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Extraction and characterization of sodium alginate from three brown algae collected from Sanur Coastal Waters, Bali as biopolymer agent

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Abstract. *Permatasari AAAP, Rosiana IW, Wiradana PA, Lestari MD, Widiastuti NK, Kurniawan SB, Widhiantara IG. 2022. Extraction and characterization of sodium alginate from three brown algae collected from Sanur Coastal Waters, Bali as biopolymer agent. Biodiversitas 23: 1655-1663.* Sodium alginate extracted algae are gaining attention as alternative biopolymer materials. This research aimed to extract and characterize the natrium alginate from three brown algae obtained from Sanur Beach, Denpasar, Bali, Indonesia. Alginate extraction was carried out using acid hydrolysis by Na_2CO_3 with further purification using isopropyl alcohol. Physicochemical characterization was carried out using FT-IR. The results showed that *Sargassum aquifolium* and *Turbinaria ornata* had higher yields (%) than *Padina australis* ($P<0.05$). There was no significant difference ($P>0.05$) in water content, protein content, fat content and pH of the three brown algae. Overall, these three types of brown algae have values that are close to the sodium alginate standard set by the Food Chemical Codex (FCC), except for the results of *Padina australis* in the yield parameter. The functional group Na which is characteristic of sodium alginate is present in all alginate isomers, but there are some functional groups that are still not available in the extraction process in this study based on FT-IR analysis. These findings indicate that sodium alginate extracts from various types of brown algae have specific characteristics as defined by the Food Chemical Codex (FCC) and show great potential to be used as biopolymer materials, especially in the biomedical field.

Keywords: Biological agent, food chemical codex (FCC), macromolecule, medical sector, polysaccharides

INTRODUCTION

Capsules are the primary components that play an essential part in drug development because they have manufacturing benefits over other medicinal preparations such as tablets, syrups, and powders (Maestri et al. 2020, Khoa et al. 2022). Gelatin is the most common substance used in the pharmaceutical industries to produce capsules (Shimokawa et al. 2018, Chuenbarn et al. 2021; Ke et al. 2021). A report from gelatin manufacturers of Europe in 2005 reported that 44.5% (136,000 metric tons) of gelatin capsules came from pork skin, 27.6% (84,000 tons) from cowhide, and 26.6% (81,000 tons) of bone (Karim and Bhat 2009). The report even limits some groups such as vegetarians, Muslims, Jews, and Hindus who limit their consumption of food, including capsules whose raw materials come from cows and pigs. The gelatin capsule shell has weaknesses, such as being less stable in an aqueous environment, so the swelling and cracking time tends to be faster and can cause drug efficacy effects (Markl and Zeitler 2017, Li et al. 2019; Liu et al. 2019; Yang et al. 2020).

Seaweed, as a culinary and medicinal component, still has to be processed into semi-finished products such as agar, alginate, and carrageenan, which may improve its added value (Ibrahim et al. 2021; Premarathna et al. 2021; Pradhan et al. 2022; Qiu et al. 2022). Some food packaging is produced from seaweed, such as sushi wrappers, gelling agents in the pharmaceutical sector's development of gelatin-free capsules in Indonesia, or ecologically friendly bioplastic solutions in the bioindustry (Sivakanthan et al. 2020). The production of seaweed in Indonesia has a high potential for serving worldwide market demands, particularly in the domains of food, health, and medicines (García-Poza et al. 2020; Rimmer et al. 2021). This is based on the content of seaweed, which supports participation in illness prevention and therapy via several modes of action (Déléris et al. 2016). Several types of seaweed are widely used as fishery commodities, including red seaweed (*Euchema* spp., *Gelidium* spp., *Gracillaria* spp.), which is known as an agar producer (Martínez-Sanz et al. 2019), brown seaweed (*Sargassum polycystum, Padina* spp., *Turbinaria* spp.) as an alginate producer (Trica et al. 2019; Darli et al. 2020).

The alginate component of brown algal cell walls may account for up to 40% of the algae's total dry weight (Costa et al. 2021; Dharani et al. 2020). The alginate content in brown algal cell walls contributes to the flexibility of the algae's tissue structure (Rabillé et al. 2019; Dharani et al. 2020; Lai et al. 2021). Although all brown algae contain alginate, only a few species of brown algae are studied and processed to make alginate products (Dharmayanti et al. 2019). Karimah, (2016), for example, conducted research on the extraction of alginate from *Sargassum* sp. as a

capsule material, and employed it as a material for drug delivery systems. Sodium alginate is derived from the Moroccan plant *Laminaria digitata* and has the potential to be used as a medicine delivery agent (Fertah et al. 2017). *Colpomenia peregrina* alginate extraction yielded alginate with a molecular weight after acid treatment of 386.4×10^3 g/mol, whereas extraction after cellulase treatment yielded the lowest molecular weight (Rostami et al. 2017). Various alkaline procedures were used to extract alginate from *Sargassum latifolium* (Fawzy et al. 2017). The chemical composition of sodium alginate extracted from S. latifolium in Egyptian waters was total sugar (41.08 %) and uronic acid (47.4 %), with protein, lipid, and sulfate content of 4.61 %, 1.13 %, and 0.09 %, respectively (Dalal et al., 2021). Alginate extraction yields from algae *Sargassum* spp. and *Padina* spp. obtained from Ghanaian sea waters ranged from 17-23% by weight of dry matter (Rhein-Knudsen et al. 2017).

Several varieties of brown seaweed, including *Sargassum aquifolium*, *Turbinaria ornata*, and *Padina australis* have the potential to be processed to create alginate raw materials in Indonesia but are currently underdeveloped. It is a habitat for the development of brown seaweed, which is abundant in the waters of Sanur Beach in the Bali Province. Cultivated seaweed is popular as a traditional food among locals and is offered at traditional marketplaces. Similarly, owing to a lack of knowledge and market demand for brown algae commodities, brown seaweed, which has prospects as an alginate producer, was still not commonly farmed by the community. Based on the above, the aim of this study was to identify the properties of sodium alginate derived from brown marine algae collected from the waters of Sanur Beach in Bali Province. The findings of this research are predicted to be developed as an alternative to the major raw material used in the production of herbal-based capsule shells from brown seaweed, hence increasing the added value of seaweed commodities. On the other hand, sodium alginate obtained from brown algae can reduce the import of gelatin, and alginate raw materials and the use of related products derived from animal sources.

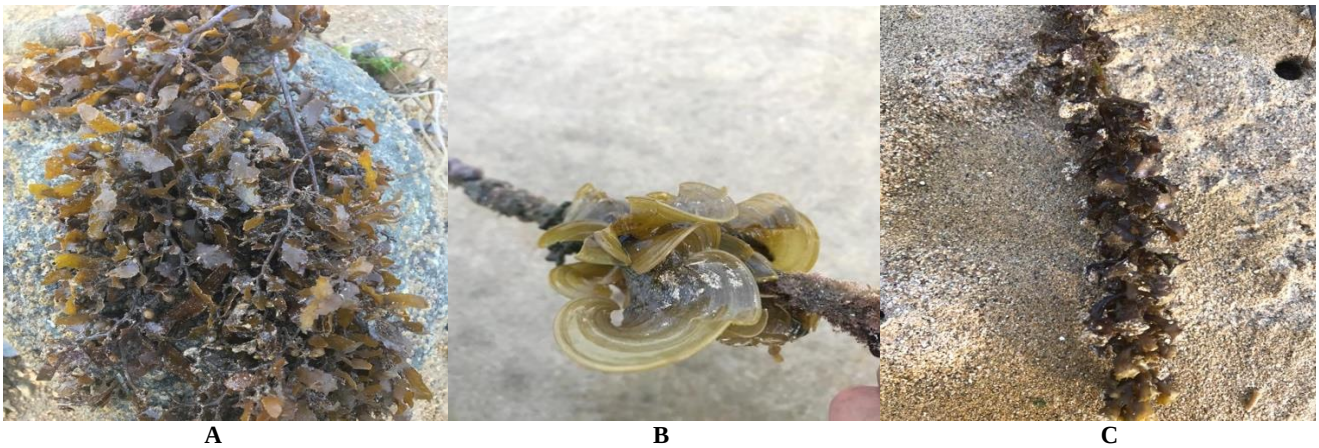
MATERIALS AND METHODS

Design and study area

The method used in this study was the descriptive and quantitative method (Widyastuti 2009). Three types of brown seaweed were used, namely *Sargassum aquifolium* (Turner) J. Agardh, 1820., *Padina australis* Hauck, 1887., and *Turbinaria ornata* (Turner) J. Agardh, 1848 (Figure 1). The three brown algae were collected in the waters of Sanur Beach, Bali, and identified at the National Research and Innovation Agency (BRIN), Jakarta with registration number: 25498. Extraction and measurement of yield (%) of sodium alginate were carried out at the Science Laboratory, Universitas Dhyana Pura, Badung, Bali. Physical and chemical characteristics tests were carried out at the Chemical Instruments Laboratory, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, East Java.

Sampling of brown seaweed

Brown algae samples were collected in the waters of Sanur Beach, Denpasar City, Bali Province (Figure 2). The samples were collected at low tide. The brown algae samples utilized were from three different species: *S. aquifolium*, *P. australis*, and *T. ornata*. The algae samples were collected and placed in sterile plastic bags before being sent to the laboratory. The sodium alginate extraction samples were cleaned with fresh water, dried, and kept in plastic bags for further extraction.



A **B** **C**

Figure 1. The brown seaweed collecting from Sanur Beach Coast Waters, Denpasar-Bali. A. *Sargassum aquifolium*, B. *Padina australis*. C. *Turbinaria ornata*

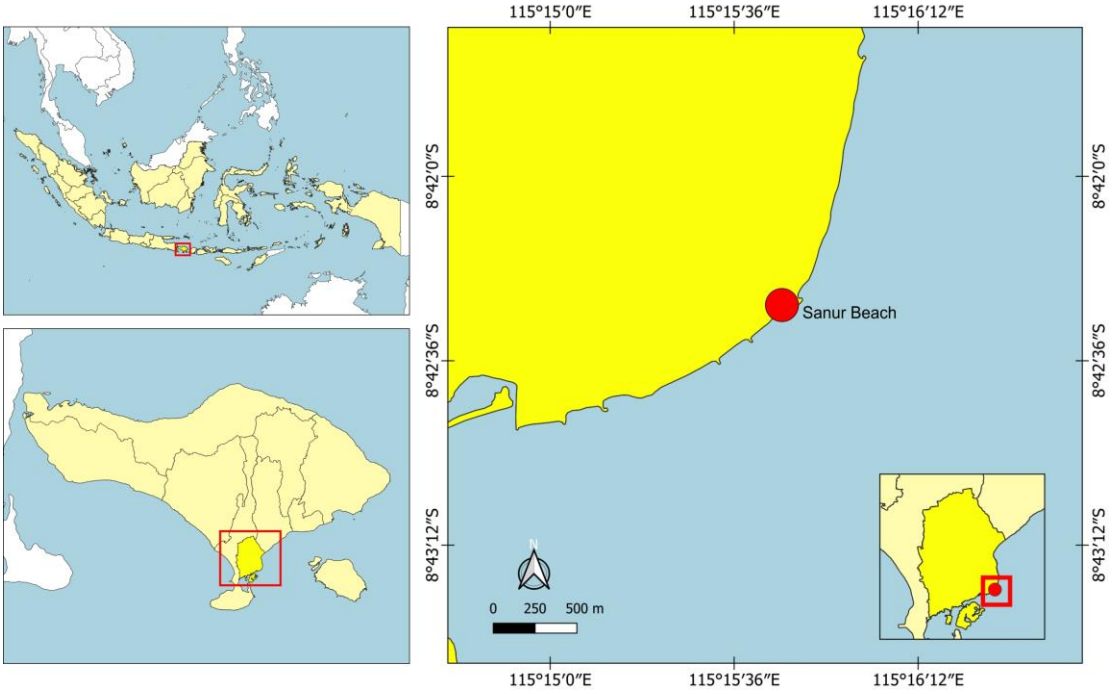


Figure 2. The sampling location for three brown algae (on the red spot) is on Sanur Beach, Denpasar City, Bali Province, Indonesia

Sodium alginate extraction procedure

Extraction was carried out using the method in Sunar, (2015). Brown algae samples were rinsed and sanitized with fresh running water before being sun-dried for three days. The dried sample was cut into tiny pieces and weighed up to 90 g. The weighted brown algae sample was then submerged for 1 hour in a 1% HCl (Merck, Germany) solution. Following the completion of the immersion time, the acid content of the sample is eliminated by adding 4% Na_2CO_3 (Merck, Germany). The immersion results were then heated for two hours at 60ºC and stirred with a IKA C-MAG MS magnetic stirrer, 230V (Sigma-Aldrich, UK). The sample was then diluted with distilled water until fully immersed and allowed to stand for 30 minutes before being filtered using filter paper (Sigma-Aldrich, UK). The filtrate was then bleached with a 12% NaOCl (Merck, Germany) solution and let to stand for two hours. The pH of the filtrate was then adjusted to 2-3 by adding 5% HCl and allowing it to stand for 5 hours until the sample produced foam lumps. After 5 hours, the foam clumps generated in alginic acid were combined with 10 mL of NaOH (Sigma-Aldrich, UK). The obtained alginic acid was then converted into sodium alginate extract by adding 99% isopropanol (C_3H_7OH) (Merck, Germany) in a ratio of 1:2. The filtrate that has been added to isopropanol is then transferred to a test tube and centrifuged to obtain deposits. The precipitate is then put into a Petri dish and put in the freezer. This process is carried out until the precipitate turns into a "gel" form. The gel formed is then placed in a freeze dryer to obtain a powder form. The resulting powder was blended and weighed for use in the FT-IR analysis (Helmiyati and Apriliza 2017).

Determination of sodium alginate yield

The determination of the sodium alginate yield (%) obtained in this research plan is calculated using the equation (Sunar 2015) :

$$Yield\ (\%) = \frac{mass\ of\ crude\ sodium\ alginate}{sample\ mass} \times 100\%$$

Chemical characteristics of sodium alginate

Determination of the moisture content

Measurement of the moisture content of sodium alginate samples using the working principle of the gravimetric (da Silva et al. 2008). In short, the sample was weighed as much as 1 gr using Kern ABT analytical balance (Sigma-Aldrich, UK) and then dried in Memmert Oven UN 55 53L for 30 minutes. The dried sample was then removed and cooled in a desiccator. Next, the sample was weighed again using an analytical balance to determine the weight after drying, and the water content was determined using the following equation:

$$Water\ content\ (\%) = \frac{(W1 - W2)}{W} \times 100\%$$

Where *W1* is the weight of the sample before drying (g), *W2* is the weight of the sample after drying (g), and *W* is the weight of the sample (g).

Determination of ash content

Measurement of ash content was carried out by putting the sample into a porcelain dish and burning it in a furnace at a temperature of 550°C, then cooling it in a desiccator.

The cooled and stable ash is then weighed so that the total ash content can be determined using the equation:

$$Ash\ content\ (\%) = \frac{(W1 - W2)}{W} \times 100\%$$

Where: *W1* is the weight of the sample after being burned (g), *W2* is the weight of the sample before being burned (g), and *W* is the weight of the sample (g) (Mokoginta et al. 2019).

Protein content

The protein content was determined using the Kjeldahl technique in three stages: destruction, distillation, and titration. In brief, 10 mL concentrated H_2SO_4 (Merck, Germany) was pipetted into a 100 mL Kjeldahl flask with 1 g of the material. A catalyst in the form of a selenium combination was also introduced to speed up the destruction process. The Kjeldahl flask was heated in an oven, then cooled gradually. When a clear green solution was achieved, the sample was saved.

The sample was cooled and diluted with distilled water to 100 mL. A total of 5 mL was pipetted into a distillation flask after being homogeneous. During distillation, 10 mL of 30% NaOH (Merck, Germany) solution is added through the walls of the distillation flask to produce a layer under the acid solution. Next, the condenser attaches the distillate flask. An Erlenmeyer reservoir tube containing 10 mL of 0.1 N HCl (Merck, Germany) was filled with steam from the boiling liquid. Distillation was stopped if the litmus paper sample was not alkaline.

It is then titrated with 0.1 N NaOH solution (Merck, Germany). A pink to yellow hue indicates the conclusion of titration. Each sample of sodium alginate was treated three times. Determination of protein content using the equation (Reyes-Tisnado et al. 2005):

$$Protein\ content\ (\%) = Nitrogen\ Content\ (\%) \times Conversion\ Factor$$

Fat content

The Soxhlet technique for determining fat content. In summary, the pumpkin fat is dried for 1 hour at 105°C. After 15 minutes in a desiccator, the fat flask was weighed (W2). It was mashed and weighed (W1), then coated with filter paper created by thimbles. From heating mantle, fat flask, and Soxhlet to condenser. The sample was then placed in a Soxhlet with 1.5 cycles of hexane solvent. The solvent is extracted for hours until it returns via the siphon into a clear-colored fat flask. The pumpkin fat extract was separated from the hexane using a rotary evaporator (50 rpm, 69°C). The hexane-separated fat is then cooked for 1 hour at 105°C. After 15 minutes in a desiccator, the fat flask weighed (W3) (Reyes-Tisnado et al. 2005). Fat content (%) is determined using the equation:

$$Fat\ content\ (\%) = \frac{W3 - W2}{W1} \times 100\%$$

Where: *W1* is the weight of the sample, *W2* is the weight of the fat flask empty (g), and *W3* is the weight of the vessel fat + extracted fat (g).

Fiber content

Determination of carbohydrate content according to Indonesian National Standard (SNI) No. 01-2891-1991. 50 mL sample was pipetted into an Erlenmeyer with 200 ml % HCl (Merck, Germany). Then heated at 100°C for 1.5 hours, cooled, then added 0.5 mL PP indicator and 30% NaOH (Merck, Germany) till a pink solution was formed. Then, 3% CH_3COOH (Merck, Germany) was added until the solution was clear. The clear solution was then poured into a 500 mL volumetric flask, 10 mL taken and 25 mL Luff Schoorl added, boiled again for 12 minutes at 200°C, cooled, and added 15 mL 20% KI and 25 mL 25% H_2SO_4 (Merck, Germany). Also, 0.1 N $Na_2S_2O_3$ (Merck, Germany) was titrated until a light-yellow color appeared, then 0.5 mL of % starch was added, then titrated again until a milky white color developed.

Determination of viscosity

A total of 100 mL sample was poured into a beaker glass, then set the viscosity tool (Brookfield Viscometer, USA) by selecting the speed (50 rpm), and the type of spindle based on the texture of the sample. Adjust the spindle size and speed on the viscometer. press on then observes % torque. Record the results of the viscosity until it shows a stable number (Herrero et al. 2006).

Determination of functional group

A determination of the functional group of sodium alginate was carried out using FT-IR Spectroscopy (Shimadzu, Japan). As much as 2 g of each sample of brown algae were mashed and mixed with KBr to form a thin plate. The sample was pressed using a hydraulic press at 10,000-15,000 psi. The thin plate was mounted in the cell and placed on the light beam (Helmiyati and Apriliza 2017).

Data analysis

The collected data was analyzed using SPSS Version 23.0 software (IBM, USA). The one-way ANOVA test was used to test the various comparison of each sample and to find the significant difference ($p<0.05$) in each sample, followed by the Duncan test with a 5% confidence interval.

RESULTS AND DISCUSSION

Chemical characteristics of sodium alginate

The yield, water content, ash content, protein content, fat content, fiber, pH, viscosity, and functional group analysis of sodium alginate extracted from three species of brown algae were all measured in this research. Table 1 summarizes the findings of the chemical characteristics of sodium alginate studies.

Yield (%)

The measurement finding showed that the yield developed by *S. aquifolium* (21.74±2.135%) and *T. ornata* (20.16±3.644%) in this research had a greater yield percentage than that produced by *P. australis* (5.89±1.537%). Duncan's test findings revealed a significant difference ($p<0.05$) in the yield significant role played by *S. aquifolium* and *T. ornata* when compared to *P. australis*. Surprisingly, the yield generated by the two brown algae was greater than that of the standard alginate (FCC). Our results vary from those of Maharani et al. (2017) who reported low yields both by acid extraction (9.95±0.31%) and calcium extraction (11.70±0.41%). on the other hand Latifi et al. (2015) reported, that alginate extraction utilizing the calcium technique produced high yields ranging from 25.02-30.01%.

The extraction temperature and comparison group might have an impact on yield results. According to Fertah et al. (2017) temperature of 40°C with a sample size of less than 1 mm suggests excellent circumstances for obtaining high yields. Further study is required to establish this, however, since each extraction process and material utilized might change the percentage of yield obtained. Furthermore, the sample locations and various seasons might have an impact on the quantity of alginate produced (Bertagnolli et al. 2014). In our study, we collected brown algae during the rainy season, with sea air temperatures in the Sanur Beach waters ranging from 30-32°C in the intertidal zone.

Moisture content

The sodium alginate water content of *S. aquifolium* (9.55±0.74%) was found to be greater than that of *P. australis* (9.07±0.10%) and *T. ornata* (9.18±0.030%). According to Duncan's test, there is no statistically significant difference ($p<0.05$) in the water content obtained by the sodium alginate extraction technique between the three species of brown algae. The low water content in the three samples is thought to be due to the acid extraction procedure used in this study. The acid solution utilized in this research is capable of attracting the water content in the sodium alginate sample, particularly during the sodium alginate purification and precipitation process (Maharani et al. 2017). Because of isopropyl alcohol's propensity to bind hydroxyl groups (-OH), which is bound to the other two carbons and the purification procedure for sodium alginate employs isopropyl alcohol solution, which is typically more efficient than ethanol (Jian et al. 2014).

Isopropanol is also used in polymer purification because of its capacity to eliminate endogenous impurities and enzymes from granular samples, increasing their mass when applied. Furthermore, isopropyl alcohol may aid in the formation of carbohydrates in the sample by promoting intramolecular interactions between air-soluble polymers through water competition (Jian et al. 2011; Navarro et al. 2000). The research also found that increasing the concentration of isopropyl alcohol improved the viscosity of the sample related to the extraction of water content by isopropyl alcohol (Zailanie et al. 2001). The moisture content may also be lowered throughout the drying process to increase the extraction quality (Herdianto and Husni 2019). Drying may be done in an oven or outside in the sun.

Ash content

The ash content of sodium alginate from *S. aquifolium* (21.90±0.51%) and *T. ornata* (21.56±1.68%) was found to be greater than that of *P.australis* (17.34±1.09%). Duncan's test findings revealed a significant difference in the ash content of sodium alginate between *S. aquifolium* and *T. ornata* when compared to *P. australis* ($p<0.05$). However, when compared to the Food Chemical Codex (FCC 1981) quality standards for the food industry, it has met the standard, which is in the range of 13-27%. The variation in sodium alginate ash concentration between the three varieties of brown algae may also be attributed to habitat conditions and the state of the seaweed developing naturally. We assume that the low ash concentration in the *P. australis* samples is due to its very thin morphology and similarities to sheets, which causes it to be degraded fast during the hydrolysis process utilizing HCl, which acts in the demineralization process. *P. australis* is a kind of brown seaweed that is often associated with a high concentration of calcium in the aquatic environment, hence enhancing the minerals in the sample (Circuncisão et al. 2018). The ash content of *S. aquifolium* and *T. ornata*, on the other hand, is greater because mineral salts attached to the seaweed and salt residues are not washed away during the cleaning phase and are carried over to the extraction process.

Protein content

The protein composition of sodium alginate did not vary significantly amongst the three species of brown algae ($p>0.05$), according to the findings. *S. aquifolium*, on the other hand, had the highest sodium alginate level (0.411±0.001%). The low levels of sodium alginate protein in the three brown algae samples could be attributed to the extraction process using strong acid (HCl), which resulted in the protein contained in the samples being decomposed into simpler compounds and carried over to the isopropyl alcohol purification process. According to another study, the protein content of sodium alginate extracted with 14% $CaCl_2$ solution was greater, ranging from 2.63 to 4.38% (Kamisyah et al. 2020). Surprisingly, the protein level in this research was greater than the FCC (FCC 1981) standard of Sodium alginate (See Table 1).

Fat content

In this investigation, the fat content generated by sodium alginate did not vary significantly amongst the three brown algae ($p>0.05$). In this investigation, the sodium alginate produced from *P. australis* had the greatest fat content (2.63±1.79). (Table 1). The fat content of the three brown algae in this study, on the other hand, was greater than that of *S. aquifolium* in Srikandi et al. (2013) which was 0.119-0.129%. The fat content findings in this investigation were not significantly different from those found in Reyes-Tisnado et al. (2005) which produced alginate from kelp *Macrocystis pyrifera* with a fat percentage ranging from 2.11-3.85%.

Table 1. Characteristics of sodium alginate extracted from three brown algae collected from the waters of Sanur Beach, Denpasar, Bali Province, Indonesia

Parameter	Brown algae species			Sodium alginates standard*
	Sargassum aquifolium	*Padina australis*	*Turbinaria ornata*	
Yield (%)	21.74±2.135[a]	5.89±1.537[b]	20.16±3.644[a]	>18
Moisture (%)	9.55±0.74[a]	9.07±0.10[a]	9.18±0.030[a]	<15
Ash (%)	21.90±0.51[a]	17.34±1.09[b]	21.56±1.68[a]	13-27
Protein content (%)	0.411±0.001[a]	0.36±0.02[a]	0.37±0.07[a]	0 -1
Fat content (%)	2.15±0.017[a]	2.63±1.79[a]	2.53±0.10[a]	-
Fiber (%)	4.17±0.133[a]	3.60±0.31[b]	3.53±0.18[b]	-
pH	7.45±0.14[a]	7.35±0.06[a]	7.63±0.20[a]	3.5-10
Viscosity (cPs)	418.73±85.49[a]	75.20±16.42[b]	296.90±107.34[a]	10-5,000

Note: Values with different notations in the same column indicate a significant difference ($p<0.05$). Alginate standard is a commercial product whose standards are adjusted to the FCC (1981)

Fiber

Fiber content contained in sodium alginate from the three brown algae samples showed results with *S. aquifolium* (4.17±0.133%) which was significantly higher ($p<0.05$) compared to *P. australis* (3.60±0.31%) and *T. ornata* (3.53±0.18%). Our results were actually higher when compared to fiber content extracted from kelp *M. pyrifera*, which ranged from 1.14-2.49% (Reyes-Tisnado et al. 2005). The fiber content in the sodium alginate material was formed due to the addition of the NaOH solution (Iryanti et al. 2018). The high fiber content in the alginate material can be processed into various products such as woven or knitted fabrics. Interestingly, alginate fiber provides better benefits for the manufacture of wound dressing raw materials because it has high absorption (Jahandideh et al. 2021). Calcium or sodium alginate fibers may ideally be generated by partially replacing calcium ions with sodium ions. Interestingly, calcium ions have significance in sustaining wet integrity in this circumstance, while sodium ions enhance water absorption (Jahandideh et al. 2021).

pH parameter

The findings revealed that the pH values generated by sodium alginate from the three brown algae were not significantly different ($p>0.05$) (Table 1). Previous research has shown that sodium alginate may be stable in the pH range of 5-10, but at higher pH values, it causes a reduction in viscosity related to β-eliminative degradation (Iryanti et al. 2018). However, if the generated pH is relatively acidic, sodium alginate will become more sensitive. and unstable related to alginate depolymerization in solution (Susanto et al. 2001; Zailanie et al. 2001). When the findings of sodium alginate in this research are compared to the value of the quality standard of sodium alginate, it can be stated that the results of sodium alginate in this study are in accordance with the quality standards of the food industry (FCC 1981).

Viscosity

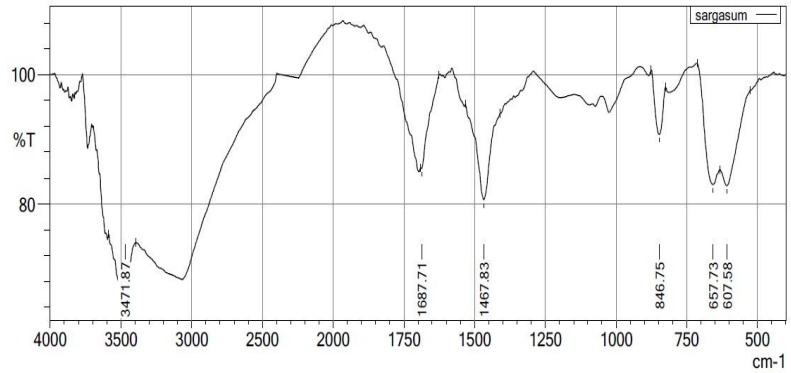
The findings indicated that *S. aquifolium* (418.73±85.49%) and *T. ornata* (296.90±107.34 %) had the greatest viscosity values among the three varieties of brown algae, while *P. australis* (75.20±16.42%) had the lowest ($P<0.05$). The variation in viscosity value might be contributed to degradation during the extraction process. This degradation decreases the molecular weight of the alginate, causing a reduction in viscosity. The bonds between polyguluronic acids, on the other hand, can be disrupted during the extraction process, resulting in a decrease in alginate viscosity (Dharmayanti et al. 2019). Interestingly, the reduced degree of purity of the alginate produced causes a large quantity of water-insoluble components and a low viscosity of the alginate (Manev et al. 2015). Sodium alginate with a low viscosity content is suitable for use in food, however, sodium alginate with a high viscosity level is suitable for use in the textile and pharmaceutical sectors (Qin et al. 2018). Differences in the environmental conditions also contribute to variations in the viscosity values generated by algae (Hamrun et al. 2018). Brown algae that focus in the tropics and at higher temperatures produce alginates with low viscosity (McHugh 1987). The high drying temperature of the collected brown algae might result in high viscosity values. According to research, drying at high temperatures causes the development of a number of sulfate esters, which improves the viscosity level (Heriyanto et al. 2017; 2018).

Functional group analysis of sodium alginate

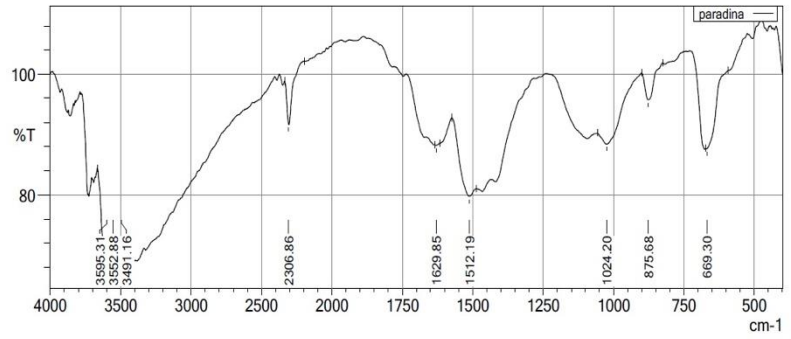
Functional group analysis of three types of brown algae collected from Sanur Beach, Denpasar, Bali is shown in Table 2. The spectrum of sodium alginate are shown by *S. aquifolium* showed the presence of a hydroxyl group (-OH), carbonyl group (-C=O), mannuronic acid, and Na in the alginate isomer as shown in the FTIR curve (Figure 3A). The spectrum of sodium alginate from *P. australis* showed the presence of a hydroxyl group (-O-H) at wave numbers 3595.31, 3552.88, and 3491.16 cm-1. Furthermore, the COO-group was asymmetric at wave numbers 1629.85 cm-1. The carboxyl group (C-O) is at 1024.20 cm-1. At 875.68 cm-1, the C-O group is stretching uronic acid. The Na group in the alginate isomer at 1512.19 cm-1 (Figure 3B). On the other hand, the alginate spectrum of *T. ornata* only showed a carboxyl group (CO) (1026.13 cm-1), uronic acid strain CO (875.68 cm-1), alkyne group (C≡C), Na in alginate isomer (1604.77 cm-1), and symmetrical COO (1415,75). cm-1) (see Figure 3.C).

Table 2. Functional group wave values

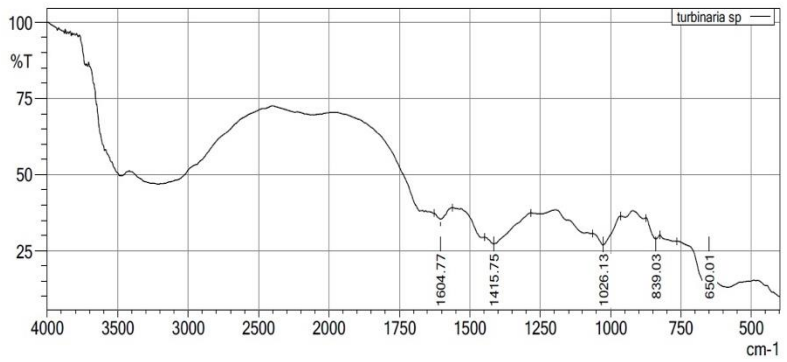
Sodium alginate *Sargassum aquifolium* (cm^{-1})	Sodium alginate *Padina australis* (cm^{-1})	Sodium alginate *Turbinaria ornata* (cm^{-1})	Wave number reference (cm^{-1})	Functional Group Interpretation (cm^{-1})
3471.87	3595.31 3552.88 3491.16	-	3600-3200	Hydroxyl group (O-H)
-	1629.85	-	1680-1600	COO- asymmetric
1687.71	-	-	1900-1650	Carbonyl Group (C=O)
-	1024.20	1026.13	1300-1000	Carboxyl group (C-O)
-	-	-	-	Guluronat
846.75	-	839.03	850-810	Mannuronat
-	875.68	-	950-700	C-O stretching uronic acid
-	-	-	3750-3000	N-H stretching
-	-	-	3000-2850	C-H stretching
-	2306.86	-	2400-2100	C≡C (Alkyne)
1467.83	1512.19	1604.77	1614-1300	Na in alginate isomer
-	-	1415.75	1415-1410	COO-symmetric



A



B



C

Figure 3. FTIR curve of sodium alginate in this study. A. *Sargassum aquifolium.* B. *Padina australis*. C. *Turbinaria ornata*

From the FT-IR spectra above, it can be seen that the sodium alginate extracted in this study has some differences with the reference sodium alginate due to the different wavenumber values. The wave numbers shown by *S. aquifolium* only show four similarities with the wave numbers shown by references such as hydroxyl groups, carbonyl groups, mannuronate, and Na in alginate isomers. The number of similar wavenumbers was also shown in *T. ornata* in the carboxyl group, mannuronate, Na in alginate isomer, and COO-symmetric. Sodium alginate *P. australis* showed the highest similarity in this study with the reference wavelength of eight functional groups. The results of our study have differences when compared to the functional group of sodium alginate *Sargassum sp*. in a study conducted by Karimah (2016), which showed the character of the functional group in accordance with commercial sodium alginate and the reference standard. Interestingly, in this study, the researchers added STPP to the starch-alginate formulation so that the P=O functional group appeared at the wave number of 1211.34 cm^{-1}. However, the sodium alginate isomer that appears identically appears in the three brown algae in this study, namely at 1467-1604 cm^{-1}. We assume that the absence of some functional groups in this study could be caused by the extraction method and process carried out.

To conclude, based on alginate standards established by the Food Chemical Codex (FCC) (1981), sodium alginate extracted from three types of brown algae collected from the waters of Sanur Beach, Denpasar, Bali Province, has ideal physicochemical characteristics and has the potential to be developed as a functional food industry requirement. The functional groups of the three alginates isolated from the three species of brown algae were all distinct but, Na in the alginate isomer was present in all three sodium alginate samples. The findings demonstrated that the three sodium alginates derived from brown algae in Sanur Beach area had the potential to be exploited as biopolymer materials. Additional research is needed, particularly regarding the cytotoxicity that may occur in sodium alginate when cell lines and experimental animals are applied. On the other hand, it is also necessary to know the important components of brown algae, through HPLC tests, gel strength, H NMR analysis, and more detailed FTIR.

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