Physicochemical characterization of kappa-iota carrageenan gel with papain enzyme

D Fransiska¹, B S B Utomo¹, M Darmawan¹, D Gozali², M N Iqbal²
¹Research Centre of Marine and Fisheries Product Processing and Biotechnology, Jl. KS Tubun Petamburan VI, Slipi, Jakarta
²Faculty of Pharmacy, Padjadjaran University, Bandung
Corresponding author: dinanomo@gmail.com

Abstract. Carrageenan is a group of galactose polysaccharides extracted from seaweed and has the ability to form a thermo-reversible gel or a viscous solution when added to the salt solution widely utilized as gelling, thickener and stabilizers in various industries such as food, pharmaceuticals, cosmetics, printing, and textiles. Only two types of carrageenan can be used in the manufacture of hydrogels which are kappa and iota. Kappa-carrageenan is produced from the tropical seaweed *Kappaphycus alvarezi* and *Eucheuma denticulatum* is the main species producing iota-carrageenan. The aim of this research is to know the composition of kappa-carrageenan and iota-carrageenan mixture as gel base to produce the optimum physical properties and to know the physical properties of optimum formula with the addition of papain enzyme for 4 weeks storage. The determination of the optimum formula of mixed carrageenan kappa and iota gel with Simplex Lattice Design method. The physical properties of the optimum formula with the addition of papain enzyme during 4 weeks storage are viscosity, pH, dispersion, and sineresis of optimum formula were statistically verified using Anova Univariate method of experimental design of two factorial with 95% confidence level. The results showed that the optimal formula of mixed carrageenan kappa and iota respectively concentrations of 0.3%, 1% and the use of 0.8% papain enzyme.

1. Introduction
Indonesia is one of the largest *Eucheuma* seaweed producing countries in the world. Based on statistical data from the *Food and Agriculture Organization* in 2014, Indonesia produces *Eucheuma* seaweed amounting to 81.4% of the total production of *Eucheuma* seaweed in the world. However, 64% of the total seaweed production in Indonesia is only exported and little use is made of the processed seaweed product.

Carrageenan is a group of galactose polysaccharides extracted from seaweed. Most of the carrageenan contains sodium, magnesium, and calcium which can be bound to the sulfate ester groups of galactose and copolymer 3,6-anhydrogalactose. Complex carrageenan is water soluble, linear chain and galactant sulfate. This compound consists of a number of galactose and 3,6-anhydrogalactose units that are bonded with sulfate groups or with α 1,3-D-galactose and β 1,4-3,6-anhydrogalactose bonds. Based on the sulfate substituent in each monomer, carrageenan can be divided into several types, namely kappa, iota, lambda, mu, nu and x-carrageenan (1). However, only two types of carrageenan that can be used in the manufacture of hydrogels, namely kappa and iota (2).

Naturally, iota and kappa types are formed enzymatically from their precursors by sulfohydrolase. While commercially, this type is produced using alkaline treatment or extraction with
lye. Currently, kappa-carrageenan is produced from tropical seaweed *Kappaphycus alvarezii*, which is known in the trading world as *Eucheuma cottonii*. *Eucheuma denticulatum* (with the trade name *Eucheuma spinosum*) is the main iota-carrageenan species. Lambda carrageenan is produced from *Gigartina* and *Condrus* species (3).

Carrageenan has the ability to form a thermo-reversible gel or a thick solution when added to a salt solution so it is widely used as a gelling agent, thickener and stabilizer in various industries such as food, pharmaceuticals, cosmetics, printing, and textiles (3,4).

Based on research(5), the amount required for the enzyme to improve the stability of carrageenan gel (300 mg papain in 50 mL final carrageenan solution). Therefore, this study was conducted to develop formulation *shelf-life* by entrapment in a cross-linked papain biodegradable ionotropically, focusing the use of enzymes which better and efficient (6). The stability of papain-containing formulations can be increased by entrapment in an ionotropically biodegradable cross-linked hydrogel. In addition, this trapped enzyme is resistant to the disturbance of the excipient formulation and is stable at higher temperatures. Therefore papain can be stored at room temperature with extended *shelf-life* (7). These advantages are used for commercial purposes for the pharmaceutical industry.

The aim of study was to investigate whether these composition formula of mixture kappa-carrageenan and iota-carrageenan as a gel base of optimal methods *Simplex Lattice Design* and study optimum formula of kappa-carrageenan and iota-carrageenan with the addition of the enzyme papain.

### 2. Method

#### 2.1. Material

The materials used in this study were kappa carrageenan and carrageenan iota, commercial papain enzyme, and distilled water.

#### 2.2. Research methods

The research method used includes optimization, evaluation of gel bases, addition of active substances in the base, evaluation of preparations, and data processing.

##### 2.2.1. Definition optimization formula

Formula gel optimization from a mixture of kappa and iota carrageenan performed with Design Expert methods *Simplex Lattice Design* version 7.0.0. In its application as a gel, the concentration of carrageenan- kappa used ranges from 0.3–1.2% and iota-carrageenan 0.3–1.0%, viscosity gel formula ranges between 2000–4000 cPs, and pH to use as topical ranges from 4-6. Profil raw material substance that kappa and iota refers HOPE V Edition (Handbook of Pharmaceutical Excipients).

Gel made by dissolving iota and kappa carrageenan in 100 ml of distilled water at a temperature of 90°C and stirred for 20 minutes. Evaluation of the draft formula made to all formulas based on the profile of raw materials formulations. The evaluations carried out include pH (Aulton, 2003) and viscosity (8).

##### 2.2.2. Addition of papain enzymes

After getting optimal formula gel, then gel adding the enzyme papain (300 mg in 50 mL papain carrageenan solution) were dissolved in distilled water at a temperature of 40°C for 15 minutes. Evaluation was carried out on the optimum formula which had been added with papain enzyme for 4 weeks of storage. Evaluations were carried out on days 1, 7, 14, 21, and 28, covering stability test at room temperature and stability test at high temperatures (Chandira, 2010). Evaluation includes: pH (9), viscosity (8), scatterplot (10), and the Cycling Test.
3. Result and discussion

3.1. Optimized gel formula

Optimization is a method for obtaining mathematical interpretation of data. The combination of ingredients in the formulation is made in such a way that experimental data can be used to predict responses in a simple and efficient manner. Optimization is done using the Design Expert method Simplex Lattice Design version 7.0.0. The results of this method will provide recommendations or concentration solutions in making gel formulas from a mixture of kappa and iota based on the material profile. The best formulation concentration results according to the selected criteria will be optimized for physical properties for 4 weeks of storage.

| No | Factor 1 A:Kappa (%) | Factor 2 B:Iota (%) | Response 1 Viscosity (cPs) | Response 2 pH (pKa) | Unit |
|----|----------------------|----------------------|-----------------------------|---------------------|------|
| 1  | 1.20                 | 0.30                 | 5708                        |                     | 5    |
| 2  | 1.20                 | 1.00                 | 6762                        |                     | 5    |
| 3  | 0.30                 | 0.30                 | 1766                        |                     | 4.5  |
| 4  | 0.30                 | 0.30                 | 1766                        |                     | 4.5  |
| 5  | 0.30                 | 1.00                 | 2820                        |                     | 5    |
| 6  | 0.30                 | 1.00                 | 2820                        |                     | 5    |
| 7  | 1.20                 | 0.30                 | 5708                        |                     | 5    |
| 8  | 1.20                 | 1.00                 | 6762                        |                     | 5    |

The results of the analysis obtained four formulas comparison kappa and iota carrageenan gel is F1, F2, F3, and F4 with the respective concentrations of F1 (1.2% : 0.3%), F2 (1.2% : 1%), F3 (0.3% : 0.3%), and F4 (0.3% : 1%). Respon data according to tests performed in all formulas, respectively (Table 1). This design has a requirement that the response value with the same ratio must be the same or not show a significant value. Results responses viscosity at F1 1.2%: 0.3%, F2 1.2: 1%, F3 0.3%: 0.3% visually show right values outside of the terms of a preparation gel is in the range of 2000-4000 cPs because the range of the gel can be spread by good (8), whereas the results of the F4 viscosity response with a kappa concentration ratio of 0.3% and 1% iota presented a response that met the requirements. The results of the pH response in all of the bases present a value that meets the criteria, namely in the pH range of good topical preparations in the pH range 4 - 7 (9). This is further strengthened by the statement that kappa-carrageenan is a polymer that can form a strong gel containing about 35% sulfate esters and 3.6-anhydrogalactose while Iota-carrageenan is a polymer that can form a weak gel because it contains sulfate esters 32 3,6% and anhydrogalactose about 30% (11). So that from the design results visually, there is only 1 formula based on the met conditions, the results of this smoothing will be clarified using design analysis.

The analysis was carried out by looking at the results of the analysis and the Effects List of the 1 viscosity response and 2 pH response. The results of the analysis showed the effect of the concentration of the mixture formula kappa and iota carrageenan on viscosity. It means that carrageenan’s viscosity depends on its concentration, temperature, other solutes and the type of carrageenan which increases exponentially. The increase in viscosity can occur with the mechanism of gel formation due to cross-linking between chains (12). At high polymer concentrations where the polymer chains are interconnected and many bonds are formed, it causes gel stiffness (13).
The results of the analysis show the pitch can influence the concentration of the mixture of formula kappa carrageenan and iota against pH. pH is very important in cosmetic preparations because a very high or low pH can interfere with the absorption of the skin and cause skin irritation, so that cosmetic preparations usually have a pH ranging from 4-8 to close to skin pH 4.5-6.5 (14). Formula F3 has a pH of 4.5 and F1, F2, F4 have a pH of 5. All formulas in this study meet pH requirements.

3.2. Viscosity
The results of the Effects List analysis are to see the effect of each ingredient between kappa and iota carrageenan which has an effect on viscosity and pH responses. The result showed that kappa-carrageenan affect the viscosity (the contribution amounted to 90, 83% ) iota-carrageenan contributes 9, 17% and mix the two ingredients do not contribute. This indicates that the smaller the sulfate content, the smaller the viscosity value, but the gel consistency increases (Moirano, 1977). The gelling ability of kappa and iota carrageenan occurs when the hot solution is allowed to cool because it contains 3,6-anhydrogalactose groups. The sulfate groups will affect the process of the gel (15).

The results of the analysis on the pH value indicate that kappa-carrageenan gives a contribution of 33.33 %, iota-carrageenan accounted for 33.33 %, and the second material has a value equal at 33.33%. then the contribution of raw materials can be said to have no effect on the pH effect.

3.3. Design Optimization Results
Optimization design has used to produce a solution optimal conditions. The results of the analysis present the best formula, with "Desirability" with a value range of 0 - 1 (red color) which indicates a very good formula.

Results based on the right treatments by the resident 's minimum or maximum limit and limit or a target range for each Respon profile raw material substance, so that optimal results unpresentable in the graph overlay plot that highlights the area of operation.

![Overlay plot](image_url)

**Figure 1. Overlay plot**

The results of the Overlay plot graph provide an optimal formula for predicting the carrageenan mixture with kappa concentration of 0.34% and iota concentration of 0.73% by estimating the viscosity response of r 4.264 cPs and pH of 4.87.

3.4. Point prediction
Operation where the results to find right and response predicted value with a confidence interval of less than 0.05. with a confidence level of 95%. The results predicted in ratio for first, kappa carrageenan
mixture 0, 3 4% and iota 0, 73% and viscosity response prediction by 4. 264 cPs and with a pH of 4.87. The result of the design of the Simplex Lattice Design version 7.0.0. is not much different from the visual observations based on the Handbook of Pharmaceutical Excipients Ed specifications V.

3.5. Formula evaluation
After obtaining the optimal formula from the Simplex Lattice Design results, a mixture of kappa and iota carrageenan gel was formulated with the addition of the enzyme papain and without enzyme administration.

The stability of carrageenan as a compound will usually slowly depolymerize in storage. However, kappa and iota carrageenan usually have gel strength and reaction strength against proteins and are not affected by the depolymerization process. Storage at room temperature for 1 year, the decrease in gel strength cannot be detected because it is too small (Winarno, 1990). This is evidenced by looking at the physical properties of carrageenan gel within 7 days of storage which presents a bad result as showed in Figure 1.

3.5.1. Stability test. Stability testing was carried out to determine the changes that occurred in the preparation during 4 weeks of storage at two different temperatures, namely room temperature (25-30 °C) and high temperature (40±2 °C). Physical stability testing is related to the resistance of the gel preparation during storage. Stability test parameters include pH, viscosity and dispersibility and cycling test.

3.5.1.1. pH. The pH test aims to determine the pH of each formula made. The test was carried out using a pH meter. pH dosage should be adjusted to a pH of between 4.5-6.5 to avoid skin irritation and may increase the acceptability of use.

Note:
F1: Kappa formula 0.3% and iota 1% to 1
F2: Formula hood pa 0.3% and 1% to 2 iota

Figure 2. Viscosity value during storage at T: 25 °C
Note:
F1: Gel formula with 0.6% papain enzyme
F2: Gel formula with 0.8% papain enzyme

Figure 3. pH value during storage at T: 25 °C (a) and T: 40 °C (b)

pH of the three formulas of gel formulation during storage at a temperature of 25 °C obtained grades that meet the criteria desired pH of between 4.5 – 6.5 adjust the pH of the skin. It can be seen from the three formulas that there are no measurement results that are outside the criteria.

pH measurements of the three formulas of gel formulation during storage at a temperature of 40 °C showed results that meet the criteria desired pH of between 4.5-6.5 in accordance with the pH of the skin. Based on the data from the pH measurement results at a storage temperature of 25 °C and a temperature of 40 °C, it can be concluded that the three formulas are pH stable during the storage period. In addition, kappa and iota carrageenan can be used as gel formers at low pH, but they are easily hydrolyzed so that they cannot be used in food processing. Decreasing pH causes hydrolysis of the glycosidic bonds resulting in loss of viscosity. Hydrolysis is influenced by pH, temperature and time. Hydrolysis is accelerated by heat at low pH (16). The analysis showed that there was no effect of increasing the concentration of the enzyme papain on the pH value.

3.5.1.2. Viscosity. Viscosity is the ability of a substance to flow. The viscosity test was carried out on the entire formula at 2 storage temperatures and was carried out every week until the 4th week.

The results showed that viscosity of F2 almost every time it decreased except at the 14th time it increased then decreased again, on the contrary, at F1 almost every time there was an increase in viscosity but at time 14 it decreased. The observation results of the preparation at 40 °C tended to be stable except for F2 which had an increase in storage on the 14th day then decreased on the 28th day. The increase in viscosity could occur because the gel base was less homogeneous and caused clumping at the bottom of the container. While the decrease in viscosity can be affected by the presence of air bubbles in the preparation which can increase the viscosity value (17). The decrease in viscosity can also be affected by an increase in temperature so that the size of the bubbles becomes large and easy to break. At the observation of the dosage at 40 °C, there was an increase and decrease in the two gel formulas with different papain enzyme concentrations. At the 6% papain enzyme concentration the viscosity shift was relatively constant, while at the 8% papain enzyme concentration the viscosity increased then decreased on day 28. The increase and decrease in viscosity can occur because of carrageenan as polyelectrolyte. Repulsive force between the negative charges along the polymer chains are sulfate groups, resulting in a molecular chain tightened. Due to its hydrophilic nature, the polymer is surrounded by movable water molecules, causing the carrageenan solution to be viscous (18). This situation is likely that the smaller the sulfate content, the smaller the viscosity value, but the gel consistency is increasing (19).
Carrageenan viscosity affects the properties of the gel, especially the gel formation point and melting point, where high carrageenan viscosity results in higher melting and gelling rates than carrageenan with low viscosity (16).

Carrageenan gel formation involves two separate and sequential steps, namely the formation of coils into the chain in the cooling and cation-dependent aggregation processes between the chains. Suitable cations, potassium, calcium and some alkaline metal ions (Li+, Na+, Rb+, Cs+) are required as important components to stimulate the gel formation process at lower concentrations (20).

Viscosity is the resistance of a liquid to flow. The viscosity of the preparation during storage tends to decrease but still meets the requirements for good gel viscosity. The viscosity value at room temperature storage is more stable than the preparation stored at high temperature. This is in accordance with the Arrhenius kinetics equation where viscosity is inversely proportional to temperature. The higher the temperature, the lower the viscosity value.

In addition to temperature, the storage time affects the stability of the preparation. The longer the preparation is stored, the lower the viscosity value will be due to the influence of the environment, namely high humidity so that the preparation absorbs moisture and causes an increase in the volume of the preparation. The results of the analysis showed that there were differences in the viscosity of each concentration of the formula.

3.6. Coverage
The spreadability test is used to determine the ease of application of a semisolid preparation to the skin (8). The spreadability is inversely proportional to the viscosity where the higher the viscosity, the lower the resulting dispersive power. Likewise, if the lower the viscosity the higher the resulting dispersive power. Good gel dispersion is about 5-7 cm and a spread of 25-49 cm² (10).
Measurement of the dispersion of the gel during the storage period at 25 °C showed that there was a change in the spreadability. This change occurs because of the shift in viscosity that has been previously seen. At the storage temperature of 25 °C, formula 1 has the addition of the spread at 24% and formula 2 has the addition of the scatter 40%.

The analysis results showed that there was an effect of storage temperature on the spreadability. The results of the measurement of the scattering power at a storage temperature of 40 °C show the changes in the spreadability experienced by the three formulas. Formula 1 has a change in the spreadability by 36%, and formula 2 with 162%.

3.7. Cycling Test
This test is carried out to see the gel syneresis that occurs. Syneresis was calculated by measuring the weight loss during storage then compared with the initial weight of the gel. The test was carried out for 6 cycles or 12 days and observed whether or not there were changes that occurred in each preparation.

| Day | Sample Weight (g) |          |          |
|-----|-------------------|----------|----------|
|     |                   | F1       | F2       |
| 0   | 100               | 100      |          |
| 12  | 97.8              | 98.3     |          |
| %   | 2.2%              | 1.7%     |          |

Table 2. Observation Results of Cycling Test for Gel Preparations

Note:
F1: kappa 0.3% and iota 1% with the enzyme papain 0.6%
F2: kappa 0.3% and iota 1% with the enzyme papain 0.8%

Syneresis is the discharge of water from the gel in which the gel shrinks so that it tends to squeeze water out of the cell. Based on the observations of the Cycling Test, the percentage value of reduction in dosage weight due to storage for 6 cycles or 12 days was obtained. In formula 1, the percentage of weight reduction is 2.2%, and in formula 2 the percentage of weight reduction is 1.7%. The decrease in water binding capacity and syneresis can be overcome by adding other stabilizers in the form of hydrocolloids or water-soluble polymers.
4. Conclusion
The results showed that the optimal kappa and iota carrageenan mixture gel formula was 0.3% kappa-carrageenan and 1% iota-carrageenan. The physical properties and characteristics of the gel mixture of kappa and iota with the addition of papain enzyme 8% were able to meet the optimal stability improvements and dosage parameters.

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