4. DISCUSSIONS

Agar is the main constituent of the cell walls from red algae and mostly extracted from macroalgae orders *Gracilaria* and *Gelidium* (Craigie, 1990). Agar is one of pollysacharide that characterized by their gelling and stabilizing ability. Agar has many application in food processing, pharmaceuticals, cosmetics, microbiology, molecular biology, and also biotechnology according to Armisen (1997). The structure of agar constituted by disaccharidic repetitive unit of D-galactose and 3.6-anhydro-L-galactose (Imerson, 2011). An alpha origin, the harvest season, and extraction process are some factors that can substitute these repetitive disaccharidic unit into sulfate, methyl, carboxyl, or pyruvate groups (Imerson, 2011; Armisen *et al.*, 2000; Nussinovitch, 1997) Extraction of agar is a process where seaweed is washed to remove foreign matter and then heated with water for several hours. There are some differences in the treatment of the seaweed prior to extraction depending on the genus used. *Gelidium* is simply washed and extracted with hot water, while *Gracilaria* must be treated with alkaline before extraction. This pre-treatment causes a chemical change in the agar. resulting in agar with an increased gel strength.

In this research, there are NaOH used as the alkaline treatment and $CaCO_3$ or calcium carbonate as the bleaching agent. Calcium carbonate is one of the most stable phase bleaching agent and commonly used in industries (Lailiyah *et al.*. 2012 *cit.* Noviyanti *et al.*. 2015). Alkaline treatment of *Gracilaria* is well known in food industry to eliminate the residue of sulfate and it forms 3.6-anhydro-L-galactose. This alkaline treatment affect the relaxation spectrum of agar gels and improve the rheological properties of agar gel (Nishinari & Watase, 1983 *cit.* Chirapart, 1995). The concentrations of both alkaline and bleaching are 2, 4, 6, 8, and 10% and 2 controls from commercial and the other is no treatment agar. The objective of this research is to find the best concentration of NaOH and $CaCO_3$ to produce agar with the best characteristic compared to the control therefore physical and chemical anaylsis are needed. Physical analysis including texture, color, and rendement, while chemical analysis including pH and water content. All the data from analysis are served in mean data with deviation standard. Deviation standard shows heterogen level of the data. The higher level of deviation standard indicates the scatter level of the data which means the data is different from the others.

4.1. Physical Analysis

4.1.1. Texture Analysis

The aim of texture analysis is to see the effect of NaOH addition to the agar gel forming process. The method of gelation process is by heating the sample and let the temperature down until it compeletely firms and set. According to Table 3 and Figure 10, the texture range of seaweed gel is between 15.33 to 91.80 gf. Agar texture and gel strength value were increasing with addition of NaOH. The addition of NaOH will increase the gel strength value because it can remove the excess sulfate inside the agar. because sulfate will inhibit the agar to bind (Disantina, 2008). The increasing of gel strength is also affected by $CaCO_3$ by creating calcium bridge due to the interaction between Ca^{2+} and carboxyl group (Bronner & Jack, 1982 *cit.* Lesmana, 2008).

Some factors affecting the gel strength are sulfate content, agarose content, temperature, and pH level (Glicksman, 1983; Selby & Wynne, 1973). The forming of agar gel is caused by three hydrogen atoms in 3.6-anhydro-L-galactose that push the molecules to form helix structures. The interaction between these helixes will form gel, therefore the higher content of 3.6-anhydro-L-galactose the higher helix structures formed and the gel will increase (Glicksman, 1983). The addition of NaOH is also important to keep the pH level on a stable point because the 3.6-anhydro-L-galactose will not work well on a acid pH level. The acid situation will hydrolyze beta-1.4 that connects D-galactose with 3.6-anhydro-L-galactose (Armisen & Galatas, 1987)

4.1.2. Color Analysis

Color analysis was done by looking at the effect of $CaCO_3$ addition to L*. a*. and b* value of sample surface. This analysis was done with chromameter. Meacock *et al.*. (1997) *cit.* Benjakul *et al.*. (2004) stated that bleaching agent will increase whiteness on the sample. Park (2000) *cit.* Benjakul *et al.*. (2004) also stated that the addition of calcium carbonate will turn the sample into white. According to Table 3, the higher level of calcium carbonate will also increase the L* value of agar from *Gracilaria verrucosa*. The lightness of agar is scattered at range 39.75 for the control with no calcium carbonate to 52.17 for 10% concentration of NaOH and $CaCO_3$. The brightest

color of agar is coming from the commercial one because the control is not only containing agar but also vanilla extract and other ingredients that makes the result is not accurate. L* value indicates brightness of the sample with range from 1 to 100. a* value divided into two parts, a* positive indicates red and the negative indicates greenish color, while b* that also divided into positive that indicates yellow and negative indicates blue (Gaurav, 2003).

Some of the agar from the extraction process is having a muddy color, this is caused by calcium that was not dissolved in the system (Lesmana, 2008). The effect of calcium carbonate addition can be seen on comparison between sample with calcium carbonate and the one without it.

4.1.3. Rendement Analysis

Agar rendement powder was calculated by ratio of the weight of dried agar and dried seaweed. The weight of dried seaweed used in this anaylsis is 45 g. The value of rendement is affected by water content, ash content, and cellulose content (Yunizal, 2004). Chapman (1980) also added that the method of extraction is also take an important role of the amount of rendement. The extraction process of this research is affected by the various pH level due to NaOH addition. The rendement will increase because the addition of NaOH as alkaline treatment that break cell walls that makes the agar flow out easily while being extracted (Linggih, 1988 *cit.* Pranata, 2004). The rendement of the agar are vary at range 1.16 for concentration 2% of NaOH and $CaCO_3$ and 4.31 for concentration 10% of NaOH and $CaCO_3$.

4.2. Chemical Analysis

4.2.1. Water Content Analysis

All the data from water content analysis are suitable to the standard from SII with maximum amount 23%. Low water content will prevent spoilage of the sample because the growth of microorganism is slow (Winarno, 1995). Water content analysis is needed to see the amount of water inside the agar powder (Kumala *et al*, 2013). There are some factors that affect the water content which are air humidity, temperature, and air flow

(Taib, *et al*., 1988). While doing the analysis, agar was dried using cabinet dryer that effective enough to dry the sample and lowering the water content (Kosasih & Suprijatna, 1967). Based on the Table 2, the water contents are various between 3.19% to 23.18% which correct based on SNI (*Standar Nasional Indonesia*) that is 24% maximum.

4.2.2. pH Analysis

The analysis of pH level is measured using pH meter (Kohlman, 2003). During this analysis, all the samples measured for 5 to 10 minutes until reach a stable number with two repetition. Based on the result, the pH level is increasing along with the increase of NaOH. The reason is because NaOH is one of strong base that usually used as alkaline treatment especially in the extraction process (Santika *et al.*, 2014). With the addition of $CaCO_3$ the value of pH is increasing as well because calcium carbonate is also a base compound. Based on the Table 2, the pH values are various at range 6.94 for control and 10.42 for 10% NaOH and $CaCO_3$ concentration. The standard of pH level for agar is at range 6 to 7 (Rosulva, 2008) which means the pH level that acceptable are between control until 4% NaOH and $CaCO_3$ concentration because it keeps increasing more than 7.99 for the next concentration.

From the physical and chemical analysis, agar treated with 10% of NaOH and 8% of $CaCO_3$ has a better result compared to the others as shown at Appendix 2. From Table 3, the texture of agar with 10% of NaOH and 8% of $CaCO_3$ is 69.79 gf, similar to the commercial agar that has 63.03 gf texture value. From Table 3, the rendement of agar with 10% of NaOH and 8% of $CaCO_3$ is 4.19 which similar to the commercial and has a better result compared to the other treatment agar that has the rendement value under 3%. From the color analysis, the color of agar with 10% of NaOH and 8% of $CaCO_3$ has a better result which is 52.08 compared to the other treatment agar that has value far from the commercial.