



Application of Agar-agar as Food Additives

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Authors' contributions

This work was carried out in collaboration among all authors. Author J designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors YA and SR managed the analyses of the study. Author GZF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This review article aims to study the types of seaweed that can be processed into agar, agar extraction methods, characteristic of agar, and application of agar as a food additive. Based on articles and other literature reviews, it can be concluded that: 1) The types of seaweed that can be extracted into agar-agar are *Gracilaria* sp., *Gelidium* sp., and *Gelidiella* sp. 2) Extraction of agar from seaweed can be done using the methods of Yolanda and Agustono [1], Wicaksono et al. [2] and Shantika et al. [3]. 3) The characteristic of agar can be reviewed from particle size, moisture content, ash content, heavy metals, pH, solubility, turbidity, gel strength, and viscosity. 4) Agar-agar as a food additive had been applied to analog rice, jelly drink, and edible film making.

Keywords: *Method; extraction; characteristic; Gracilaria sp.; Gelidium sp.*

1. INTRODUCTION

The type of seaweed in the Rhodophyceae class that is commonly used are *Gracilaria*, *Sargassum*, and *Gelidium* sp. [4]. Based on data from The Ministry of Marine Affairs and Fisheries

(KKP), it is known that the amount of seaweed production in Indonesia in 2019 reached 9.655.534,22 tons, while in 2020 there was a decrease in production which only reached 4.088.629,82 tons. Of the three seaweeds used for agar extraction such as *Gracilaria* sp.,

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Gelidium sp., and *Gelidiella* sp., the amount of production of *Gracilaria* sp. is one of the largest. In 2009 it reached 35 tons, absorbed by the national industry by 81% and the rest is absorbed by foreign industries [5]. The locations of seaweed cultivation in Indonesia are scattered around the islands of Sulawesi, Bali, West Nusa Tenggara, East Nusa Tenggara, and Papua with an indicative area of land utilized reaching 769.452 ha with a total number of seaweed cultivators 442.070 Fishery Households (RTP) [6]. In Indonesia, the processing of agar is usually still at the semi-traditional stage with the results are in the form of sheets, bars, and flour. This agar consists of two fractions, namely agarose and agarpectin. Agarose is a component that can form gel and contain sulfate, while agarpectin is a component that cannot form gel and does not contain sulfate [7]. Agar-agar has properties like gelatin, which is soluble in hot water. At a temperature of 35-39°C it is solid and at 85-95°C can form gels [8].

In the food industry, agar is used as a gelling agent because of its ability to form a very hard gel at very low concentrations. Gel on agar can be formed in a solution with an agar concentration of 1% [9]. Gel agar is rigid, brittle, easy to form, and has a certain melting point [10]. Agar is widely used as an emulsifier, stabilizer, gelling, suspension, coating, and inhibitor [4], all of these benefits are included in food additives. Alginate and carrageenan produced from seaweed can also be used as food additives. Alginate is usually produced from types of seaweed such as *Sargassum* sp. and *Turbinaria* sp. which are found in many waters in Indonesia [11]. Meanwhile, carrageenan can be produced from types of seaweed such as *Euचेuma spinasum* and *Euचेuma cottonii* which have high levels of carrageenan, namely around 62-68% of the dry weight [12]. Alginate and carrageenan can be used as gelling agent, thickener, stabilizer, and emulsifier. Agar that can be used in the industrial sector, namely agar which has high gel strength, has low levels of sulfate, ash, and water, also has a white and bright color [13]. With the wide use of agar as a food additive, it is very important to review the types of seaweed that can be processed into agar, agar extraction methods, characteristic of agar, and application of agar as a food additive.

2. AGAR RAW MATERIALS

Agar is the main product that is produced from seaweed, especially from the Rhodophyceae

class, such as *Gracilaria*, *Gelidium*, and *Gelidiella*. *Gracilaria* sp. is a group of red algae (Rhodophyceae) [14]. The main product of *Gracilaria* sp. according to FAO (Food and Agriculture Organization) [15] is a raw material for making agar-agar. *Gracilaria* sp. is widely cultivated in ponds and has been successfully cultivated in Indonesia [16]. Generally, the agar content in *Gracilaria* ranges from 16-45% [17].



Fig. 1. *Gracilaria* sp.
Source: Othman et al. [18]

The classification of *Gracilaria* sp. according to Anggadiredja et al. [2] is as follows:

Phylum	: Rhodophyta
Class	: Rhodophyceae
Order	: Gigartinales
Family	: Gracilariaceae
Genus	: <i>Gracilaria</i>
Species	: <i>Gracilaria</i> sp.

Various types of *Gelidium* sp. in Indonesia are used as raw material for domestic agar factories and as an export commodity [19]. The agar content ranges from 12-48%, but depending on the type. The distribution of *Gelidium* sp. is affected by the type of substrate, salinity, waves, current, and tide [20]. The taxonomy of *Gelidium* sp. is as follows according to Hatta and Dardjat [21]:

Phylum	: Rhodophyta
Class	: Rhodophyceae
Order	: Gelidiales
Family	: Gelidiaceae
Genus	: <i>Gelidium</i>
Species	: <i>Gelidium</i> sp.



Fig. 2. *Gelidium* sp.
Source: Smith [22]

Gelidiella (*G. Acerosa*) grows attached to the rock. This algae appears on the surface of the water at low tide and experiences drought. This alga is one of the agar resources that are traded. The Gelidiaceae family, although the taxonomy of red algae is currently in the process of being revised [23], and includes *Gelidium*, *Ptilophora*, and *Gelidiella* [24,25]. The taxonomy of *Gelidiella* is as follows:

Phylum	: Rhodophyta
Class	: Florideophyceae
Order	: Gelidiales
Family	: Gelidiellaceae
Genus	: <i>Gelidiella</i>
Species	: <i>Gelidiella acerosa</i>



Fig. 3. *Gelidiella* sp.
Source: Serdiati and Samliok [26]

Gracilaria gave the largest contribution (>90%) for agar raw material rather than the other *agarophytes* genus. This is because *Gracilaria* is

widely cultivated in ponds, while the other *agarophytes* are still harvested from the nature [27].

The results of the desk study show that most of the Gelidiaceae used are still harvested from natural populations, and as stated by Friedlander [28], information about the cultivation of this species in Central Sulawesi water or other equatorial waters. According to various sources, some members of the Gelidiaceae family prefer temperatures that are lower than the average surface temperature of the waters in Central Sulawesi Province [29,25], but several species that have a natural distribution in Indonesia are likely to be cultivated in Central Sulawesi, such as the species *Gelidiella acerosa*.

Another type of seaweed from a group of red algae that is commonly used by the agar industry as a raw material for agar is *Hypnea* sp. However, the utilization of *Hypnea* sp. still not optimal, only a few are traded and not developed in cultivation [30]. *Hypnea saidana* species can grow and are found in the waters of Haruku Island and is a producer of carrageenan and agar, as for which it is sold raw in the form of dried seaweed [31].

3. AGAR EXTRACTION METHOD

The extraction method can affect the quality of the resulting agar. The type of solvent, time, temperature used in the agar extraction method are variables that greatly affect the quality of the agar [1]. Furthermore, there are also other factors that can affect the quality of agar, such as weight ratio of the material to the volume of solvent, temperature, stirring, extraction time, and the soaking process [2]. Extraction production costs for 1 kg agar can reach approximately Rp.54.000 [32]. Here are some of the methods for extracting agar:

3.1 Extraction of Agar *Gracilaria verucosa* by Yolanda and Agustono [1]

This agar extraction method was taken from research conducted by Yolanda and Agustono [1] which used a descriptive method with primary data collection in the form of observation, interviews, and active participation, as well as secondary data that could be obtained from reports or documentation. Of the various methods discussed, the extraction of agar based on Yolanda and Agustono [1] is an extraction of agar which is carried out by one of the agar industry factories in Indonesia, which produces a

fairly high amount of agar, production costs are also higher than the other extraction methods discussed in this article.

The raw material used is *Gracilaria* sp. The extraction process is conducted on a laboratory scale, but in general, the process is the same as on an industrial scale. First, 15 kg of *Gracilaria* sp. seaweed is prepared. In this process, the manufacture of agar powder begins with alkaline soaking using NaOH in *Gracilaria verucosa* which has been previously dried. 15 liters of 1,5 N NaOH solution were used, with a ratio of alkaline and 1,5 kg *Gracilaria verucosa* is 1:10. Soaking is done using a mini extractor at a temperature of 110°C for the heating process of 1 hour. Then, wash using 17 liters of freshwater for 20 minutes and carried out 6 times until the pH became neutral.

After the washing process, it is followed by soaking in a solution of HCl and AC02 (*oxalic acid*) dissolved in 15 liters of RO (*reverse osmosis*) water. Acid soaking process for 20 minutes, the purpose of this process is to clean the components that interfere with or stick to the seaweed and to facilitate the extraction process because the hydrolysis process will occur during soaking which makes *Gracilaria* soft. Then, wash again by soaking in water (17 liters) for 20 minutes, this process is carried out 3 times.

The soaking stage for *Gracilaria* is called *bleaching* stage for 30 minutes using 5 grams of chlorine in 15 liters of RO (*reverse osmosis*) water. This stage is the process of bleaching or removing pigments in seaweed, due to this pigments can affect the color of the resulting agar powder. Furthermore, wash using water (17 liters) for 20 minutes 3 times to dissolve the remaining bleaching soaking water and neutralizing the pH. Then, the second acid soaking using HCl for 20 minutes to obtain more crushed seaweed to facilitate the extraction process. Wash using 17 liters of RO water to bind the pollutant compounds, and carried out for 20 minutes 2 times to neutralize the pH.

The extraction process of *Gracilaria verucosa* at temperature of 110°C for 1 hour using 12 liters of RO water. RO water can dissolve minerals contained in *Gracilaria verucosa*. Extraction of agar is when neutral conditions so as not to reduce the strength of the gel, because if it is in acidic conditions there will be a hydrolysis process which can reduce gel strength. During the extraction process, stirring continuously so that it is evenly heated until the seaweed

becomes powder. Furthermore, the filtration process manually using a filter to separate the extract from the dregs which dissolved in water. The results of the filtration are put into the container and stored for the cooling process. The gelling process occurs during the cooling process which lasts for 12 hours at room temperature. Then, the membrane press process manually by inserting the agar into a high-density cloth and placing it on the hydraulic jack table. For the pressing results in the form of agar-agar sheets, fill evenly and pressed slowly, also repeatedly until there are no pores or trapped water.

The next process is to dry chips to facilitate the milling process, which is a process that uses a miller tool with a speed of 3.450 rpm and mesh size on it. In this process, the sieve using mesh size 60. This tool is equipped with two cloth containers to accommodate the agar powder produced from the milling process, as well as to keep the powder from scattering out. Then, insert the chips into the hole on the miller slowly. The milling process aims to obtain agar powder that can fulfill the quality standards desired by the company.

The advantage of this extraction method is that when testing the physical and chemical quality of the agar powder produced, as a whole, it meets the standard value. Although, there are still drawbacks such as the high value of gel strength which is influenced by alkaline treatment which can reduce sulfate levels. The higher the NaOH concentration, the higher the gel strength value. The resulting turbidity value is also low, because it is influenced by the sodium salt content obtained from the alkaline NaOH solution.

3.2 Extraction of Agar *Gracilaria verucosa* by Wicaksono et al. [2]

Research by Wicaksono et al. [2] was conducted to determine the effect of immersion time on the physicochemical characteristics of *Gracilaria verrucosa* agar by analyzing several parameters such as gel strength, gelling and melting point, viscosity, sulfate content, and infrared spectra. The materials used for agar extraction in this method are *Gracilaria verrucosa*, CaO 0,5% and NaOH. The soaking time treatment obtained an advantage in the resulting agar which has a higher physicochemical value than the untreated agar, with the best results being the soaking time of 1 hour deep extraction of this agar. However, the gelling point parameter obtained the same value between treated agar and control agar.

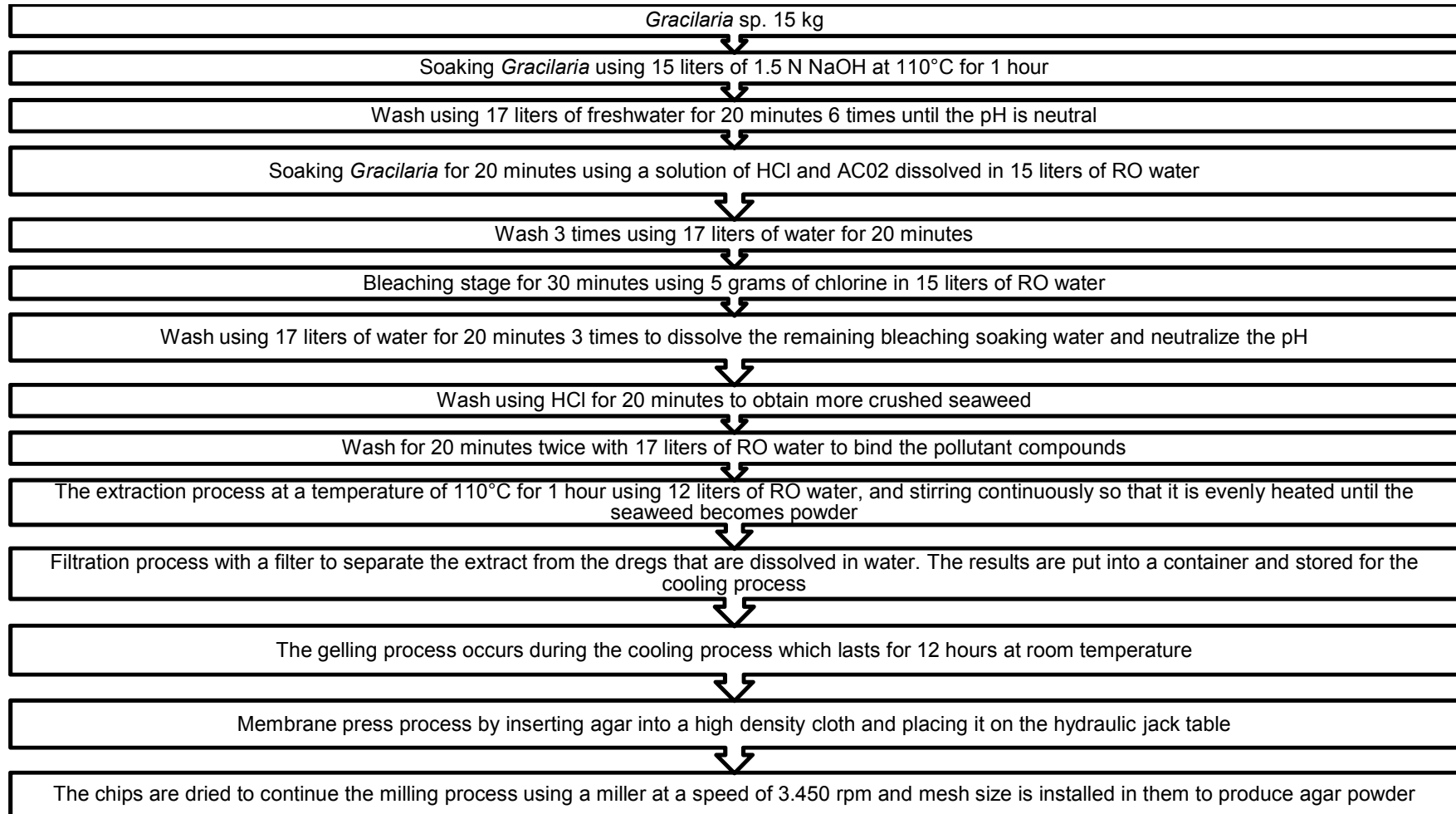


Fig. 4. Extraction method of *Gracilaria verucosa* by Yolanda and Agustono [1]

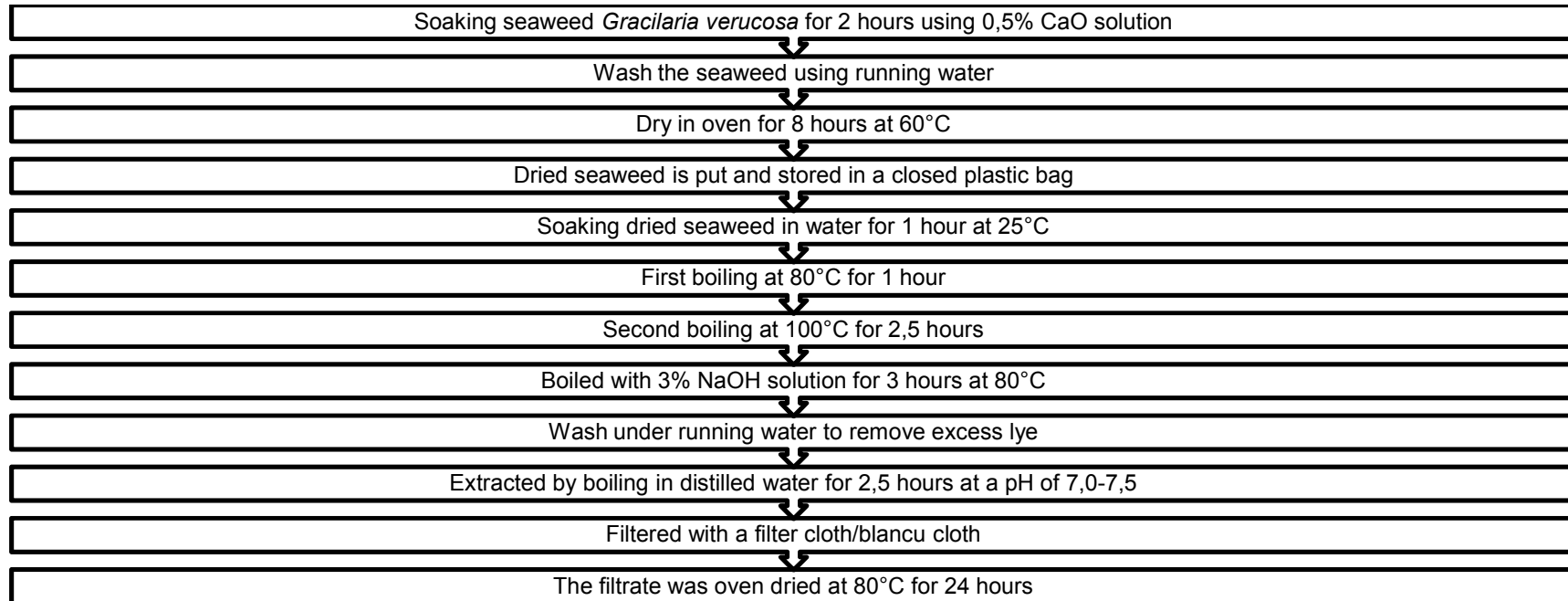


Fig. 5. Extraction method of Agar *Gracilaria verucosa* by Wicaksono et al. [2]

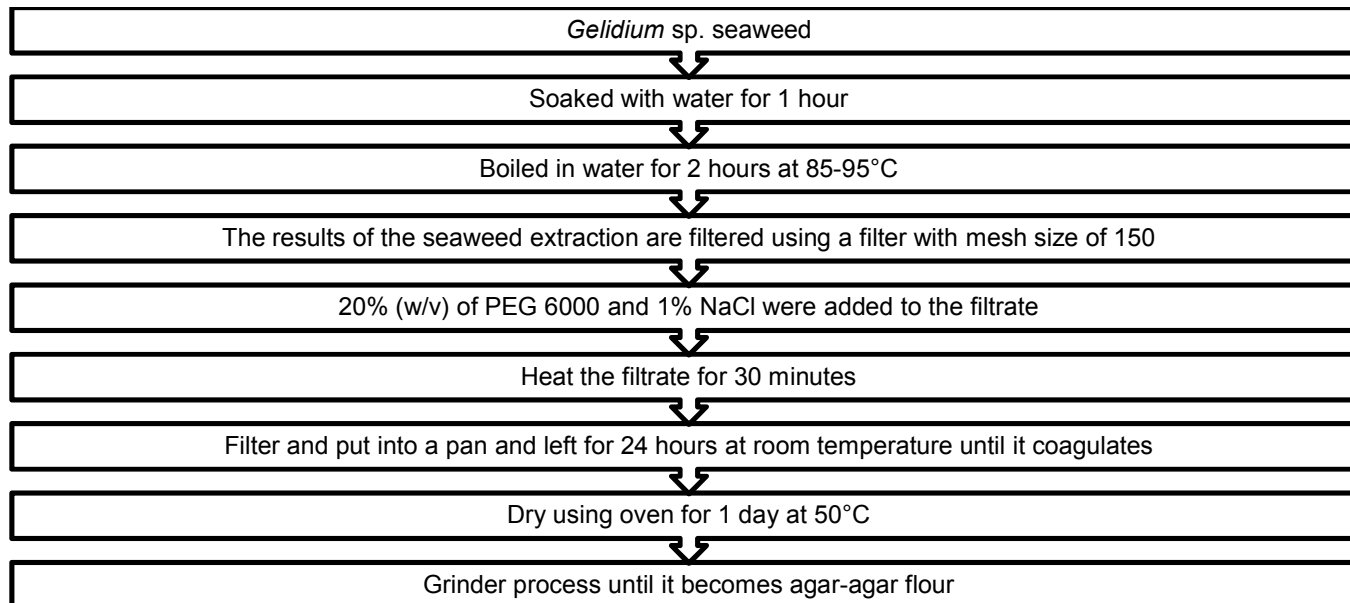


Fig. 6. Extraction method of *Gelidium* sp. by Shantika et al. [3]

Before extraction, *Gracilaria verucosa* seaweed was soaked for 2 hours using 0,5% CaO solution. Then, wash with running water and then oven-dried for 8 hours at 60°C. The dried seaweed obtained is put and stored in a closed plastic bag and ready for extraction.

The extraction process begins with soaking the dried seaweed in water for 1 hour at 25°C. After boiling with water, do it 2 times. The first boiling is at 80°C for 1 hour and the second is at 100°C for 2,5 hours. Then, boiling was continued using 3% NaOH solution for 3 hours at 80°C. The next process is washing. Wash under running water to remove excess lye. The next stage is extraction. Extract by boiling it in distilled water for 2,5 hours at a pH of 7,0-7,5. After that, filter using a filter cloth/blancu cloth. The filtrate obtained was dried for 24 hours in an oven with a temperature of 80°C.

3.3 Extraction of *Gelidium* sp. by Shantika et al. [3]

This agar extraction method was taken from the research of Shantika et al. [3] who extracted agar using *Gelidium* sp. in making bakto agar as a compactor for microbial growth media. This research includes the manufacture of agar, determination of agar yield, determination of water content, protein, fat, carbohydrate content, determination of gel strength, and total plate count testing.

Seaweed *Gelidium* sp. immersed in water for 1 hour, the ratio used is 1:20, weight/volume. Furthermore, boil a seaweed in water for 2 hours at a temperature of 85-95°C. The extraction results from seaweed are filtered using a sieve with mesh size of 150. Then, 20% (w/v) of PEG 6000 and 1% NaCl are added to the resulting filtrate and then reheat for 30 minutes. After that, filter again and put into the pan and left at room temperature for 24 hours until it coagulates. Then, dry it for 1 day at 50°C using an oven and continue with the grinder process until it becomes agar-agar flour.

The advantage of using the agar extraction method is that it can produce bio-agar which is within the limits of the commercial quality standard for bio-agar based on the values of several parameters observed such as moisture content, ash content, protein, fat, carbohydrates, sulfate content and gel strength, although overall, the values of these parameters were still below the yield of the bakto grade to commercial standards.

4. CHARACTERISTIC OF AGAR

The quality of agar from seaweed can be affected by several factors such as the quality of seeds, the choice of location for seaweed cultivation, cultivation methods, harvest age, maintenance, and post-harvest handling and processing [33]. The Physico-chemical of a phycocolloid such as agar is an important indicator of the quality product to be accepted by the market.

The characteristic of agar-agar are rigid, brittle, malleable, and have a certain melting point. Acidity (pH) greatly affects the strength of agar gel. When pH decrease, the strength of agar gel is getting weaker to a pH of 2,5 [35].

Agar with different physico-chemical properties will have different functions in its use in the market and will greatly determine the price of the product. Based on the results of research by Yolanda and Agustono [1] on the physical and chemical characterization of agar powder *Gracilaria* sp. at PT. Java Biocolloid Surabaya obtained the following results:

Table 1. Agar-agar Characteristic Standard

Component	Spesifikasi
Particle size	80-100 mesh
Water content	<18%
Ash content	<6,99%
Heavy metal	<10 ppm
Arsenic	<3 ppm
pH	6,8 – 7,0
Solubility	Dissolves at 100°C

Source: Poncomulyo et al. [34]

Agar powder produced by PT. Java Biocolloid has a standard value in determining the physico-chemical characteristic of agar powder. Granulometric value of 100% pass mesh size 60, standard mesh size for agar is 60 or 2,5 mm siever. The size of powder can affect the pH and the amount of mineral content contained in it [36]. Turbidity 18-21 NTU, according to Wenten [37] a good turbidity for agar is around 25-35 NTU. pH 6,4-6,8, according to Rosulva [9], the pH value of agar powder is 6-7. Gel strength 8000-1000 g/cm². Viscosity 17-19, the standard viscosity value set by the FAO is a minimum of 15 cP (centipoises) [27]. Also, 8-11% moisture content, a maximum of 18%. Moisture content affects the shelf life of agar powder and shows the stability and quality index of ingredients. Agar powder with low moisture content is less easily damaged than agar powder with high moisture

content. The value of the moisture content of agar powder produced by PT. Java Biocolloid shows a lower value than the commercial standard value.

Another research by Utomo and Satriyana [38] regarding the physico-chemical properties of agar from *Gracilaria chilensis* seaweed extracted with different amounts of water, resulted in an analysis of the raw material showing that dried seaweed *Gracilaria chilensis* has a moisture content about 19,26%. The maximum moisture content required by SII for dry seaweed ranges from 15% to 23% [39]. CAW content of seaweed is 46,18%, this CAW content shows the purity of seaweed is the cleanliness of seaweed from impurities such as sand, rocks, or other seaweed mixtures. The yield, gel strength, and sulfate content of agar from the extraction of *Gracilaria chilensis* stated that the amount of extracting water 10 times of weight dry seaweed, it will cause the yield to decrease to 10,04% [40].

The average value of gel strength produced in this study ranged from 98,57 g/cm². The average value of agar sulfate content in this study ranged

from 1,77% to 2,55%. The results of the analysis of variances showed that the handling of different amounts of extracted water did not have a significant effect on the sulfate content of agar. The average value of 3.6 anhydro-galactose levels in this study ranged from 36,55% to 37,04%. The results of the analysis of variance showed that each handling with the addition of carrageenan iota with different concentrations did not have a significant effect on the levels of 3.6 anhydro-galactose.

Utomo and Satriyana [38] is research results can be concluded that the amount of extracted water as much as 20 times gives agar results with the highest yield of 20,21%. The amount of extracted water handling did not significantly affect the parameters of sulfate content, gel strength, 3.6 anhydro-galactose content, gel formation temperature, and gel melting temperature from the resulting agar. The use of 20 times the amount of extracted water is also relatively easy to carry out the extraction, where filtering the filtrate is quite easy and the results are easy to clot.

Table 2. Agar Powder Characteristic Test Results

No.	Physico-chemical	Percentage	Standard
1.	Granulometry	100% pass mesh size 60	60 mesh size
2.	Turbidity	18 – 21 NTU	25-35 NTU
3.	pH	6,4 – 6,8	6-7
4.	Gel Strength	800 – 1000 g/cm ²	150-600 g/cm ²
5.	Viscosity	17 – 19 cP	15 cP
6.	Moisture content	8 – 11%	15 – 21%

Source: Yolanda and Agustono [1]

Table 3. Yield, Gel strength, and Sulfate content of Agar

Parameter	The amount of extracting water		
	15 times	20 times	25 times
Yield (%)	17.32±1.22 ^a	20.21±1.05 ^b	19.94±0.99 ^{ab}
Gel strength (g/cm ²)	112.14±11.99 ^a	119.28±11.94 ^a	98.57±11.95 ^a
Sulfate (%)	2.28±0.54 ^a	1.77±0.53 ^a	2.55±0.55 ^a

*description: numbers on the same line followed by a different superscript letter (a, b, etc.) show significantly different

Source: Utomo and Satriyana [38]

Table 4. Anhydro-galactose content, Gel formation temperature, and Gel melting temperature

Parameter	The amount of extracting water		
	15 times	20 times	25 times
3.6 Anhydro-galactose content (%)	36.55±0.99 ^a	36.68±1.13 ^a	37.04±0.97 ^a
Gel formation temperature (°C)	33.36±0.58 ^a	34.33±0.58 ^a	34.53±0.5 ^a
Gel melting temperature (°C)	84.43±1.32 ^a	85.63±1.76 ^a	83.56±0.76 ^a

*description: numbers on the same line followed by a different superscript letter (a, b, etc.) show significantly different

Source: Utomo and Satriyana [38]

5. AGAR APPLICATION FOR FOOD ADDITIVES

Research by Aini et al. [41] regarding the characteristics of analog rice from corn-red bean flour using agar as a binder, this research is developing on agar by utilizing it as a binder for brown rice from corn and red bean flour. In this study, agar is used as a binder because it is easy to obtain rather than the other binders such as gum or carrageenan. Molecules and water of agar-agar will move freely when heated in water to form a gel.

According to Bourekoua [42] agar can be like a soft solid with many pores in the inside when dissolved in hot water and also cooled so that it will have a chewy texture. Because of this, it is expected that the elasticity level and sensory value of the analyzed analog rice can be improved by adding agar to the analog rice. Based on the results of research, it can be seen that analog rice made from corn flour and red bean flour with a ratio of 70:30, and with the addition of 1,5% agar is the best result.

Agar can also be used in the manufacture of green grass jelly drink to reduce the level of syneresis, which is the time when water leaks from the grass jelly gel due to new bonds formed between the polymers in the grass jelly gel structure [43]. If syneresis level is high, it can damage the gel in product, so that a hydrocolloid substitution is needed which can help bind water to the product and reduce the level of syneresis in gel [44]. Ramadhan and Trilaksana [45] say that agar is suitable for use as a single gelling agent in formulations, because it has good gel resistance at low pH conditions, as well as the binding capacity to the moisture content of the material. Based on research by Hardoko et al. [46] regarding the substitution of agar in the manufacture of green grass jelly drink (*Cyclea barbata*) to reduce syneresis, the results showed that agar can reduce the level of syneresis in green grass jelly drink, with a 20% substitution of agar is the best result. However, in making jelly drink, a formulation is needed so that the texture of jelly drink is not too hard or soft, because the jelly has a strong gelling character but breaks easily.

In making edible film, agar-agar also can be added because it is very easy to obtain, but has similar properties to carrageenan. Besides that, edible film that has been added with agar can also be used as packaging for wrapping jenang food. Such as the research by Kasfillah et al. [47]

which analyzed the characterization of edible film from jackfruit seed flour and agar as jenang food wrapper, the result show that the added agar could affect characteristic of edible film included thickness with a value of 0,126 mm, tensile strength of 3,502 Mpa, elongation of 1,904%, moisture content of 7,08%, and water resistance. This edible film is also classified as environmental friendly and can protect food products, also can be eaten directly and safe for the environment [47].

Hydrocolloid that can make edible film such as gel, soy protein, corn protein and wheat protein, where the edible film made from hydrocolloid has the advantage of being able to protect products well against oxygen, carbon dioxide, as well as desire mechanical properties and enhancing the structural integrity of product. However, there is a deficiency such as the edible film solution with variations of agar has a high thickness. This can occur because the shape and texture of edible film solution that using agar is not good.

6. CONCLUSION

Based on result from articles and references that had been review, it can be concluded that: 1) Extractable types of seaweed into agar are *Gracilaria* sp, *Gelidium* sp, and *Gelidiella* sp. 2) The agar extraction from seaweed could be done by Yolanda and Agustono [29], Wicaksono et al. [2], and Shantika et al. [3] methods. The agar characteristic can be reviewed by particle size, moisture content, ash content, heavy metal, pH, solubility, turbidity, gel strength, and viscosity. 4) Agar-agar as food additives had been applied to analog rice, jelly drink, and edible film making.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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