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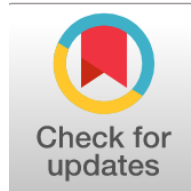
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Characteristics of Agar Powder (*Gracilaria verrucosa*) trough Acid – Base Pre - extraction

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Abstract

General Background Seaweed represents a vital fishery commodity with extensive applications across the food, pharmaceutical, and cosmetic industries. **Specific Background** *Gracilaria verrucosa*, a prominent red algae species, yields agar molecules whose functional characteristics depend heavily on extraction conditions. **Knowledge Gap** Standard extraction pretreatments utilizing weak acids often fail to maximize agar recovery and functional parameters from algal cell structures. **Aims** This study evaluates the combination of strong alkali (NaOH) and strong acid (HCl) pretreatments on the physical characteristics of *Gracilaria verrucosa* agar powder. **Results** The empirical findings demonstrate that a pretreatment using 7.5% NaOH and 0.040% HCl delivers optimal structural outcomes, recording a yield of 13.82%, gel strength reaching 1032.5 g/cm², viscosity of 18.32 cP, and reduced syneresis at 0.96%. **Novelty** Synergistic strong acid-base pretreatment replaces traditional weak acid steps, efficiently cleaving sulfate esters while preserving structural polysaccharide integrity. **Implications** These standardized parameters offer an efficient industrial approach to produce high-grade agar powder meeting commercial standard specifications.

Key Findings Highlights

Pretreatment with combined strong alkali and strong acid solutions significantly improves physical properties of agar powder.

Increasing concentrations of sodium hydroxide and hydrochloric acid reduce syneresis while elevating viscosity and gel strength.

The treatment using 7.5% alkali and 0.040% acid concentration yields the highest extraction recovery and optimal structural performance.

Keywords : Agar Powder, *Gracilaria Verrucosa*, Acid-Base Pretreatment, Gel Strength, Extraction Yield

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Introduction

Seaweed is a fishery commodity that has high economic value. Seaweed is widely used in the paper, food, cosmetics and pharmaceutical industries [1]. Seaweed contains polysaccharides, proteins, carotenoids, phenolic compounds, a small amount of fat, vitamins and minerals, most of which are sodium and potassium salt compounds [2,3,4,5,6,7].

Gracilaria verrucosa is a type of red seaweed that is very suitable for processing as gelatin flour because it has very high agarophytes compared to other types of red seaweed. Agarophyte is a characteristic of red algae with homogeneous viscosity and gel strength [8].

The sulfate composition contained in the seaweed *Gracilaria verrucosa* will be reduced by NaOH through hydrolysis, resulting in high gelatin gel strength, however, the addition of NaOH has an impact on the low yield of gelatin flour, so an acidic pretreatment is needed as a balancer. Generally, the agar processing industry uses acetic acid in the extraction pretreatment, but acetic acid is not able to extract the maximum agar content from seaweed. The acid solution that is able to properly remove the gelatinous content from the cell walls of *Gracilaria verrucosa* is HCl [9,10].

HCl solution with a concentration of 0.025% pre-treatment before extraction of the seaweed *Gracilaria verrucosa* produces high yield values [11]. The combination of a strong base solution and a strong acid before extraction will produce agar powder with high gel strength, high yield value, and increases the viscosity of the agar [12]. Based on the problems above, this research aims to determine the effect of NaOH and HCl concentrations on appropriate pre-extraction treatment to obtain gelatin flour with the best quality.

Materials and Methods

The experiment was conducted at the Laboratory of Food Technology, Science and Technology, Universitas Muhammadiyah Sidoarjo. *Gracilaria verrucosa* was obtained from the Tanjung Sari, Sidoarjo. This research used a factorial randomized block design (RBD) with 2 factors which were repeated 4 times. The first factor is NaOH (N) in (%) with 3 treatments, namely 3.5% NaOH solution (N1), 5.5% (N2), and 7.5% (N3). The second factor is the concentration of HCl (H) in (%) with 2 treatments, namely: HCl 0.035% (H0) and 0.045% (H1)

The research procedures were: (1) washing was carried out 3 times; (2) soaked in NaOH solution with concentrations of 3.5%, 5.5%, and 7.5% with a ratio of 1:10 in a mini extractor at a temperature of 110 °C for 60 minutes; (3) rinsed with 17 L of water with 6 repetitions for 20 minutes; (4) soaking 15 L in HCl solution with a concentration of 0.035% and 0.040% for 20 minutes and checking the pH; (5) rinsed with 17 L of water with 3 repetitions until the pH is neutral; (6) soaked in 15 L of NaOCl with a concentration of 4% for 30 minutes; (7) rinsed with 17 L water with 3 repetitions until the pH is neutral; (8) extraction using 12 L of distilled water at a temperature of 110 °C for 1 hour while stirring; (9) filtering using calico cloth; (10) drying for 12 hours at room temperature; (11) pressing to form gel sheets; (12) the gel sheet is ground to form a smaller size; (13) drying in an oven at 85 °C for 60 minutes; (14) flour into the miller machine at a speed of 3450 rpm.

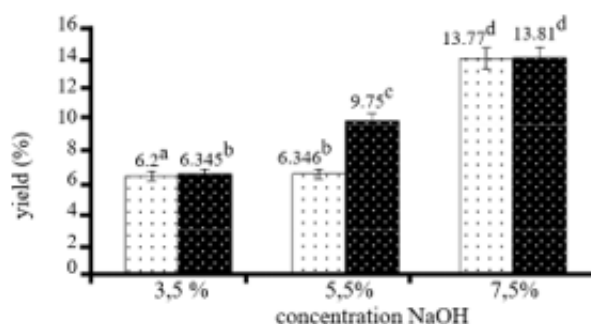
The variables observed include: yield, gel strenght, viscosity, and sineresis. The data obtained were analyzed using analysis of variance in SPSS 23 software. If the results of the analysis showed significant differences, it was continued with Duncan's multiple range test at a 95% confidence interval ($\alpha=0.05$).

Result and Discussion

3.1 Yield

In this research, the percentage yield of gelatin flour produced ranged from 6.375% to 13.86%. Analysis of variance $\alpha=0.05$ shows that pretreatment with NaOH and HCL and the interaction between the two have an effect on the yield of gelatin flour. Duncan's further test showed that the 3.5% NaOH and 0.040 HCl pretreatment was not significantly different from the 5.5% NaOH and 0.035% HCl pretreatment but was significantly different from the other pretreatments. The histogram of the average yield of gelatin flour can be seen in Figure 1.

The results in this research resulted in a yield without alkali and acid pretreatment during extraction which resulted in a yield value of <3%. Meanwhile, in research conducted by Peng where the seaweed *Gracilaria verrucosa* was extracted by pretreatment with a basic solution, namely NaOH and an acid solution, namely acetic acid with the lowest concentration, which could produce a yield of 5% [13]. In this research, the resulting yield level was directly proportional to the concentration of the alkali and acid solutions used in the pretreatment before extraction. Where in this research the pretreatment with the lowest concentration, namely 1.5% NaOH and 2% acetic acid produced.



Notes: Numbers followed by the same letter indicate that they are not significantly different based on the 5% HSD test.

Figure 1. Average histogram of gelatin flour yield test.  hydrochloric acid 0.035 %,  hydrochloric acid 0.040 %.

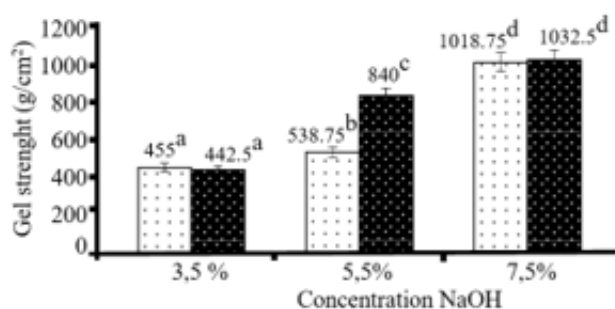
Figure 1.

The agar flour yield was 5%, while the highest pretreatment with 4.5% NaOH and 4% acetic acid produced an agar flour yield of 6.5%. This is directly proportional to the yield levels produced in agar flour in this study. In this research, the use of a combination of strong base and strong acid solutions, namely NaOH and HCl, produced maximum yield levels compared to previous studies.

Pre-treatment of NaOH 7.5% and HCl 0.040% was the pre-treatment chosen in this study because the average yield value of gelatin flour which is included in the SNI quality specifications for agar flour is as large and has the greatest value compared to other pre-treatments, namely 13.8175%.

3.2 Gel strenght

The value of the gel strength of agar flour in this study ranged from 420g/cm² to 1040g/cm². Based on the analysis of variance $\alpha=0.05$, pretreatment with NaOH and HCl and the interaction between the two have an effect on the gel strength of agar flour. The highest mean gel strength was found in 7.5% NaOH and 0.040% HCl pretreatment, while the lowest mean gel strength was found in 3.5% NaOH and 0.040% HCl pretreatment. The Duncan test results show that the 3.5% NaOH and 0.035% HCl pretreatments are not significantly different from the 3.5% NaOH and 0.040% HCl pretreatments but are significantly different from the other pretreatments. The histogram of the average gelatin flour gel strength test can be seen in Figure 2.



Notes: Numbers followed by the same letter indicate that they are not significantly different based on the 5% HSD test.

Figure 2. Histogram of the average gelatin flour gel strength test.  hydrochloric acid 0.035 %,  hydrochloric acid 0.040 %.

Figure 2.

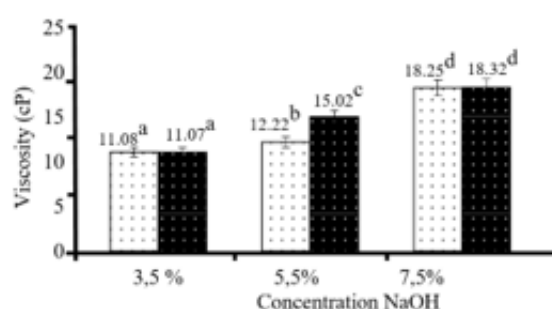
According to research conducted by Liu *et al.* the gel strength of agar flour tends to increase in line with the combination of the concentration of alkaline or acid solution used [14]. In this study, *Gracilaria verrucosa* seaweed extracted with 2% NaOH and 0.025% H₂SO₄ pretreatment produced a gel strength of 250g/cm² whereas with pretreatment of 4% NaOH and 0.03% H₂SO₄ resulted in a gel strength of 600g/cm². These results are directly proportional to the gel strength produced by agar flour in this study. Where the increase in the concentration of the base solution, namely NaOH and the acid solution, namely HCl, is in line with the increase in the strength of the resulting gel.

Pre-treatment of 5.5% NaOH and 0.040% HCl was the chosen pre-treatment because the average gel strength was within the agar powder specifications according to SNI, namely 840 g/cm². The gel strength of agar powder is directly proportional to the concentration of the acid or base solution used in pretreatment before extraction [15]. The higher the concentration of NaOH and HCl used, the higher the gel strength of the agar produced.

3.3 Viscosity

In this research, the viscosity value of the gelatin flour produced ranged from 11.01 cP to 18.42 cP. The histogram of the average viscosity test can be seen in Figure 3. The analysis of variance ($\alpha=0.05$) shows that pretreatment with NaOH and HCl as well as the interaction of the two solutions has an effect on the viscosity of agar flour. In research conducted by Uju *et al.* on agar flour, the viscosity value was in line with the use of alkali and acid solutions used in extraction pretreatment, the higher the concentration of alkali and acid solutions used, the higher the viscosity value produced in the agar flour product. so that [16].

In this research, pre-treatment before extraction using the lowest concentration, namely 3% KOH and 0.5% acetic acid, resulted in a viscosity value of 6%, while in the highest pre-treatment with a concentration of 5% KOH and 1% acetic acid, a viscosity value of 14% was obtained for agar flour. so that. The results of the viscosity values in the research conducted by Uju *et al.* is directly proportional to the results of the viscosity values in this study [16].



Notes: Numbers followed by the same letter indicate that they are not significantly different based on the 5% HSD test.

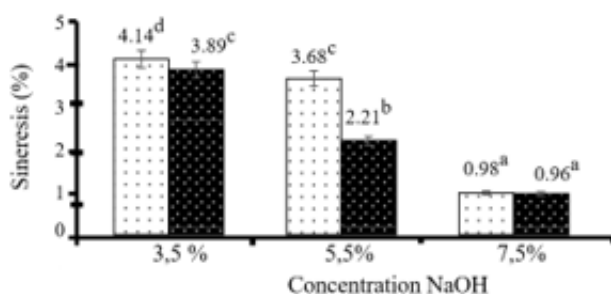
Figure 3. Histogram of the average viscosity test for agar flour.  hydrochloric acid 0.035 %,  hydrochloric acid 0.040 %.

Figure 3.

The viscosity of agar powder is directly proportional to the gel strength value [17]. This is because the higher the gel strength, the friction force on the agar also increases and thus the viscosity of the agar also increases [15]. Pretreatment with 5.5% NaOH and 0.040% HCl was the selected pretreatment because the average viscosity was within the SNI standard for agar flour.

3.4 Syneresis

The analysis of variance $\alpha=0.05$ shows that extraction pretreatment with NaOH and HCl solutions and the interaction between the two have an effect on the syneresis of gelatin flour. The syneresis level in agar flour ranges from 0.86% to 3.3%. The highest mean syneresis was found in 3.5% NaOH and 0.035% HCl pretreatment and the lowest mean syneresis of gelatin flour was found in 7.5% NaOH and 0.040% HCl pretreatment. In the Duncan further test, it was seen that the 7.5% NaOH and 0.035% HCl pretreatment was not significantly different from the 7.5% NaOH and 0.040% HCl pretreatment but was significantly different from the other pretreatments. The average histogram of the syneresis test is shown in Figure 4.



Notes: Numbers followed by the same letter indicate that they are not significantly different based on the 5% HSD test.

Figure 4. Average histogram of gelatin powder syneresis test.  hydrochloric acid 0.035 %  hydrochloric acid 0.040 %.

Figure 4.

The syneresis index produced in research conducted by Villanueva *et al.* which did not use acid and base pretreatment before extraction, showed that the resulting syneresis index was >16%, which is greater than the syneresis index produced in this study. NaOH and HCl, which are alkaline solutions and strong acids, play a very reactive role in maintaining and strengthening the polymerization of the gel contained in agar flour in this study so that the resulting syneresis index is also low. This is directly proportional to the higher the concentration of NaOH and HCl used, the lower the syneresis index produced [18].

Based on the SNI standard, gelatin flour pretreated with NaOH 5.5% and HCl 0.040% is the selected pretreatment because the average syneresis value that falls within the standard is 2.21%. The higher levels of syneresis contained in agar flour cause less stability of the polymer in the gel during the storage process [11].

Conclusion

The results of the research can conclude that pre-extraction treatment using different concentrations of NaOH and HCl has an influence on the yield, syneresis, gel strenght, and viscosity of agar powder.

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