Comparison of Gel Preparation Methods on Gel Strength Measurement of Carrageenan

Fateha¹, N Ulya¹, Asmanah¹ and Agusman²

¹ Physical Laboratories of Research Center for Marine Fisheries Product Processing and Biotechnology
² Researcher in Research and Development Center for Marine and Fisheries Product Processing and Biotechnology, Jalan KS. Tubun, Petamburan VI, Slipi, Central Jakarta 10260, Indonesia

E-mail: fateh_1504@yahoo.com

Abstract. This study aims to develop a carrageenan gel strength test method with an emphasis on the carrageenan gel preparation method for measurement. The gel was prepared by comparing the two heating temperatures (80 °C and 90 °C), heating devices (waterbath and hotplate) and measurement (with container and without container). The results showed that there were no difference between cooking temperatures of 80 °C and 90 °C, but heating at 80°C obtained a lower deviation standard. The use of hotplate and waterbath were not significantly different resulted in gel strength, but the use of hotplate had a slightly larger variation than waterbath. Measuring the strength of the gel that was still in the crystal container and removed from the container were not significantly different, but measurements with the gel condition were still in the container obtained more accurate test results. Based on the results of this study it can be concluded that the carrageenan gel preparation technique by heating at a temperature of 80 °C using a waterbath or hotplate is the best preparation test method. Meanwhile, the gel does not need to be removed from the container before being measured using a texture analyser. The development of this method can be used as a standard gel preparation method for measuring the strength of carrageenan gel.

1. Introduction

Indonesia is the largest seaweed producing country in the world which is the main commodity in the fisheries sector. In Indonesia, the source of caraginophytes comes from seaweed Eucheuma and Kappaphycus. Seaweed production in Indonesia reached 38.7% of the total world seaweed production in 2016 [1]. The abundant amount of production opens up opportunities for seaweed and caraginophyte utilization in various industrial fields including food and beverage, chemical, textile, pharmaceutical and others [2–5]. The widespread application of carrageenan has made carrageenan in great demand and has a high economic value.

Carrageenan is a polysaccharide obtained through the extraction of several species of red seaweed (Rhodophyta) such as Eucheuma cottonii and Eucheuma spinosum [6]. Commercial carrageenan is divided into 3 types, namely kappa-, iota- and lambda-carrageenan [7]. The three types of carrageenan have different properties. Kappa carrageenan has high gel-forming ability but has a tendency to undergo syneresis, carrageenan iota has a weak gel-forming ability and carrageenan Lambda has the ability to form high viscosity solutions [8].
Gel strength is the ability of the gel to withstand loads per unit area. The quality of carrageenan is identical to gel strength [9,10] including those that are directly related to its characteristics and functional properties as a thickener, film forming, texture forming and gelling agent. The gel strength is also a physical property of carrageenan that determines the application of its use in the industrial sector to the determination of its selling value [2,11,12].

The gel strength testing method includes several stages, namely preparation, heating, clotting and measuring the texture. Testing of carrageenan gel strength generally uses samples with a concentration of 1% - 1.5% (w/v), and potassium chloride as much as 0.2%, temperature variations of 80 °C - 90 °C [10,13–15]. [16] stated that KCl (potassium chloride) interacts better than ammonium salt and sodium salt and is effective in increasing the strength of carrageenan gel. However, no research has yet compared the preparation technique with heating devices, temperature or measurement with a container or without a container. The variation in preparation methods and measuring the gel strength of carrageenan is one of the bases for conducting this research used to obtain more validated measurement data. In addition, the study of the preparation method and gel strength measurement is expected to support the development of quality standardization of seaweed quality in Indonesia.

2. Method

2.1. Material
The material used in this study is a commercial pure carrageenan type (kappa refined carrageenan) obtained from PT. Centram (Surabaya, Indonesia). The chemical aids used are KCl (potassium chloride) pure analysis (1,04936 Merck).

2.2. Equipment
The equipment used includes waterbath (Memmert), hotplate (Multiple Stirring Hotplate, Velp Scientifica, Milano, Italy), refrigerator and texture analyser (TAXTplus Stable Micro System, Goldamig, Surrey, UK).

2.3. Carrageenan gel preparation
The method of preparation of carrageenan gel strength [17]. The experiment to test the gel strength value begins with a comparison of the waterbath heater and the hotplate, the best heating device used in the test experiment by comparing temperatures of 80 °C and 90 °C. The best test experiments of heating and temperature devices are then used in the experiment using containers and without containers at the time of measuring the gel.

2.3.1. Effect of heating device on the measurement results of carrageenan gel strength
A total of 3 g of carrageenan flour was added to 197 g of distilled water using a 250 ml baker, then stirred with a magnetic stirrer until homogeneous. As much as 0.6 g of KCl is added and heated with waterbath (Memmert) or hotplate (Multiple Stirring Hotplate, Velp Scientifica, Milano, Italy) until dissolved at 80 °C [17]. The stirring process in the hotplate heater uses a magnetic stirrer, while the waterbath heater uses a spatula. The solution was keep at 80 °C ± 2 °C for 15 minutes. Then the gel liquid was poured to the brim into a glass container (7 mm in diameter, 4 mm in height) and then covered with a glass cover (8 mm long x 8 mm wide) in order to obtain a flat surface and avoid water evaporation during the clotting process. After the solution has stalked (1-2 hours), the glass container is turned over and stored in the refrigerator at 10 °C for 12-15 hours. Seven carrageenan gel samples were prepared for each experiment. Then the gel strength was measured with a Texture Analyser (TAXTplus Stable Micro System, Goldamig, Surrey, UK). The best gel strength analysis results using a heating device will be used in the experiment to see the effect of temperature on the gel strength value.
2.3.2. **Effect of heating temperature on the gel strength of carrageenan**

Observation of the effect of heating temperature (80 °C ± 2 °C and 90 °C ± 2 °C) on the measurement results of carrageenan gel strength following the test procedure as shown in point 2.3.1 using one of the best heating devices (waterbath or hotplate) based on the results statistical analysis. The best gel strength analysis results were then used in the experiment to see the effect of the container on the gel strength measurement value.

2.3.3. **The effect of the crystallization container on the results of measuring the gel strength of carrageenan**

The best experimental results are at point 2.3.1. and 2.3.2 (heating and temperature apparatus) were then used in the preparation to compare the gel strength measurement experiments to see the effect of the container (without and with the container). The rest of the preparation and measurement steps are the same as before.

2.3.4. **Measurement of gel strength with Texture Analyser**

The lid of the container gel was removed, and then the gel strength was measured by a Texture tool (TAXT Plus Stable Micro Systems, Goldaming, Surrey, UK) using a probe with a diameter of 12.5 mm at a constant speed of 1 mm/s until broken gel with a penetration depth or a distance of 15 mm. The value that appears on the monitor is the gel strength in grams per square centimeter (g/cm²) [18].

2.4. **Statistical analysis**

The statistical analysis of the results of this study was carried out using SPSS version 22 software. The data obtained were calculated the mean value, standard deviation (SD) and coefficient of variance (CV) to see the precision of the test. The t test is a test that measures the level of significance of the mean difference between a group of data with a mean value [19]. Comparison of the results of preparation and measurement of gel strength with various experiments (heating equipment, heating temperature, container) using the t-test and data distribution is displayed in the form of a box plot.

3. **Result and Discussion**

3.1. **Comparison of the results of measuring gel strength based on heating devices**

The heating device used as a comparison is a waterbath and hotplate equipped with a magnetic stirrer. Both of these heating devices are commonly owned by laboratories so they have no difficulty in their application. Waterbath and hotplate are laboratory equipment that function as bathers with different media, namely the waterbath conducts heat through the water medium while the hotplate conducts heat through the plate. The principle of waterbath and hotplate heating is more or less the same, namely providing heat and temperature that can be adjusted as desired [20,21].

Table 1 showed the comparison of the results of measuring the gel strength between the waterbath heater and the hotplate. The measurement results show that the gel strength with a waterbath heater ranges from 1206.05 g/cm² - 1379.06 g/cm², while the hotplate heater ranges from 1188.61 g/cm² - 1352.13 g/cm². The results of the calculation with the t-test statistic showed that the two tests were not significantly different (p = 0.289), meaning that the use of water and hotplate would produce relatively the same gel strength test values. Furthermore, from the calculation of the value of standard deviation (SD) and coefficient of variance (CV) from 7 times the test, it was found that the use of the hotplate gave a slightly greater value for SD (67.32) and CV (5.31) than waterbath. This showed that the precision of using waterbath is better than using hotplate. The distribution values are presented on the boxplot graph (Figure 1).
Table 1. Comparison of the strength values of carrageenan gel based on heating devices

| Carrageenan Flour | Gel strength (g/cm²) | Waterbath | Hotplate |
|-------------------|----------------------|-----------|----------|
|                   |                      |           |          |
| 1                 | 1300.43              | 1352.13   |          |
| 2                 | 1379.06              | 1349.81   |          |
| 3                 | 1231.67              | 1311.8    |          |
| 4                 | 1346.89              | 1210.56   |          |
| 5                 | 1206.05              | 1273.16   |          |
| 6                 | 1342.2               | 1188.61   |          |
| 7                 | 1356.79              | 1187.97   |          |
| Average           | 1309.01              | 1267.72   |          |
| SD                | 61.39                | 67.32     |          |
| CV                | 4.69                 | 5.31      |          |

Figure 1. Box plot of waterbath and hotplate

3.2. Comparison of the results of measuring gel strength by container
Clotting in the container is one of the stages of the preparation of the carrageenan gel test, but at the time of measuring the gel, there was no information that explained whether the gel was released from the container or not (2-4 mm) [10,22]. The nature of kappa carrageenan, which can form a rigid gel, is easier to remove from the container. In contrast to the iota type carrageenan which tends to be weaker than kappa which causes difficulty in releasing the gel from the container. This is due to the characteristics of iota gel which is not hard, soft, elastic and tends to be stable without syneresis [23]. Rigidity is related to the elasticity of a product where an increase in the value of rigidity will result in a product that is increasingly inelastic or stiff, and vice versa [11]. The gel formation that varies from the type of carrageenan is the basis for performing gel measurement experiments without using a container and with a container.
Table 2 showed the results of measurements of gel strength with container and without container. The range of measurement results for gel without container ranged from 1146.38 g/cm$^2$ - 1318.84 g/cm$^2$, while the measurement for gel with a container ranged from 1160.22 g/cm$^2$ - 1266.07 g/cm$^2$. The further calculation of the two experiments was statistically with the t-test, the results were significantly different ($p = 0.026$) between the gel measurements without container and with the container. Calculation of SD and CV values based on 7 (seven) times measurement of gel with a container, obtained SD (31.64) and CV (2.58) values which are smaller than the measurement of gel without container. This showed that the use of a container when measuring gel has a better precision value than measuring gel without a container. The value of the spread is further presented on the boxpot chart (Figure 2). In addition, the use of a container provides convenience when measuring the gel so that it can avoid damage to the gel texture caused by physical treatment before the test or the gel texture which has low gel value.

**Table 2.** Comparison of the gel strength values of carrageenan based on use without container and with container

| Carrageenan Flour | Gel Strength (g/cm$^2$) | Without Container | With Container |
|------------------|-------------------------|-------------------|---------------|
| 1                | 1318.84                 | 1221.92           |
| 2                | 1272.92                 | 1160.22           |
| 3                | 1272.66                 | 1266.07           |
| 4                | 1204.74                 | 1220.69           |
| 5                | 1383.29                 | 1256.62           |
| 6                | 1146.38                 | 1235.55           |
| 7                | 1159.68                 | 1230.73           |
| Average          | 1251.22                 | 1227.32           |
| SD               | 79.71                   | 31.64             |
| CV               | 6.37                    | 2.58              |

**Figure 2.** Box plot comparison of gel strength between without container and with container
3.3. Comparison of the results of measuring gel strength based on temperature

Temperature is one of the factors that influence the solubility of carrageenan [24]. [25] states that the ability to form gels from kappa carrageenan occurs when the hot solution becomes cold, because it contains 3,6-anhydro-D-galactose groups and is reversible. The temperature of the gel preparation used by the researchers generally ranges from 80 °C to 90 °C at which point the carrageenan begins to dissolve completely [14,26]. Temperature plays an important role in determining the utilization of carrageenan in addition, too high a temperature can hydrate kappa and iota types of carrageenan. High temperature and low cation level contribute to increased autohydrolysis. Along with the cooling temperature, the autohydrolysis process of the gel can be prevented by the formation of potassium ions which combine with the sulfate groups in carrageenan [27].

Table 3 showed the comparison of the gel strength values between using a temperature of 80 °C and a temperature of 90 °C. The range of gel strength values at 80 °C ranged from 1146.38 g/cm² - 1383.29 g/cm² and at 90 °C ranged from 1218.15 g/cm² - 1346.37 g/cm². Furthermore, the data was calculated statistically with the t-test and the results were not significantly different with (p = 0.064) between the use of temperatures 80 °C and 90 °C. This means that the gel strength test at a temperature of 80 °C and 90 °C will produce relatively the same values. Furthermore, from the calculation, the SD (31.64) and CV (2.58) values at 80 °C were lower than at 90 °C. This showed that using a temperature of 80 °C has a better precision than that of 90 °C. The value of the distribution of the data is further presented in the boxplot graph (Figure 3).

| Carrageenan Flour | Gel strength (g/cm²) |
|-------------------|----------------------|
|                   | 80 °C    | 90 °C    |
| 1                  | 1318.84  | 1264.9   |
| 2                  | 1272.92  | 1221.13  |
| 3                  | 1272.66  | 1267.83  |
| 4                  | 1204.74  | 1259.74  |
| 5                  | 1383.29  | 1235.55  |
| 6                  | 1146.38  | 1346.37  |
| 7                  | 1159.68  | 1218.15  |
| Average            | 1227.32  | 1259.10  |
| SD                 | 31.64    | 40.35    |
| CV                 | 2.58     | 3.21     |

4. Conclusion

Research on the comparative method of measuring the strength of carrageenan can be ignored that the experiment using a heating device, either a waterbath or hotplate, is feasible because it gives no significant difference. Meanwhile, the temperature of 80 °C and the use of a crystal container are the best temperature and method of preparation for the strength of carrageenan gel.
Figure 3. Box plot of gel strength preparation at 80 °C dan 90°C

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