1. INTRODUCTION

1.1. Background of Research

In Indonesia, seaweed is one of the biggest marine commodity. Based on *Direktorat Jenderal Perikanan Budidaya* (2011), seaweed production has been increasing every year around 30,57%. At first, there are not much seaweed cultivation in Indonesia, mostly only at South Sulawesi, but now increasing since the cultivation methods are getting easier. There are two big types of seaweed cultivated in Indonesia which are *Euchema cotonii* and *Gracilaria sp*. The difference between them two is the cultivation media. *Euchema cotonii* cultivated in the sea while *Gracilaria sp.* in the fishpond. The agar from these seaweeds mostly used in food industry as emulsifier, microbe media, and stabilizer (Chapman, 1980).

Red algae or Rhodophyta is one of the oldest groups of eukaryotic algae and some of them are known as agarophyte that produce hydrocolloid agar in their cell walls. Agarophyta divided into three big orders which are Gelidiaceae (*Gelidium, Pterocladia, Gelidiella*), Gracilariales (*Gracilaria*) and Ahnfeltiacea (*Ahnfeltia*). *Gracilaria* is the largest source to produce agar for extraction. Seaweed or algae is commonly used as ingredients of a lot of products such as cosmetics, textile, farmacy, and foods. In seventh century food industry, seaweed from *Gelidium sp.* mostly used to produce agar. Since the production of agar is began to increase, *Gracilaria sp.* was employed to complement the lack of *Gelidium sp.* in nature. *Gracilaria sp.* mostly cultivated in Chile, China, Taiwan, and other countries on a very large scale.

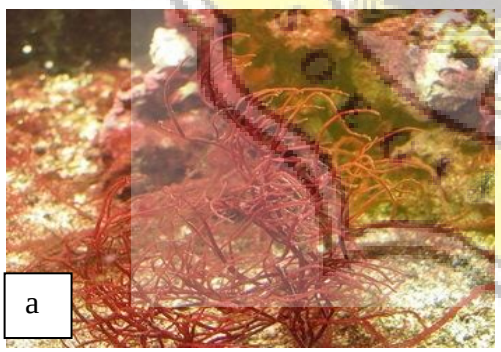
The problems of using *Gracilaria sp.* as the ingredient of agar are the quality of its gel that still low such as the strength of gel, texture of gel, and also the low amount of agar rendement. Glicksman (1983) *cit.* Heri (2015) stated that these problems are caused by the appearance of ester sulfate inside the agar (at C-6 atom) that bend the structure of agar and inhibit the gelling process. According to Guiseley *et al.*, (1980) using alkaline such as NaOH can remove the C-6 atom of L-galactose and idealize the structure of agar. The conventional extraction process of agar mostly produce agar with non-

appealing color that makes it hard to be accepted by the market. The addition of bleaching agent such as $CaCO_3$ or calcium carbonate will make the color of agar brighter, clearer, and accepted by the market. Therefore, the additon of NaOH and $CaCO_3$ in this study focusing in solving these problems of *Gracilaria verrucosa*.

1.2. Literature Review

1.2.1. Seaweed

Seaweed is macro algae that classified as low level plant and based on the classification, seaweed is included in *Thallophyta* division. As a low level plant, the morphology of seaweeds are thallus, stems, and leaves (Meiyana, *et al.*, 2001). *Thallophyta* divided into four big classes which are Red Algae (*Rhodophyceae*) (a), Blue Algae (*Cyanophyceae*) (b), Brown Algae (*Phaephyceae*) (c), and Green Algae (*Chlorophyceae*) (d) (Glicksman, 1982) (Figure 1.a, b, c, d). Green algae and blue algae mostly live in fresh water while brown and red algae grow in the sea. Red and brown seaweed are important for marine life because both containing polysaccharide in a big amount that other plants do not. The polysaccharides are algine, carageenan, funoran, and agar.



Source: Indonesia.alibaba.com

Source: ucmp.berkeley.edu/greenalgae

Source: pinterest.com

Source: https://www.asissludge.com

Figure 1. Red Algae (*Rhodophyceae*) (a); Blue Algae (*Cyanophyceae*) (b); Brown Algae (*Phaeophyceae*) (c); Green Algae (*Chlorophyceae*) (d)

From the classification by Dawson (1966), *Gracilaria sp*. included in *Rhodophyceae* class. The color of *Rhodophyceae* is red to green and its affected by biliprotein substances (phycocyanin and phycoerythrin) (Goodwin,1974). Kadi & Atmadja (1988) added that chlorophyll and carotene are also affecting the color of *Rhodophyceae*. The amount of chlorophyll contained in *Rhodophyceae* is 0,3-2,0% (Meeks, 1974). Based on the living habitate, *Gracilaria sp.* as one of *Rhodophyceae* can also cultivated in fishpond after 60 days (Ahda, *et al.,* 2005 *cit.* Melindasari, 2013). The characteristic of *Gracilaria sp* is having flat or cylindrical thallus with smooth surface (*Badan Penelitian dan Pengembangan Pertanian,* 1990). *Gracilaria sp* is one of the most potential seaweed to produce agar. Seaweed from *Gracilaria sp* commonly used because the price is low and is easy to get

The chemical content of *Gracilaria sp* is different based on type, growth location, age, and cultivation methods that affects its quality and price. Based on Kadi & Atmadja (1988) the agar content from *Gracilaria sp* is various from 16-45%. The contents of *Gracilaria sp* are shown at Table 1

Table 1 Content of *Gracilaria sp.*

	Maximum	Minimum
Gelation temperature	39°C	32°C
Gel melting temperature	--	85°C
Moisture	20%	--
Ash	6.5%	--
Ash, acid-insoluble	0.5%	--
Foreign organic matter	1.0%	--
Foreign insoluble matter	1.05%	--
Foreign starch	0	--
Gelatin	0	--
Water absorption	--	5 times its weight
Arsenic	3 ppm	--
Lead	10 ppm	--
Other heavy metals	40 ppm	--

Source: Selby & Whistler (1993)

1.2.2. Agar

Agar is hydrophilic colloid that extracted from sea algae class *Rhodophyceae* (Peterson & Johnson, 1978). Agar is ester sulfate substance that is not soluble in cold water (Putro, 1991 *cit.* Pipih, 2009) and the chemical structure of it is 3,6-anhydro L-galactose (Furia, 1975 *cit.* Utomo, 2006). The structure of agar divided into two main parts which are agarose and agaropectin with various amount (Glicksman, 1983). The sugar units of agarose are D-galactose, L-galactose, 3,6-anhydrogalactose, D-xilose, while the sugar units of agaropectin are D-galactose, L-galactose, 3,6-anhydrogalactose, D-xilose, sulfate galactose, and pyruvate acid. Agarose is a neutral gel former component with no sulfate content (Furia, 1975). Peterson & Johnson (1978) also added that agarose is composed by repeated agarobiosa unit. Agaropectin is sulfate polysaccharide that composed by agarose with sulfate acid ester, D-glukoronat acid and pyruvate acid (Peterson & Johnson, 1978). The structures of agar are shown below at Figure 2.

Agar that distributed into the market are various, there are granule, powder and other forms of agar. In Indonesia, the quality standard of agar has been included in *Standar Industri Indonesia* (SII) as shown in Appendix 1. Agar specifications are also described in Food Chemical Codex (1981) that included arsenic, ash content, gelatin, heavy metal, drying loss, and water holding capacity.

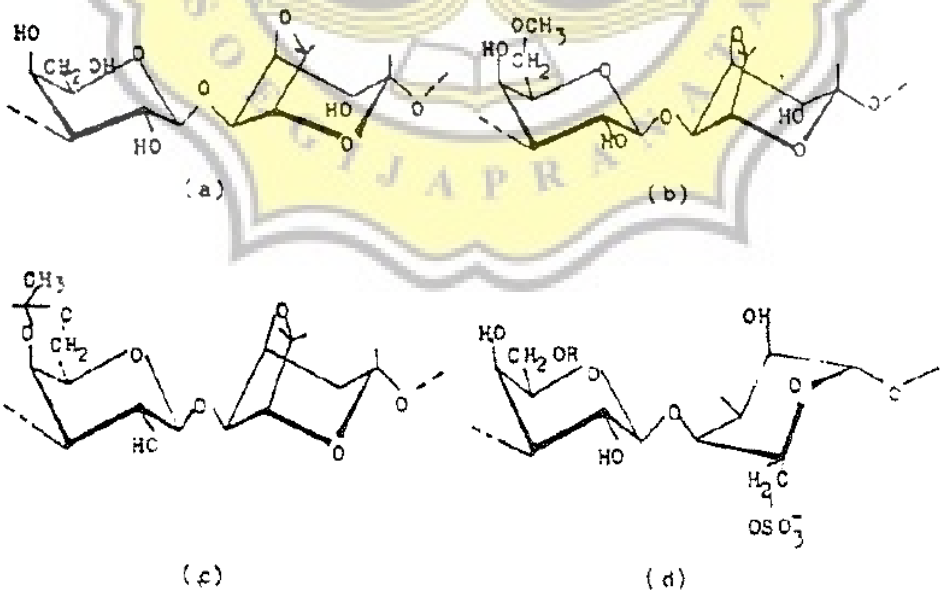


Figure 2. Agar Structure: (a) agarose, (1-3) d-galactose and (1-4) anhydro-L-galactose; (b) metil agarose (1-3) 6-0-metil-d-galactose and (1-4) anhydro-L-galactose; (c) pyruvated agarose; (d) sulfate galactant (Chapman and Chapman (1980)

1.2.3. Process of Making Agar

The processing of seaweed are divided into cleaning, washing, soaking, bleaching, alkaline treatment, extraction process, filtering, freezing, drying, and thawing (Indriany, 2000).

a. Cleaning and Washing

Cleaning and washing process needed to remove all the dirts, stones, mud, and other debris. Right after cleaning and washing process, the seaweed must be dried to prevent a fermentation process that decrease the quality and colloid content (Putro, 1991). The drying process can be done under the direct sunlight or using cabinet dryer/oven. This drying process will also help the removal of seaweed's color (Putro, 1991).

b. Soaking and Bleaching

Soaking the seaweed is needed to remove all the debris and to make the tissue soft. It also to ease the extraction process (Indriany, 2000). In the bleaching process, stirring the seaweed is needed with soaking time around 30 minutes. The washing process needed to be done to remove the smell the bleaching agent.

c. Alkaline Treatment

The usage of agar is mostly for gelling agent in a solid media. It is important to avoid the interaction between the media and the component such as meat extract, peptones, proteins, sugars, pigments, and many more. (Armisen, 1987) To obtain the agar, the seaweed need to get extracted. One of the steps is alkaline treatment. This step is well known as a technique for gelling and being used in the food industry to eliminate the sulfate residues. Pretreatment using sodium hydroxide affect the relaxation spectrum of agar gels (Santos, 1990) and the rheological properties of agar gel. Tagawa & Kojima (1972) *cit.* Armisen (1995) stated that agar isolated from *Gracilaria* is suitable for commercial purposes only after the treatment with alkali. The yield and physical properties of agar such as gel strength determine its commercial value. The extraction methods using alkaline treatment increasing the strength of gel and the quality of the agar (Tagawa, 1963).

d. Extraction Process

A high temperature of water is needed in seaweed extraction based on the solubility characteristic of agar that only soluble in hot water (Furia, 1980). The temperature of water is around 90 to 150^{o}C that followed with filtration and freezing process (Wheaton & Lawson, 1985). When extracting the seaweed, the pH must be in neutral condition to make it easier with 100^{o}C for 1 to 4 hours. One of the factor of extraction process is time. The more extraction duration, the hydrolytic degradation would happen that will decrease the quality of gel of agar (Matsuhashi, 1977 *cit*. Priatama, 1989).

Clear water or aquadest is used in the extraction process (Winarno, 1990) and the amount of the water is various based on the type of the seaweed, seaweed from *Gelidium sp*. is need more water than *Gracilaria sp*. because of its hard texture, water is needed to break the cell wall (Sukamulyo, 1989). Extraction time is also various, Nasran (1993) stated that *Gracilaria sp.* usually needs 1.5 to 2 hours of extraction. After being extracted, the agar solution is filtered in a hot temperature with a little pressure (Chapman, 1970). The filtrate then freezed and thawed in the next day (Kosasih & Suprijatna, 1967)

e. Drying

Drying process of agar divided into two parts which are freezing-thawing methods or pressure drying (Aveline in Glicksman, 1969 & Mitsuhashi, 1977). Drying using oven is better to fasten the drying process and lowering the water content of agar (Kosasih & Suprijatna, 1967).

1.3. Objectives

The objective of this research is to find the best alkaline and bleaching solution concentration to produce agar powder that has the best gel characteristics such as texture, color, aroma, and pH compared to the commercial agar in the market.