Production and characterization of the healthy brown rice milk with sodium alginate addition from brown algae Sargassum binderi as emulsifier

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Abstract. Brown rice milk, a plant milk, is potential to cure beriberi disease. Alginate was used as an emulsifier and increased the nutrition of brown rice milk. Alginate from Sargassum binderi was extracted by nonacidic treatment. The yield of alginate was 6.25 %. The moisture and water content of alginate were 18.27 % and 12.2 %, respectively. The density and viscosity of 0.1 % alginate in aqueous solution were 1.007 g·mL\textsuperscript{-1} and 7.651 \times 10^{-3} kg·m\textsuperscript{-1}s\textsuperscript{-1}, respectively. The characteristics peaks of alginate appeared at 3,477; 1,633 and 1,419 cm\textsuperscript{-1}, corresponding to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH), respectively. The best composition of alginate addition in brown rice milk was 0.2 %. The viscosity of the 0.2 % alginate in brown rice milk was 1.206 \times 10^{-2} kg·m\textsuperscript{-1}s\textsuperscript{-1} and showed a texture of small particle with closer spaces between the particles. The addition of alginate in brown rice milk also inhibited the process of sedimentation of milk which showed in the first order. The nutrition composition of the best brown rice milk was 40.21 mg·mL\textsuperscript{-1} carbohydrate; 1.3 mg·mL\textsuperscript{-1} protein; 0.158 mg·mL\textsuperscript{-1} fat and 13.1 mg·mL\textsuperscript{-1} total dietary fiber.

1. Introduction
Milk is called the fifth source that makes perfect nutrition [1]. The growth of raw milk production every year reflecting the increased in consumer demand for milk. The main components of milk are proteins, fats, sugars (lactose), minerals (solid state) and water [2].

Originally, milk is referred to the product released from the mammal gland of the animal; however, the liquid extract from a plant that has a similar consistency to animal milk is also called as milk, such as soy milk and rice milk. In this paper, we called this type of milk as plant milk. The composition of plant milk is similar to cow's milk, in terms of protein, and is usually consumed as a substitution to cow's milk by those who are allergic to cow's milk. Plant milk is an alternative for people lacked lactase in their digestive tract that is not able to digest lactose contained in cow's milk. Therefore, the development of plant milk product by food industries aims to target this type of consumers.

Brown rice is an example of an ingredient that is used in the development of plant milk product. Utilization of brown rice as a beverage offers a good alternative to protein sources for a wide range of consumers, from children to the elderly [3]. The content of vitamin and mineral in brown rice was 2–3 times higher than white rice. Brown rice has a low glycaemic index (low starch and high complex carbohydrates) that can reduce the risk of type-2-diabetes [4].

In this study, we used alginate as an emulsifier in the production of brown rice milk. Moreover, alginate was used to increase the nutrition of brown rice milk and improve the texture of milk. Alginate was extracted from brown seaweeds (Sargassum binderi) obtained from Sayang Heulang
Beach, Pameungpeuk, Garut, West Java. Alginate is the main content of brown algal cell walls. Alginate is a polysaccharide composed of β-D-mannuronic acid and α-L-guluronate linked by 1,4-glycosidic bond. Alginate levels in brown alga are about 20–27 % of the total dry weight [5]. This research aimed to use the sodium alginate extracted from brown algae Sargassum binderi as an alternative emulsifier for the production of brown rice milk. Milk products are expected to have a good stability, high nutritional value and have a long shelf life.

2. Methods

2.1. Algae collection
Brown algae were collected from Sayang Heulang Beach, Pameungpeuk, Garut, West Java of Indonesia. The species were in family Sargassaceae: Sargassum binderi. Brown algae were washed in distilled water, dried overnight at 60 °C in an oven. The dry weights were obtained after drying overnight at 105 °C.

2.2. Extraction of alginate
The samples were suspended in 2 % (w/v) CaCl₂ for 2 h, washed with deionized water and treated with 40 % formaldehyde for 2 h to cross-link phenolic compounds. Alginate was extracted according to a method of Haug [6]. The samples were then washed three times with deionized water and the alginate was extracted by addition of an aqueous solution of 3 % Na₂CO₃ for 48 h. In other treatment, samples were also suspended in a mixture of 1 M Na₂CO₃ and 0.5 % EDTA, and the pH of the suspension was adjusted to pH 11 [6]. This suspension was then filtered through muslin cloth and precipitated in ethanol as the sodium salt. The precipitate was separated by centrifugation and dried overnight at 60 °C. The yield of alginate was expressed as percentage/dry weight.

2.3. Purification of sodium alginate extracts
Sodium alginate was purified according to the method of Gomez et al. [7]. An aqueous solution of sodium alginate obtained in the extraction step was directly precipitated, under stirring, by addition of ethanol until reaching a proportion of 1:1 in volume, respectively. Thus, the insoluble polymer was separated and then exhaustively washed with acetone by soxhlet for 100 h. Finally, the biopolymer was dried at room temperature under vacuum condition until constant mass was reached.

2.4. FT-IR analysis of alginate
Fourier-transform infrared spectroscopy (FT-IR) analysis of the samples was carried out using an FT-IR spectrophotometer in ITB to identify the functional groups. The dry sample was mixed with potassium bromide and pressed into a plate for measurement. The FT-IR spectrum was then recorded.

2.5. Analysis of moisture content of alginates
One gram of sodium alginate was accurately weighed and dried in a vacuum oven at 70 °C for 24 h. The sample was allowed to cool to room temperature in vacuum desiccators and re-weighed. The moisture content of the alginate sample was determined from the weight difference and expressed as a percentage of original weight.

2.6. Production of brown rice milk
Brown rice milk was prepared by mixing brown rice with water in the ratio of 1:15 (w/v), homogenized and processed to reach the consistency of porridge. Then the mixture was filtered using a calico cloth to obtain brown rice milk. Then, alginate was added to milk with the concentration of 0.05 %, 0.1 % and 0.2 % (w/v). The milk was pasteurized at 85–95 °C. Each treatment was replicated three times. Then, milk texture was observed under a microscope with 400 times magnification.
2.7. Analysis of the rate of deposition of brown rice milk
One mL of sample was put into a flask, diluted to 10 mL by aquades. The transmitter’s measurement of the suspension was performed at a wavelength of 656 nm. The transmittance results were then converted into a turbidity value with the equation below.

\[ S = -\log \left( \frac{T}{100} \right) \]  

(1)

Where:
S = turbidity
T = transmitters

2.8. Analysis of carbohydrate, protein, fat and fiber content
Nutrition analysis of milk was performed according to BSN [8], on carbohydrate, protein, fat and fiber content. Carbohydrates, protein, fat and fiber content were analyzed using Luff Schroll method, Kjeldahl method, gravimetric method and enzymatic-gravimetric method, respectively.

3. Results and Discussion

3.1. Sargassum binderi
Seaweed was the main ingredient used in this study. It was obtained from the growing area in Sayang Heulang Beach, one of the coral beaches in the southern part of Java Island. The coordinate sampling points were 7°40'12"S and 107°41'37"E.

![Sargassum binderi](image)

Figure 1. Sargassum binderi.

*Sargassum binderi* had a characteristic of the thallus (± 1.5 cm), smooth/slippery, the height reached about 60 cm (figure 1). Regular branching, opposite (left-right), main branch adjacent to each other, arises on the main short stem (± 1–2 cm) above holdfast. Leaves were oval, serrated edge, length of 5 cm, the width of 1 cm and a pointed tip. The bladder was round, rounded or pointy tip, 1 cm long-winged and 0.4 cm in diameter. The reproductive organs formed a special stalk, scratch, flatten and jagged. Leaves and bladders were shrunk.

3.2. Alginate extraction
Brown seaweeds (*Sargassum binderi*) from Indonesia were collected for extraction and purification of sodium alginate. The yield of alginate is 1.2 g from dry weight *Sargassum binderi*. The moisture and water content of alginate extracted from seaweed sample was 18.27 % and 12.2 %, respectively. The density and viscosity of 0.1 % (v/v) alginites in aqueous solution were 1.007 g·mL⁻¹ and 7.651 × 10⁻³ kg·m⁻¹s⁻¹, respectively, while the viscosity of commercial brown rice milk (without the addition of alginate) is 2.45 × 10⁻³ kg·m⁻¹s⁻¹. The high viscosity of alginate indicates that it can be applied to thicken the consistency of brown rice milk.
3.3. FT-IR of alginate
The FT-IR spectrum of sodium alginate is given in figure 3. As the alginate is a carbohydrate, the characteristic peaks of alginate appeared at 3,477; 1,633 and 1,419 cm\(^{-1}\), corresponding to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH), respectively. This result indicates that these three peaks were appeared in FT-IR spectrum obtained from sodium alginate extracted from brown alga *Sargassum binderi* localized in Indonesia. The results of the study are in accordance with the results of previous study [9] which reported that the characteristic peaks of alginate appeared at 3,429; 1,630 and 1,428 cm\(^{-1}\), corresponding to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH), respectively.

3.4. The rate of deposition of brown rice milk
The effect of alginate addition on the stability of brown rice milk was evaluated by measuring the turbidity value of the final product. The turbidity value was obtained by the measurement using the UV-vis spectrophotometer at \(\lambda = 241\) nm. The rate of precipitation of brown rice milk with various alginate concentrations was tested kinetically by using the integral method on the order of 0, 1 and 2.
3.4. Alginate Addition to the Stability of Brown Rice Milk

Figure 4. Variation of alginate addition to the stability of brown rice milk.

Based on the kinetics analysis shows that the rate of precipitation of brown rice with the addition of alginate was of order one. This result indicates that the addition of alginate two-fold from the initial quantity resulted in the four times increase of stability. This result also indicates that alginate could increase the stability of brown rice milk so that the resulting brown rice milk does not quickly settle or separate into two phases. Alginate interacts with brown rice milk dispersed in the liquid phase so that the milk is kept awake.

3.5. Texture of Alginate

Figure 5. Texture of brown rice milk with alginate addition 0.05 % alginate (a); 0.1 % alginate (b) and 0.2 % alginate (c).

Analysis of milk texture was performed under a microscope using 400 times magnification. Brown rice milk with 0.2 % alginate addition had a denser particle constituent and clearer particle transparency properties compared to other treatments. Particles of brown rice milk with the addition of alginate formed an emulsion. This is because the effect of the addition of alginate is a process of incorporating hydrocolloid polymers or polymer chain interactions resulting in a larger and more stable aggregate polymer. When mixed with brown rice milk, the alginate polymer will form a surface-active compound that converts the suspension into a smaller emulsion resulting in a softer milk texture.
3.6. Analysis nutrition of brown rice milk

3.6.1. Analysis of carbohydrate in brown rice milk

Table 1. Carbohydrate content in brown rice milk.

| Treatments       | Carbohydrate Content (mg·mL<sup>-1</sup>) |
|------------------|-------------------------------------------|
| Milk + 0.05% Alginate | 38.00 ± 0.034 × 10<sup>4</sup>          |
| Milk + 0.1% Alginate  | 38.97 ± 0.042 × 10<sup>4</sup>          |
| Milk + 0.2% Alginate  | 40.21 ± 0.048 × 10<sup>4</sup>          |

The composition of carbohydrate in brown rice milk with the addition of alginate was analyzed using Luff Schroll method. Addition of 0.2% alginate had the highest carbohydrate content (40.21 mg·mL<sup>-1</sup>) (table 1). The carbohydrate content of brown rice milk in this study was higher than commercial brown rice milk (without the addition of alginate) which contained carbohydrate of 38.3 ± 0.047 × 10<sup>4</sup> mg·mL<sup>-1</sup>. Increased levels of carbohydrates were influenced by the addition of alginate in milk because alginate was a carbohydrate polysaccharide.

3.6.2. Analysis of protein composition in brown rice milk

Table 2. Protein content in brown rice milk.

| Treatments       | Protein content (mg·mL<sup>-1</sup>) |
|------------------|--------------------------------------|
| Milk + 0.05% Alginate | 1.01 ± 0.045 × 10<sup>5</sup>        |
| Milk + 0.1% Alginate  | 1.20 ± 0.033 × 10<sup>5</sup>        |
| Milk + 0.2% Alginate  | 1.30 ± 0.027 × 10<sup>5</sup>        |

Determination of protein content was done by the Kjeldahl method. This method principally determined the total nitrogen contained in the sample. The addition of alginate in the processing of brown rice milk affected the amount of protein in milk. This is shown by the increasing amount of protein in milk with the addition of higher concentration of alginate (table 2). The increase of protein content in brown rice milk was influenced by the interaction that occurs between alginate monomers through hydrogen bonds. The presence of these hydrogen bonds trapped and bound the protein contained in brown rice milk. The protein content of brown rice milk in this study was higher than commercial brown rice milk (without the addition of alginate) which contained protein of 0.97 ± 0.033 × 10<sup>5</sup> mg·mL<sup>-1</sup>.

3.6.3. Analysis of fat composition in brown rice milk. Fat content in brown rice milk was analyzed by gravimetric method. Table 3 shows that the fat content in brown rice milk increased as the addition of alginate into milk. The highest fat content was in the addition of 0.2% alginate with a fat content of 0.158 mg·mL<sup>-1</sup>. 

| Treatments       | Fat content (mg·mL<sup>-1</sup>) |
|------------------|----------------------------------|
| Milk + 0.05% Alginate | 0.15 ± 0.045 × 10<sup>5</sup>    |
| Milk + 0.1% Alginate  | 0.19 ± 0.033 × 10<sup>5</sup>    |
| Milk + 0.2% Alginate  | 0.21 ± 0.027 × 10<sup>5</sup>    |
Table 3. Fat content in brown rice milk.

| Treatments               | Fat content (mg·mL⁻¹) |
|--------------------------|------------------------|
| Milk + 0.05 % Alginate   | 0.124 ± 0.022 × 10⁻⁴   |
| Milk + 0.1 % Alginate    | 0.128 ± 0.035 × 10⁻⁴   |
| Milk + 0.2 % Alginate    | 0.158 ± 0.038 × 10⁻⁴   |

Alginate can increase the dissolved nutrient content of brown rice milk. Alginate forms a hydrogen bond that is a carboxyl group with a hydroxyl group of glycerol in an edible film. The existence of this interaction causes an esterification reaction in the process of fat hydrolysis causing the molecule is nonpolar which can be extracted by nonpolar solvent also in hexane. So the fat content produced from brown rice milk with the addition of alginate is increasing. The fat content of brown rice milk in this study was higher than commercial brown rice milk (without the addition of alginate) which contained fat of 0.97 ± 0.033 × 10⁻³ mg·mL⁻¹.

3.6.4. Analysis of total food fibers in brown rice milk. Dietary fiber is a component of plant tissue that is resistant to the hydrolysis process by enzymes in the stomach and small intestine [10]. Fiber is the main component of cell walls in various vegetables and fruits. Chemically the cell wall consists of several types of carbohydrates such as cellulose, hemicelluloses and pectin. Fiber is a polysaccharide that linked to the β-glucoside bond that will not be degraded by α-amylase and also stomach acid. The content of fiber in brown rice milk with the addition of alginate is presented in table 4.

Table 4. Total food fiber content in brown milk.

| Treatments               | Mass of total fiber/mL milk | Mass of Crude fiber | Mass of Food fiber |
|--------------------------|----------------------------|---------------------|-------------------|
| Milk + 0.05 % Alginate   | 13.5 mg                    | 7.8 mg              | 5.7 mg            |
| Milk + 0.1 % Alginate    | 13.6 mg                    | 6.7 mg              | 6.9 mg            |
| Milk + 0.2 % Alginate    | 18.9 mg                    | 5.8 mg              | 13.1 mg           |

Table 4 shows that the highest fiber content was in brown rice milk with the addition of 0.2 % alginate. The addition of alginate increased the total fiber content in milk. This indicates that the fibers digested by human digestive enzymes are more numerous and can be used as energy sources.

4. Conclusion

Sargassum binderi was collected from Sayang Heulang Beach, Pameungpeuk, Garut, West Java with a yield of alginate of 6.25 %. The characteristics peaks of alginate appeared at 3,477; 1,633 and 1,419 cm⁻¹, corresponding to hydroxyl (OH), carbonyl (C=O) and carboxyl (COOH), respectively. The best composition of alginate addition in brown rice milk was 0.2 % (w/v) in 100 mL of the brown rice milk.

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