4.44 ± 0.8c          | 4.45 ± 0.09c         |

(Value represent mean ± SD from triplicate determination)

3.4 pH value of Fermented Seaweed (Sargassum sp.)

The pH data is presented in the Table 4. The pH reduction after being fermented for 24 hours. The reduction in pH is due to the production of lactic acid by the LAB starter [18]. The pH reduction of seaweed extract with L. acidophilus was higher than the pH reduction with L. plantarum. It means that the production of acid by L. acidophilus was higher than L. plantarum. In other words, L. acidophilus had higher survival rate in the seaweed extract (Sargassum sp.) than L. plantarum.

Table 4. pH value of fermented Sargassum sp.

| Fermentation time (Hours) | Starter |
|--------------------------|---------|
|                          | L. plantarum | L. acidophilus |
| 0                        | 7.40 ± 0.08c | 7.23 ± 0.34c   |
| 24                       | 6.33 ± 0.12b | 5.37 ± 0.21a   |

(Value represent mean ± SD from triplicate determination)

3.5 Phenol Content

Data on fermented seaweed extract phenol content are presented in Table 5. The phenol content of seaweed extract with the addition of both starters at the beginning of the fermentation and the end of fermentation was not significantly different. This might be because the growth of the starter is not too good because the media is not too supportive. It could also because the growth phase of the starter cell for 24 hours has not yet reached the phase where the cell will excrete its metabolites in the form of phenolic compounds.

Table 5. Phenol content of fermented Sargassum sp.

| Fermentation time (Hours) | Starter |
|--------------------------|---------|
|                          | L. plantarum | L. acidophilus |
| 0                        | 62 ± 36.51a  | 79.67 ± 50.56a |
| 24                       | 105.67 ± 36.83a | 156.28a         |

(Value represent mean ± SD from triplicate determination)
Phenolic compound is a secondary metabolite that are not essential for the growth of organism. It has antioxidants action because it can donate hydrogen atom. It can give protection against adverse factors. Organism produce secondary metabolite include phenolic compound are observed in ecologically disadvantaged. Usually microorganism produce secondary metabolites like phenolic compound in the late growth phase because the the quality of environmental at that phase is decreased [19]. Microbial secondary metabolites synthesis can be influenced significantly by manipulating formulation of media [20].

3.6 Antioxidant activity

Data antioxidant activity presented in Table 6. At the beginning of the fermentation, samples with L. acidophilus starter had higher antioxidant activity. This difference might be caused by the presence of starter media which was added during the inoculation of the starter. During starter production, MRSB was used as culture medium and incubated for 24 hours. During the incubation of L. acidophilus, antioxidant compounds might be produced and the concentration or antioxidant activity was greater than the starter L. plantarum. At the end of the fermentation, it was noted that the addition of L. acidophilus gave greater antioxidant activity than the addition of L. plantarum. Hunaefi et al. [23] reported that during fermentation, percentage scavenging activity (%) in Lactobacillus acidophilus is greater than Lactobacillus plantarum.

| Fermentation time (Hours) | L. plantarum | L. acidophilus |
|--------------------------|--------------|---------------|
| 0                        | 45364.33± 775.59<sup>a</sup> | 43498.33 ± 249.55<sup>b</sup> |
| 24                       | 40918.33 ± 1011.88<sup>a</sup> | 39940.67 ± 443.4<sup>a</sup> |

(Value represent mean ± SD from triplicate determination)

4. Conclusion

Sargassum sp. extract fermented with Lactobacillus plantarum and Lactobacillus acidophilus showed a pH reduction, enhancement of sugar reduction, TPC, viability of LAB starter, and antioxidant activity.

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