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INCREASE OF STABILITY SHELF LIFE AND KINETICS STUDY OF TYPE 1 BROWN RICE MILK THROUGH ADDITION OF ALGINATE EXTRACT FROM *Sargassum Binderi*

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ABSTRACT

Brown rice milk is milk from brown rice. It is used lactose intolerance and low fat milk. This research aimed to use the sodium alginate extracted from *Sargassum binderi* as an stabilitazion and degradation of brown rice milk. *Sargassum binderi* seaweed taken from Sayang Heulang beach, Pameungpeuk Garut, West Java. The purpose of this research is increase the stability of milk and extend shelf life of brown rice milk. The method used was extraction alginate from *Sargassum binderi* and analysis of brown rice milk. The analysis were dye degradation, deposition rate and shelf life of brown rice milk. Result of this research was constant pH value of the composition of brown rice milk from various variations of the addition alginate until the 15 days to various compositions of adding alginate to milk. Then, the sedimentation rate of the effect addition alginate was in order 1. The addition of 1.2% alginate had the lowest deposition rate constant at 0,0171. The constant rate of deposition brown rice milk samples with the addition of 1.2% alginate which is 0.0022 was the best milk sample because it successfully suppressed the degradation rate of dyes up to 95.79% compared to those without the addition alginate. The number of microbes that appear in milk samples did not exceeded the maximum limit of microbial contamination.

Keywords: Brown rice milk, *Sargassum binderi,* alginate, stability

INTRODUCTION

Milk is something we all know has a very important place in our daily diet (Gomez et al., 2009). This gives us the essential calcium needed for growth and maintenance of the body. Many people need nutrition in their body to supply energy and nutrition (Battaglini et al., 2009). Nutritional benefit in combination with acceptability in food products is a significant opportunity for food industry (Molkentin et al., 2013). People have started to drink vegetable milk instead of cow's milk.

The reasons also variety, from trying to be a vegetarian, because they have lactose intolerance, to looking for lower fat milk choices. One of the vegetable substitutes for cow's milk which is now widely consumed is brown rice milk (Moongngarm et al., 2010).

Self-made brown rice milk contains almost no trans fat and cholesterol. In some finished products, fat and cholesterol levels may be present as a by product of the production process as well as flavorings and or added sugar. That is, the content of trans fat from rice milk is the least compared to all vegetable milk substitutes for this cow's milk. Therefore, good brown rice milk is consumed for people who want a diet low in cholesterol and low in fat (Durand et al., 2003). Because it is low in fat and cholesterol, drinking vegetable milk can also nourish the heart. Moreover, the magnesium content in rice milk is also efficacious for controlling blood pressure.

Brown rice milk is a substitute for cow milk which is very good and healthy (Alvarez et al., 2005). The benefits of brown rice for the body include rice milk extracted directly from rice grains, it is lactose-free milk as opposed to cow's milk. It really helps people who are lactose intolerant. Secondly, brown rice milk contains higher levels of carbohydrate than cow's milk. Third, the level of cholesterol in rice milk is zero. This makes it very healthy, especially for people with heart conditions. Fourth, brown rice milk is also very low in fat, so everyone who maintains weight can drink it without worry (Prado et al., 2008).

In this study brown rice milk combined with alginate from chocolate algae (Murdinah et al., 2009). The brown algae used is *Sargassum binderi.* One of the potential biological resources from Indonesian marine waters is seaweed with various types (Shirosaki et al., 2011). Seaweed is part of aquatic plants, which are included in the macro class of algae (Aseer et al., 2009). Alginate is a linear copolymer consisting of two monomeric units, namely D-mannuronic acid and L-guloronic acid (Vauchel et al., 2008).

The ratio of the monomers of alginate is important in relation to its bioactive properties and the structural properties of the gel (Siew-Ling et al., 2011). This research aimed to use the sodium alginate extracted from *Sargassum binderi* as an stabilitazion and degradation of brown rice milk. In this research, a kinetic analysis was carried out to determine the level of milk stability.

MATERIALS AND METHODS

Tools and Materials

The research materials is used brown rice from supermarkets. The brown rice was included in the cigeulis variety which resistant to WCK biotype 2,3 HDB strain IV. Brown rice is planted in lowland rice fields (less than 500 m above sea level). *Sargassum binderi* seaweed taken from Sayang Heulang beach, Pameungpeuk Garut, West Java. In this research, reagents was $CaCl_2$, aquabides, formaldehyde, EDTA, Na_2CO_3 and chloroform.

Method

Extraction Alginate from *Sargassum binderi*

Two grams of samples was immersed in a 2% $CaCl_2$ (w/v) solution for 2 hours, then washed with aquabides. The sample was soaked in a formaldehyde solution for 2 hours and washed again with aquabides 3 times. The extraction process was carried out by adding 3% (w/v) 1 M Na_2CO_3 and 0.5 gram EDTA at pH 11. Then it was filtered a muslin cloth and was precipitated ethanol as sodium salt. The precipitate was separated by centrifugation and dried in an oven at 60^{o}C (Latifi et al., 2009).

Analysis of Brown Rice Milk Dye Degradation

One mL of sample is inserted into 2 mL microtube. Then it is added 1 mL chloroform and is stirred by vortex. The sample is centrifuged at a speed of 12,000 rpm for 30 minutes so that it will separate into two phases. The top phase is decanted and absorbance measurements are made at λ = 241 nm based on the maximum wavelength scan in the range of 200-400 nm.

Analysis of Brown Rice Milk Deposition Rate

One mL of sample is put into a measuring flask. Then it is demarcated to 10 mL by aquades. Then tranmitan measurements are carried out at a wavelength of 656 nm. The transmittal results are converted into turbidity values with the equation below.

$$S = -\log\left(\frac{T}{100}\right) \quad (1)$$

Information : S = Turbidans
T = Transmitans

Self Life of Brown Rice Milk

The pH value is measured by a pH meter. Samples have been pasteurized and packaged in vial bottles of 10 mL, each pH value is measured on day 0 to day 15 using a pH meter. Before being used the pH meter was calibrated using a buffer solution of pH 4, 7 and 10.

RESULTS AND DISCUSSION

Production of Brown Rice Milk

Brown rice milk is milk obtained from rice. This is obtained by processing the rice through processing. For this process, the most preferred rice is brown rice. Brown rice goes through several long processes, after the final product of rice milk and husk is separated. In order to make rice milk, brown rice is crushed and milk is extracted. Brown rice milk in this study is SBM Type I. Brown rice milk comes from direct brown rice which is processed into milk comparison of the composition used in making brown rice milk between brown rice/brown rice with water is 1:15 (w/v). The following is the result of processed brown rice milk, SBM Type I.

Figure 1. Type of I brown rice milk

Physically SBM type I milk has a physical appearance red color. The red color produced from the milk comes from aleurons that contain genes that produce anthocyanins (red pigment pigments that act as antioxidants).

Sargassum binderi Seaweed Samples

Seaweed is the main ingredient that used in this study. Seaweed was obtained from Sayang Heulang beach, Pameungpeuk District, Garut, West Java. The beach is one of the coral beaches in the southern region of Java. Seaweed in this research is *Sargassum binderi* which grows naturally, without cultivation process. Seaweed samples were taken in February 2016 during low tide, where the depth of the sea water was only 10-30 cm. The coordinates of the sampling point are 7^{o} 40 '12 "LS and 107^{o} 41" 37 "BT which are located around the main gate of Sayang Heulang Beach.

Sargassum binderi samples are found at a distance of 30-70m from the shoreline. *Sargassum binderi* seaweed is chosen still alive, fresh and brightly colored. The sample is separated from impurities such as sand, stone or pieces of coral, then packaged in a plastic filled with water. The following is a sample of *Sargassum binderi* seaweed which is the result of sampling at Pantai Sayang Heulang.

From the Figure 2, it can be seen that *Sargassum binderi* has the characteristics of a flat thallus (±1.5 cm), smooth/slippery, reaching a height of about 60 cm. The alternate branching is regular, opposite (left right), the main branches close together, arising on the short main stem (± 1-2 cm) above holdfast. Oval leaf, serrated edge, length=5 cm, width=1 cm, sharp tip. Round bladder, rounded or pointed tip, winged 1 cm long, 0.4 cm in diameter.

Figure 2. Photo sample of *Sargassum binderi* from Sayangheulang Beach, Garut, West Java

Reproductive organs form special stalks, slashing, flattened, jagged. The leaves and bladder are shrink. It's habitat grows on rock sytrates, generally in flat reef areas, near the outer edges that are affected by relatively strong and constant water movements (DG of Fisheries and Aquaculture, 2009).

Alginate from *Sargassum binderi*

Sodium alginate in this study is yellowish brown. Brownish color is the result of reactions from the presence of phenolic compounds that are still contained in alginate. Density and viscosity of sodium alginate with a concentration of 0.1% (w/v) are 1.01 g/mL and 7.65×10^{-3} kg.m^{-1}s^{-1}. Sodium alginate water content is 12.2% (w/w).

Figure 3. Na-alginate from *Sargassum binderi* (100 mesh)

Sodium alginate water content is in the range of 5-20%, still in the range of water content obtained in sodium alginate (Mushollaeni, 2011). While the amount of sodium alginate water content determined by the Food Chemical Codex (1981) is a maximum of 15% and the maximum sodium alginate water content for food ingredients is 13% (Cottrell and Kovacs, 1977). Next is the structure of sodium alginate.

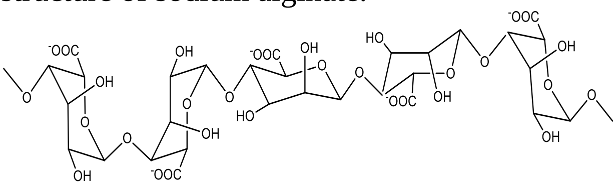

Figure 4. Structure of sodium alginate (Draget, 2000)

In the spectrum of figure can be seen the absorption peaks of alginates that have been characterized by FTIR. The presence of peaks in the area of about 3,500-3,200 cm^{-1} indicates the presence of hydroxyl groups (O-H) that bind to hydrogen. Wave numbers 1,680-1,600 cm^{-1} indicate the presence of a carbonyl group (C=O) as an aromatic group, 1,500-1,100 cm^{-1} indicates the presence of a carboxyl group (COOH).

Sodium in the alginate isomer is located at the peak of absorption of 1,523 cm^{-1}. Absorption peaks of 900-890 cm^{-1} indicate typical areas of guluronate fingerprints, while 850-810 cm^{-1} indicate typical areas of fingerprints of mannuronates. The presence of a typical fingerprint area of guluronate and mannuronate is a marker that the sample under study is an alginate compound.

Table 1. FTIR Sodium Alginate

Wave number (cm^{-1})	Interpretation of functional groups
3.310	Hydroxyl group (OH)
1.625	Carbonyl group (C=O)
1.216	Carboxyl group (COOH)
1.523	Na in alginate

Effect of Addition of Alginate to the pH Brown Rice Milk

Milk has nutritional content and contains various benefits that are needed by the body to be carried out storage that can maintain the conditions and nutrients present in milk. Milk storage capacity or known as shelf life can be defined as the time of production and packaging of a product with the point of time at which the product becomes fit for consumption. According to Sumaprastowo (2000) storability is always expressed by the environmental conditions that are used to store a material, whether food, drink, or other objects. A good storage is a system that can be regulated in conditions such as room temperature so that it can inhibit the growth of microbes in food and drinks.

The effect of adding alginate to brown rice milk was also seen on the pH value in the composition of brown rice. The following is the pH value of brown rice milk with various variations in the composition of the addition of alginate in milk with a storage temperature of 4°C. An analysis of the effect addition of alginate on the pH was carried out at room temperature. The following is the pH data of the brown rice milk.

Based on figure 6, it appears the pH value of the composition of brown rice milk from various variations of the addition alginate is constant until the 15 days to various compositions of adding alginate to milk. In the variation of the addition of 1.0% and 1.2% alginate has the best pH and managed to maintain the stability of milk at a neutral pH that is at pH 7.00. The highest pH is the addition of 0.4% alginate in the composition of brown rice milk which is at a pH of 7.04. The addition of 0.8% alginate in the composition of brown rice milk also has a pH value that manages to maintain the quality of milk at pH 7.01.

The decrease in pH of brown rice milk during storage is caused by the acid production that is increased of producing bacteria from day to day. During storage is profitable which will improve product quality to opportunities for microorganisms to move mainly bacteria. The stability of the pH value is influenced by the presence of edible film factors that found in brown rice milk.

Edible film functions is a barrier against mass transfer (oxygen, lipids and other solutes). The fatty acids is contained in the sample fully oxidized because which are blocked by edible film so the acidification process is not optimal.

Effect of Addition Alginate to the Stability Brown Rice Milk

The effect of alginate enhancers into brown rice milk was carried out by measuring the turbidan value of brown rice milk by using a UV-vis spectrophotometer at λ = 241 nm. Sedimentation rate of brown rice milk with various variations of alginate concentration was tested by kinetics using the integral method in order 0, 1 and 2. Based on the turbid value obtained then graphed the value of the turbidan on the y axis with respect to time (t) on the x axis. Of the three types of graphs obtained the highest R^2 value is in order 1.

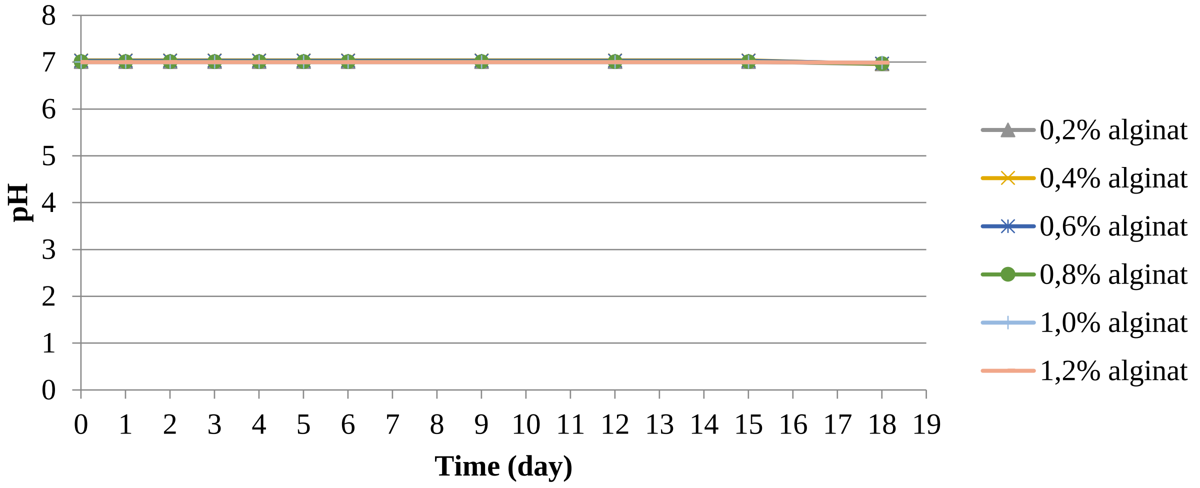

Figure 5. Effect of variations in the addition alginate on the pH with a storage temperature of 4°C

The sedimentation rate of the effect addition alginate is in order 1. This shows that alginate is able to increase the stability of brown rice milk so that the brown rice milk produced does not quickly settle or separate into 2 phases.

Alginate interacts with brown rice which is dispersed in the liquid phase so that the brown rice milk is maintained its stability. Based on the plot of the graph Ln S to get the constant rate of deposition of each milk sample.

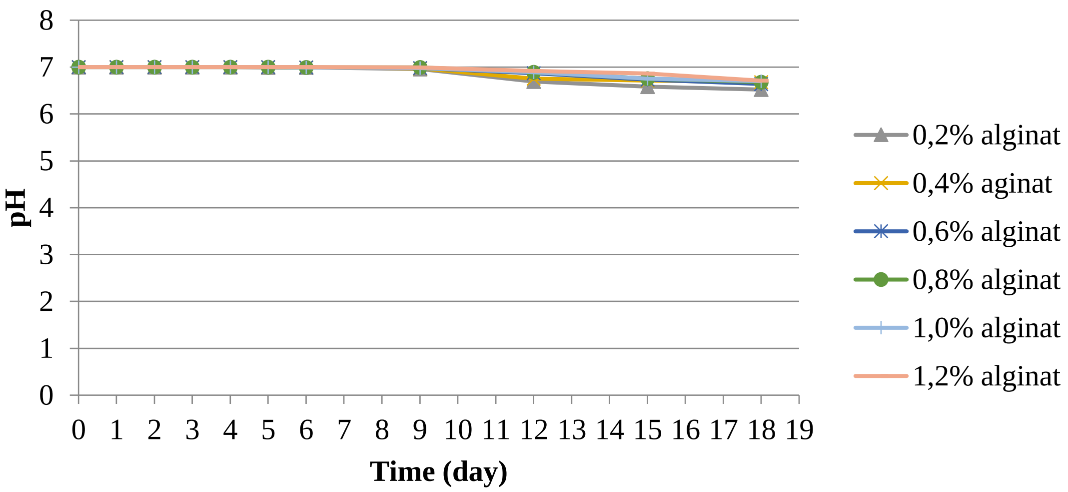

Figure 6. Effect of variations in the addition alginate at room temperature storage

Based on the Table 2, it can be seen that the highest rate of deposition constant is in the addition alginate with a concentration of 0.05%. Whereas the addition of 1.2% alginate has the lowest deposition rate constant. This shows that the higher the concentration of alginate added to milk, the stability of brown rice milk is maintained. From the data half-life of milk shows that brown rice milk has the highest stability that is 40 days 12 hours. The stability of brown rice milk with the addition alginate produces more stable milk quality than without the addition of alginate.

In the emulsion system, there is a difference in the boundary field stresses between the dispersed phase and the continuous phase in unmixed soymilk. The voltage occurs between two phases is called the boundary plane voltage. The higher voltage differences that occur in the boundary plane cause the two phases to be more difficult to mix. The addition alginate can reduce the surface tension that occurs in the boundary plane so that the two phases will be easily mixed (Kurniasari and Fithri, 2010). The higher surface tension in an area will cause two different phases to be difficult to mix (stable) due to the formation of new surfaces.

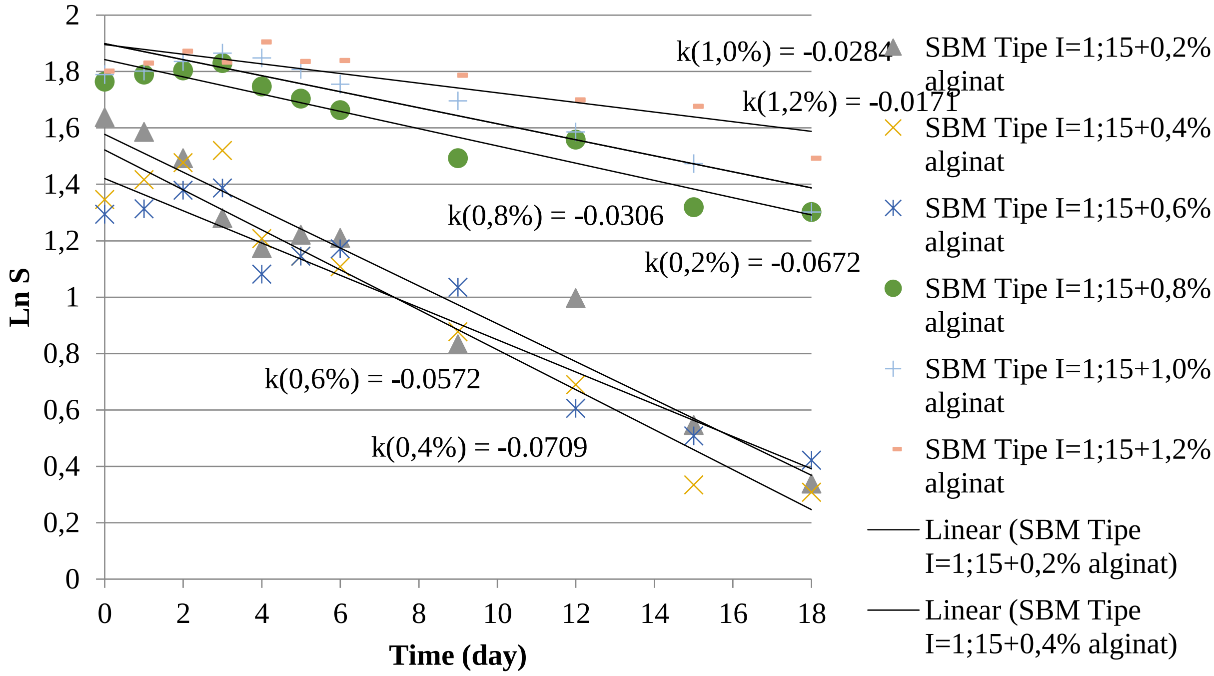

Figure 7. Plot graphic variations of the addition alginate to the stability of brown rice milk in order-1

Table 2. Rate and the half-life of the effect of addition alginate to the rate of precipitation of milk

Types of Milk	k (days^{-1})	Half time
SBM tipe I = 1:15+0,2% alginate	0,0672	10 days 7 hours
SBM tipe I = 1:15+0,4% alginate	0,0709	9 days 16 hours
SBM tipe I = 1:15+0,6% alginate	0,0572	12 days 2 hours
SBM tipe I = 1:15+0,8% alginate	0,0306	22 days 14 hours
SBM tipe I = 1:15+1,0% alginate	0,0284	24 days 19 hours
SBM tipe I = 1:15+1,2% alginate	0,0171	40 days 12 hours

Degradation Analysis of Brown Rice Milk Dyes

Determination of the maximum wavelength of brown rice milk was carried out at a wavelength scan (λ) at 400-700 nm. The following is a wavelength scan of a sample brown rice milk.

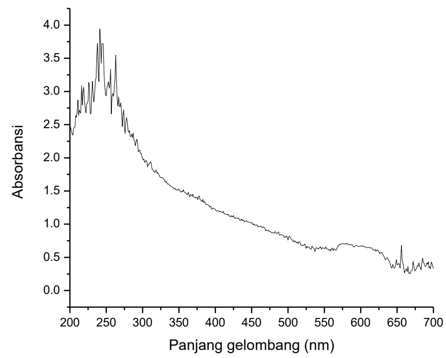

Figure 8. Wavelength of brown rice milk

From the figure 8, it appears that the maximum wavelength obtained from brown rice milk at wavelength 656 nm with an absorbance of 0.48. λ at 656 nm shows the anthocyanin pigment content contained in brown rice milk. The anthocyanin pigment is in the range of wave length 510-700 nm. Anthocyanins are a class of flavonoid compounds, which are the largest group of natural pigments in red, water-soluble browns which are responsible for giving red rice a red color.

Dyestuff degradation was analyzed by measuring the absorbance value of the milk by UV-vis spectrophotometer at 656 nm. Kinetic analysis to determine the rate of degradation dyes from brown rice milk by using the kinetic analysis of the integral method based on the law of the rate equations in order 0.1 and 2. The rate equations are used:

Orde 0: $A - A_0 = -kt$ (2)

Orde 1: $\ln A - \ln A_0 = -kt$ (3)

Orde 2: $\frac{1}{A} - \frac{1}{A_0} = kt$ (4)

Information : A = sample absorbance
k = rate constant
t = time

Kinetic analysis of the dyes degradation rate on brown rice milk samples with the addition alginate. Based on the results of the reaction kinetics, the degradation rate of brown rice milk dyes is in order 0. This shows that alginate can maintains the stability and resistance of brown rice milk. This means that the addition alginate does not affect the degradation of milk sample dyes or inhibit the rate precipitation of brown rice milk as shown below.

Based on the constant rate of deposition brown rice milk samples with the addition of 1.2% alginate which is 0.0022 is the best milk sample because it successfully suppresses the degradation rate of dyes up to 95.79% compared to those without the addition alginate.

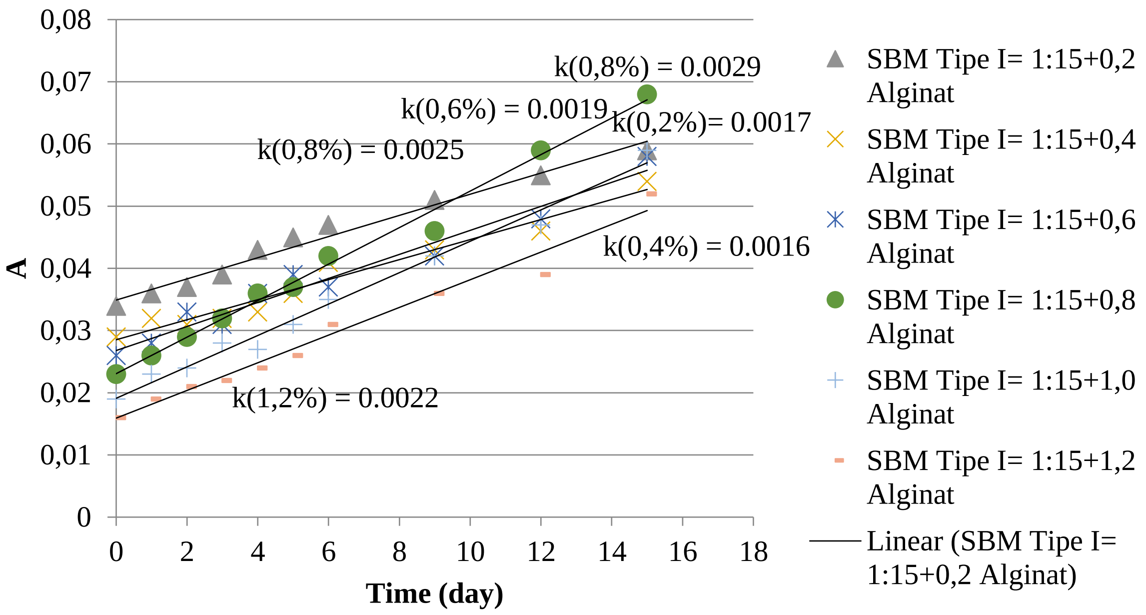

Figure 9. Plot graph degradation rate kinetic of brown rice milk dyes with the addition of alginate in order-0

Table 3. The constanta of the degradation rate of milk dyes and the half-life of the effect of addition alginate to the precipitation rate of milk

Types of Milk	k (Abs/days^{-1})	Half time
SBM tipe I = 1:15+0,2% alginate	0,0017	7 days
SBM tipe I = 1:15+0,4% alginate	0,0016	9 days 1 hours
SBM tipe I = 1:15+0,6% alginate	0,0019	6 days 19 hours
SBM tipe I = 1:15+0,8% alginate	0,0029	3 days 21 hours
SBM tipe I = 1:15+1,0% alginate	0,0025	3 days 19 hours
SBM tipe I = 1:15+1,2% alginate	0,0022	3 days 14 hours

Based on showing that the degradation rate of milk dyes has decreased to order 0. This shows that alginate is able to maintain the stability of the dyes of brown rice milk. Then determined the constant rate of deposition of milk and half-life of brown rice milk.

From the table 3 shows that the deposition rate of milk is in order 0, where this shows that alginate can inhibit the degradation of brown rice milk dyes. The half-life data shows that the most stable brown rice milk is the addition of 1.2% (w/v) alginate with a half-life of 3 days 14 hours.

Analysis of Microbial Number of Brown Rice Milk by Addition Alginate

Microbial analysis of brown rice milk was carried out by a dilution method. The medium used is LB medium with incubation temperature of 4°C and room temperature. Brown rice milk in the image incubated at 4°C did not show any microbes that appeared. Then the milk was observations from day to day on the number of microbes that appear in milk. From observations up to the 15th day milk is still in a safe condition and free from microbial contamination. But milk incubated at room temperature appears as microbes appear.

The table 4 in below shows the presence of microbial activity during storage at room temperature. The number of bacteria produced increases from day to day. But from the data obtained the number of microbes that appear in milk samples has not exceeded the maximum limit of microbial contamination as required by SNI 7388 (2009). Where the maximum amount of microbial contamination in milk samples is $5x10^4$ cfu/mL. This shows that milk with storage at room temperature has a resistance of up to 3 days.

Table 4. Analysis of the number of incubation microbes at room temperature

Sample type	The number of microbes (cfu/mL)		Average number of microbes (cfu/mL)
SBM Tipe I = 1:15	$10,2 \times 10^2$	$10,3 \times 10^2$	$10,25 \times 10^2$
SBM Tipe I = 1:15 + 0,2% alginate	$9,4 \times 10^2$	$9,4 \times 10^2$	$9,40 \times 10^2$
SBM Tipe I = 1:15 + 0,4% alginate	$9,6 \times 10^2$	$9,5 \times 10^2$	$9,55 \times 10^2$
SBM Tipe I = 1:15 + 0,6% alginate	$7,2 \times 10^2$	$7,3 \times 10^2$	$7, 25 \times 10^2$
SBM Tipe I = 1:15 + 0,8% alginate	$6,8 \times 10^2$	$6,4 \times 10^2$	$8,6 \times 10^2$
SBM Tipe I = 1:15 + 1,0% alginate	$6,1 \times 10^2$	$6,2 \times 10^2$	$6,15 \times 10^2$
SBM Tipe I = 1:15 + 1,2% alginate	$4,3 \times 10^2$	$4,4 \times 10^2$	$4,35 \times 10^2$

CONCLUSION

Sargassum binderi as alginate can increase the stability of brown rice milk. Besides being able to extend milk endurance and prevent acidification at the milk pH. The number of microbes that appear in milk samples has not exceeded the maximum limit of microbial contamination as required by SNI 7388.

REFERENCES

Alvarez, V.B., Wolters C.L., Yodovotz Y., and Ji T. 2005. Physical properties of containing milk protein concentrates. Journal of Dairy Science. Vol. 88: 862-871.

Aseer M., Sugathan S., George S.K., Joseph S., Chippu S., Gandhimathi R. and Nataraja Panikkar M.V. 2009. Bio potentials of seaweeds collected from southwest coast of India. Journal of Marine Science and Technology.Vol. 17 : 67-73.

Battaglini L., Renna M., Garda A., Lussiana C., Malfatto V., Mimosi A., Bianchi M. 2009. Comparing milk yield, chemical properties and somatic cell count from organic and conventional mountain farming systems. Ital. J. Anim. Sci., Vol. 8 :384-386.

Durand A., Franks G., and Hosken R. 2003. Particle size and stability of UHT bovine, ceral and grain milks. J. Food Hydrocll. Vol 17 : 671-678.

Draget, K.I. (2000): .Alginates, Book of Hidroclloids, 379-395.

Gomez C .G., Lambrecht M.V.P., Lozano J.E., Rinaudo M., and Villar M.A. 2009. Int. J. Bio. Macromal. Vol. 44 : 365–371.

Latifi A.M., Ehsan S.N., and Hamid B. 2015. Comparison of extraction different methods of sodium alginate from brown alga Sargassum sp. localized in the Southern of Iran. Journal of Applied Biotechnology Reports. Vol. 2 : 251–255.

Molkentin J. 2013. Applicability of organic milk indicators to the authentication of processed products. J. Food Chem.Vol. 137:25-30.

Moongngarm, A., and Saetung N. 2010. Comparison of chemical compositions and bioactive com-pounds of germinated rough rice and brown rice. J Food Chem. Vol. 122: 782-788.

Murdinah F., Ghifar S.Y., Cholid. 2009. Processing of edible coating made of alginate using CaCl2 and Ca-lactete as gelling agents. Journal of Marine and Fisheries Postharvest and Biotechnology. Vol. 7 : 59-65.

Prado, F.C, Parada J.L., Pandey A., and Soccol C.R., 2008. Trends in non-dairy probiotic beverages. J Food Research International. Vol. 41: 111-123.

Shirosaki., Miyuki., Koyama., Tomoyuki. 2011. Laminaria japonica as a food for prevention ofobesity and diabetes. J. Adv. Food Nutr. Vol. 64 : 199-212.

Siew-Ling H., Lesley-Lean E., and Wong C.L. 2011. Adsorption of reactive blue 4 onto the chemically modified red seaweed Amphiroa foliacea: Equilibrium, kinetics and modeling studies. International Journal of the Physical Sciences.Vol. 6 : 7171-7182.

Vauchel P., Kaas R., Arhaliass A., Baron R. and Legrand J. 2008. A new process for extracting alginates from Laminaria digitatareactive extrusion J. Food and Bioprocess Technology. Vol. 1 : 297-300.