International Journal of Environment, Agriculture and Biotechnology
Vol-6, Issue-5; Sep-Oct, 2021

Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Effect of Concentration of *Sargassum polycystum* and Fermentation Time on thickness and Yield of Nata de Sargassum

Dewi Utami[1], Kasmiati[2], Metusalach[2*]

[1]Fisheries Science Study Program, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia
[2]Department of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia
*Corresponding author

Received: 10 Sep 2021; Received in revised form: 14 Oct 2021; Accepted: 21 Oct 2021; Available online: 31 Oct 2021

Abstract— *Sargassum polycystum is one type of brown seaweeds that is abundant in Indonesian waters, including in Bone Bay, South Sulawesi. S. polycystum is an underutilized species and considered by coastal communities as trash that polluting the sea. Nevertheless, it contains carbohydrates which are essential in the nata making process. This research aimed to determine the concentration of S. polycystum and the fermentation time that produce the best thickness and yield of nata de sargassum. Th research was conducted at the laboratory of Fisheries High School (SUPM) Bone, South Sulawesi. Sample of S.polycyctum was collected from Tanjung Palette's water, Bone Bay. A completely randomized factorial design was used with 2 factors,namely concentration of S.polycystum and fermentation time, ang each factor consisted of four levels. The concentrations used were 2, 3, 4 and 5% (w/v) and the fermentation time were 7, 10, 13 and 16 days. Each treatment was carried out in three replicates. Results showed that the concentration of S. polycystum and fermentation time imparted a significant effect ($p<0.05$) on the thickness and yield of the nata de Sargassum. The thickness (5.6 mm) nata produced was at a concentration of 3% and a fermentation time of 16 days. While the largest yield (32.2%) was produced at a concentration of 5% with a fermentation time of 16 days.*

Keywords—***Brown algae, nata de sargassum, underutilized, Acetobacter xylinum, carbohydrates.***

I. INTRODUCTION

Sargassum polycystum is one of the abundant brown seaweeds in Indonesian waters, including Bone Bay, but so far is still underutilized. Although many studies on its contents and benefits, the existence of *S. polycystum* tended to be ignored and considered as trash that pollutes the ocean[1],[2], Dried *S. polycystum* contains 17.69% water, 24.51% ash, 0.50% fat, 3.65% protein, 53.66% carbohydrates, 3.81% crude fibre, and minerals such as magnesium (Mg) 89.9 mg/g, iron (Fe) 0.50 mg/g,potassium (K) 26.9 mg/g, sodium (Na) 22.23 mg/g andcalcium (Ca)18.06 mg/g [3]. According to [4],[5],materials containing high carbohydrate are good source of nata production. With over 50% carbohydrate content, *S. polycystum* may therefore be a potential material for the production of nata de seaweed.

Nata de seaweed is an alternative product processed from seaweeds that is well known and favoured by many communities, especially Japanese. Nata was first produced in the Philippines[6] from coconut water, and the product is worldwide known as *nata de coco*. Nata is a layer of extracellular polysaccharide obtained through fermentation using *Acetobacter xylinum*. Nata has a chewy texture, white gel-like appearance and floats on the surface of aqueous solution containing sugar and acid [7],[8]. Nata is essentially a mass of cellulose formed through polymerization of glucose by *A. xylinum* during fermentation process.

The key factors influencing the success of nata making are substrate concentration and fermentation time.

[9]studied the production of nata de seaweed using *Kappaphycusalvarezii* and reported that the optimum concentration of *K.alvarezii* was 2% with a fermentation period of 16 days. The longer time of fermentation the thicker the nata, but too long fermentation time affects the quality of the nata[4],[5].

The common indicator used in evaluating the success of the nata making is by measuring the thickness and yield of the nata produced. Both the thickness and the yield are indicators of how effective and efficient are the conversion of sugar (glucose) into cellulose (nata). Therefore, this paper describes characteristic of the thickness and yield of the nata de Sargassum produced at different concentration and fermentation time.It may be worth to note that this is the first study to use brown seaweed *Sargassum* as a substrate for the production of nata de seaweed.

II. MATERIALS AND METHODS

2.1. Materials and Equipment

Materials used to make nata de Sargassumwere *S. polycystum*obtained from the waters of Tanjung Palette, Bone Bay South Sulawesi, distilled water, vinegar, sugar, *Acetobacter xylinum*, ammonium sulphate, magnesium sulphate.Equipment used were plastic jar, measuring cup, stainless sieve, stove, stainless steel pan, wooden stirrer, stainless steel knive, blender, balance, newsprint, rubberbands.

2.2 Procedures of nata de Sargassum making

2.2.1.Equipmentand *S. polycystum*preparation

Equipment used, except balance and rubber bands, must beclean and sterile[10],[11].All the equipment were washed with soap, thoroughly rinsed under running tap water, dried, and then sprayed with 70% ethanol solution. *S. polycystum* was also thoroughly cleaned and washed to remove mud and other undesired materials attached to the thallus, and if necessary soaked in fresh water. Upon drained, the seaweed sample was then chopped to reduce its size to 1-2 cm long.

2.2.2. Preparation of *A. xylinum*starter

The pre-chopped *S. polycystum* was homogenized in the distilled water using a commercial house blenderat a ratio of 1:50 (w/v). The homogenized sample was then filtered through a stainless steel sieve to separate the solid and the filtrate. Furthermore, the filtrate was boiled in a boiling pan, and then sugar(10%, w/v), vinegar (1%, v/v),ammonium sulphate(0.5%, w/v), magnesium sulphate(0.5%, w/v) of the filtrate volume were added. After boiling, the solution (substrate)waspoured into a bottle, close tightly and let the solution to stand at room temperature for about 8 hours until cool.Then, 100 ml of *A. xylinum* stock was added into 1000 ml of substrate. The substrate was allowed toferment until cellulose layer was formed (about 6 days). This fermented substrate, also known as starter, was then used in the natade Sargassum making process.

2.2.3. Preparation of nata de Sargassum substrate

Fresh *S. polycystum* was washed thoroughly until the fishy smell disappeared.After draining the seaweed was homogenized in distilled water using a commercial homogenizer with the ratio of water:seaweedof 1,000:20, 1,000:30, 1,000:40, and1,000:50. Then the homogenized seaweed samples were filtered over a stainless sieve andthe filtrates were poured into separate boiling pans. Further, 10% (w/v) ofsucrose, 0.5% (v/v) of vinegar, 0.5% (w/v) of ammonium sulphate and 0.5% (w/v) of magnesium sulphate, all by volume of the filtrate, were added into each boiling pan.The mixtures were stirredevenly and then boiled.

2.2.4. Fermentation process of nata de Sargassum

Separately, as much as 500 ml of the boiled filtrates (substrate)of each treatment ratio were immediately transferred into a pre-assigned sterilized fermentation containers, covered with newsprint, and then tied with rubber bands. Allow the substrate to cool and let to stand for approximately 8 hours. Then, by opening the newsprint coverslightly, 50 ml of *A. xylinum starter solution* were poured into each of the fermentation containers without stirring. The newsprint cover was then immediately reclosed to prevent contamination. Process was done at room temperature (27-28^{0}C) for 7, 10, 13 and 16 days.

2.2.5. Harvesting of nata de Sargassum

At the end of each of the fermentation period, the nata formed was harvested, cleaned by removing its top layer (the epidermis), washed, and then cut into desired shape. The nata was then squeezed using a calico cloth until it resembles a sheet of paper. To get rid of the sour smell, then at Awa soaked in fresh water for 3 days.

2.3 Parameter measurement

2.3.1. Determination of the Nata thickness

The thickness of the nata was measured using a caliper and the thickness value was expressed asthe average of five measurements.

2.3.2. Determination of thenata de Sargassum yield

After soaking in the fresh water, the nata was allowed to drain and measured for its weight. The yield was then calculated using aformula as follow:

$$\text{Yield} = \frac{\text{Weight of the nata (g)}}{\text{Weight of ingredients (g)}} \text{ X } 100\%$$

2.4. Experimental design

The experiment was carried out by employing a completely randomized factorial design with two treatments factors, namely the concentration of the *S. polycystum* and the fermenting time. The concentration used was 2, 3, 4 and 5%, whereas the fermenting time was 7, 10, 13 and 16 days. Al treatments were repeated three times.

2.5. Data Analysis

Data of the thickness and yield of the nata de Sargassum were analysed using two-way ANOVA at 95% level of confidence. Since analysis of variance (ANOVA)indicated the presence of significant difference, the analysis was proceeded with a Tukey-test to determine which treatment has the significant difference.

III. RESULTAND DISCUSSION

3.1 Nata Thickness

The thickness of the nata de Sargassum produced in this study ranged from3.2 mmto 5.6 mm, being thinnest for 3% (w/v) concentration of 7 days fermentation and thickest for the 3% (w/v) concentration with a fermentation time of 16 days (Table 1).According to Indonesian National Standard(SNI) 01-4317-1996[12], nata that meets the quality requirements must have a thickness of about 1-1.5 cm, thus the nata de Sargassum resulted in this study, werestill far below the standard thickness by the SNI.

Table-1.The thickness of the nata de Sargassum produced at differentS. polycystum substrate concentration and fermentation time.

Consentration of *S.policystum* (%)	Thickness (mm)			
	7 days	10 days	13 days	16 days
2	3.46 ± 0.04^{ax}	4.53 ± 0.12^{xy}	4.86 ± 0.09^{x}	5.06 ± 0.09^{x}
3	3.2 ± 0.16^{ax}	4.7 ± 0.08^{y}	4.7 ± 0.08^{x}	5.6 ± 0.16^{y}
4	3.26 ± 0.09^{ax}	4.66 ± 0.09^{y}	4.76 ± 0.12^{x}	5.16 ± 0.16^{x}
5	3.73 ± 0.24^{ax}	3.9 ± 0.65^{x}	4.8 ± 0^{x}	5.2 ± 0.14^{xy}

Valuesfollowed by the samesuperscriptin the same row(a,b,…)or column (x,y…) show no differenceat 95% probability level($p>0.05$).

Results of ANOVA indicated that the concentration of *S. polycystum* and fermentation time exerted significant effects on the thickness of the nata de Sargassum. The Tuckey test showed that the3% (w/v) concentration with afermentation time of 16 days produced a significantly thicker nata as compared to the other treatments, except with that of the 5% concentration of the same fermentation time. Regardless of the absence of significant difference between the 3 and 5% concentration for the 16 days of fermentation, the tendency of the higher thickness of the nata for the 3% concentration may indicate that 3% of *S. polycystum* is the best and optimum concentration for the production of the nata de Sargassum.

The smallest thickness value of the nata obtained at a concentration of 3% of*S. Polycystum* and 7 days of fermentation time was presumably due to an inadequate fermentation duration, so thatthe capacity of *A. xylinum* bacteria to form the nata layer has not reached its plateau. Meanwhile, the thickest Nata produced at 3% concentration of *S. polycystum*ata fermentation time of 16 days indicated that *A. xylynum* has reached its maximum capacity in converting carbohydrates to cellulose. Based on the above trends, it is clear that the conversion of carbohydrates(glucose)by *A. xylinum* bacteria in the fermentation substrate into celluloseis a function of fermentation duration, thus the thickness of the nata is, up to a certain time period, a fermentation time-dependent.[4],[13],[14]reported that the longer the fermentation time the thicker the nata will be.It was also noted in this study that fermentation time was the most influencing factor in the synthesis of cellulose, and therefore on the thickness of the nata de Sargassum.

The addition of carbohydrates as a carbon source must be in an appropriate amount. Excessive carbohydrates will cause the work of *A. Xylinum* bacteria become not optimal due to part of carbohydratesis converted into acid, thusdecreasing the pH of the fermentation medium[15]. Excessive carbohydrates may also cause brown colour due to Maillard reaction. On the other hand,too little carbohydrate added will affect the thickness of the nata layer produced because of the lack of carbon source forthe *A. xylinum* to form the nata layer [16].

3.2. Yield

The yield of nata de Sargassum at various concentrations of *S. polycystum* and fermentation time ranged from 27.9 to 32.2% (Table 2). The shortest fermentation time produced the lowest yield, regardless of the concentration of the substrate. The result of Anova showed that the concentration of *S. polycystum* did not impose any effect on the yield of the nata de Sargassum while the fermentation time did. Further, the Tukey test showed that the fermentation time of 7 days produced significantly lower yield of the nata de Sargassum as compared to the other fermentation times, while the fermentation times of 10, 13 and 16 days produced a similar yield of the nata de Sargassum.

Table-2. Yield of nata de Sargassum at various concentrations of S. polycystum and fermentation time.

Concentration of S. polycystum(%)	Yield (%)			
	Day 7	10 Day	13 Day	16 Day
2	$28.53 \pm 0,35^{ax}$	$30.48 \pm 0,27^{bx}$	$30.87 \pm 0,48^{bx}$	$31.58 \pm 0,12^{bx}$
3	$27.94 \pm 0,32^{ax}$	$30.73 \pm 0,26^{bx}$	$30.61 \pm 0,22^{bx}$	$32.12 \pm 0,15^{bx}$
4	$28.05 \pm 0,12^{ax}$	$30.51 \pm 0,27^{bx}$	$31.01 \pm 0,30^{bx}$	$31.77 \pm 0,16^{bx}$
5	$28.88 \pm 0,77^{ax}$	$29.33 \pm 1,24^{abx}$	$30.78 \pm 0,22^{bx}$	$32.24 \pm 0,42^{bx}$

Values followed by the same superscript in the same row (a,b,..) or column (x,y,.) show no difference at 95% confidence level ($p>0.05$)

Raw materials play an important role in the process of formation of cellulose/nata[17]. Materials containing high carbohydrates can be used as ingredients for making nata. Besides high content of carbohydrate, *S. polycystum*also provides sufficient macronutrients and micronutrients for *A.xylinum* bacteria to grow and develop, thus may increase the yield of the resulting nata. In addition to the raw materials, the length of fermentation timeis also a crucial determining factor to the yield of the nata. Table 2 above shows thatthe fermentation time of longer than 7 days significantly ($p<0.05$)increases the yield of the nata *de Sargassum*.[18] stated that during fermentation process there is a breakdown of sugar into simpler components namely glucose and fructose, and the formation of cellulose-forming carbohydrates. Therefore, longer fermentation time provide a continuous accumulation of glucose which is then converted cellulose to form the nata.

However, the similar yield of the nata obtained for all substrate concentration at any of the same fermentation time may raise a question of whether the bacterial count of *A. xylinum* in the starter solution is sufficient to convert most, if not all, the carbohydrates (sugar) in the fermentation substrate. Logically, if most or all sugar has been converted to cellulose, then a higher substrate concentration should produce a higher yield of the nata as well.So, there is a possibility that the similar yield obtained from different substrate concentration is due to insufficient concentration of *A. xylinum* used in the process. The same is applicable to the similar thickness of the nata de Sargassum as well. Therefore, further researchers are needed to determine the best or optimum conditions for the production of good characteristics of the nata de Sargassum.

IV. Conclusion

This study is the first to use*S. polycystum*as araw material to produce nataand proves that this type of brown seaweed can be used to produce nata *de Sargassum*. Both the thickness and the yield of the nata de Sargassum were fermentation time-dependent; longer fermentation time produces thickerand higher yield of the nata *de Sargassum.* Therefore, there is no urgency to use the concentration*S. polycystum* substrate of above 3% in the production of the nata de Sargassum. However, since the concentration of the bacterial starter was not varied in this study, it is still unknown whether different or higher concentration of *A.xylinum* bacterial starter imposes any effect on the characteristics of the nata de Sargassum; a subject commands a further study.

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