

A Preliminary Study on the Agar Content and Agar Gel Strength of *Gracilaria manilaensis* Using Different Agar Extraction Processes

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Abstract: The aim of this study was to investigate the agar content and agar gel strength from intact and squeezed thalli of *Gracilaria manilaensis* collected from the earthen pond at Pantai Merdeka, Kedah, Malaysia. Agar content and agar gel strength of different agar extraction processes, i.e. native extraction and NaOH (5% w/v NaOH) treated of intact and squeezed thalli at different time intervals, were evaluated. The results showed that agar yield of intact thalli was highest in native extraction (13.5%); whereas, agar yield of squeezed thalli was highest when treated with 5% w/v NaOH for 3 hrs (9.4%). For intact thalli, agar yield of alkali-treated samples were less than native extraction. For squeezed thalli, agar yield of alkali-treated samples were not much different when treated at different time intervals except for 1hr treatment which showed that agar yield of intact thalli was lower than squeezed thalli. The gel strength was very low (< 60 g) for native extraction in both intact and squeezed thalli, but higher after treated with alkali. Analysis of variance of gel strength showed significant difference ($p < 0.05$) between native extraction and 3 hrs alkali treatment of intact thalli, while squeezed thalli showed significant difference ($p < 0.05$) between native extraction and 6 hrs, 12 hrs and 24 hrs of alkali treatment. The results demonstrated that alkali treatment did not affect agar yield; instead, it affected the gel strength of extracted agar under squeezed condition.

Key words: *Gracilaria manilaensis* • Intact thalli • Squeezed thalli • Alkali treatment • Agar Gel Strength

INTRODUCTION

One of the oldest groups of eukaryotic algae is the Rhodophyta (red algae). Some Rhodophyta known as agarophyte produce hydrocolloid agar in their cell walls. The taxonomic classification of agarophytes in the class of Florideophyceae is divided into three orders, Gelidiaceae (*Gelidium*, *Gelidiella*, *Pterocladia*), Gracilariales (*Gracilaria*) and Ahnfeltiaceae (*Ahnfeltia*) [1]. The order Gracilariales (*Gracilaria*) is the largest world-wide agar source for agar extraction [2-4]. McHugh [5] reported that the amount of agar produced from *Gracilaria* and *Gelidium* was the largest in the world which were 53% and 44%, respectively, compared to other agarophytes like *Gelidiella* and *Pterocladia*

which only produced a small quantity (3%) of agar. The best quality agar is extracted from the genus *Gelidium*, but because of the high cost and insufficient wild stock, *Gracilaria* has now been subjected to numerous structural studies of its agar due to the excellent substitute for *Gelidium* agar in the food industry.

The structure of agar from *Gracilaria* consists of repeating units of (1,3) linked-D-galactose and (1,4) link 3,6-anhydro-L-galactose [6]. However, *Gracilaria* spp. contain several structures with different substituents such as sulfate esters, methoxyls and pyruvic acid [7]. The amount of these substituents affects the physical properties of the gel. Craigie [8] stated that the molecular structure of agar polysaccharide from genus *Gracilaria* is appearing as species-specific, particularly the type and

location of sulfate esters. Armisen [1] reported that the alkaline hydrolysis treatment was able to convert L-galactose-6-sulfate to 3,6-anhydro-L-galactose, in which sodium hydroxide acted as a medium for improved agar quality of *Gracilaria*. The amount and position of sulfate group, as well as the amount of 3,6-anhydro-L-galactose affected the gel properties of *Gracilaria* [9].

To date, no comparative studies have been reported on the agar content and agar gel strength from intact and squeezed thalli of *G. manilaensis* particularly in Malaysia. The objectives of this study were to investigate the agar content and agar quality (gel strength) of intact and squeezed thalli of *G. manilaensis* from Pantai Merdeka, Kedah. Meanwhile, the effect of alkali treatment at 5% concentration of sodium hydroxide (NaOH) in the pre-extraction alkaline treatment at various duration times of 1 hr to 48 hrs in both intact and squeezed samples was evaluated, in order to contribute more information about the quality of agar from *Gracilaria* sp.

MATERIAL AND METHODS

Gracilaria manilaensis was collected from Pantai Merdeka, Kedah. The samples were cleaned from epiphyte and any dirt. For intact thalli preparation, the cleaned *G. manilaensis* was sun dried under open space and for squeezed thalli, they were cut into small pieces, pressed under a hydraulic press and sun dried. The dry intact thalli and squeezed thalli were stored for later extraction.

Alga Extraction and Analysis: The extractions were performed on 30g dry weight of intact and squeezed thalli of *G. manilaensis*. For native agar extraction, hot water extraction method was used. The algae were soaked in 1L water and then extracted by boiling the thalli for 1 h 30 min. Alkali treatment was carried out using 5% w/v NaOH. For the treatment, the samples were soaked in 1 L of 5% w/v NaOH solution at different lengths of time (1 hr, 3 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs). Excess NaOH was removed by rinsing the algae with running tap water three times. The algae were then soaked for 15 min with diluted H₂SO₄ solution to neutralize the alkali and then H₂SO₄ was drain and rinsed three times with tap water. The sample was then treated with 6% w/v chlorine to bleach the thalli and the chlorine was removed by again rinsing three times with tap water. For the agar extraction the thalli was boiled for 1 h 30 min and a filter aid was

added before the agar was squeezed through the three layers of muslin cloth. The filtrate was then frozen, thawed, oven-dried at 60°C for 24 h and weighed. Quantity of agar was determined in terms of agar yield expressed as the percentage of dry weight (%):

$$\text{Agar yield (\%)} = \left[\frac{\text{Agar dry weight (g)}}{\text{Seaweed dry weight (g)}} \right] \times 100\%$$

Rheological Analysis (Gel Strength): The gel strength (g) was measured by Brookfield CT V 1.2 Buld 9 Texture Analyzer (Brookfield Engineering Labs., Inc) using a cylindrical probe (TA3/100 mm diameter) with 4500 g loading cell at crosshead speed of 0.5 mm/s. Cylindrical samples of the gels were prepared from agar solution (1.5% w/v in water, 100 ml), then allowed to gel overnight at 4°C. All analyses were done in triplicates by compression test of this instrument.

Statistical Analysis: Analysis of variance (ANOVA) of data was done using PASW Statistics 18 (SPSS., Inc). Mean and Standard deviations were calculated using Microsoft Excel 2007 software. One-way ANOVA test was conducted for significant differences (when $p < 0.05$) between native and alkali-treated agar for gel strength from *G. manilaensis*. Tukey's honest significant difference (HSD) test was used for the comparison of means.

RESULTS

Agar Content: The agar yields expressed as percentage of seaweed dry weight under two conditions of thalli (intact and squeezed) in both native agar and alkali treated agar at different range of times (1 hr, 3 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs) are shown in Fig. 1. The agar content of native and alkali-treated intact thalli ranged from 7.1% to 13.5%. The highest agar yield was found from native extraction and the lowest from alkali-treated thalli at 1 hr treatment. Among the alkali-treated thalli under intact condition, 3 hrs treatments showed the highest agar content.

For squeezed condition, the agar content ranged from 9.4% to 8.2%, with the highest value found at 3 hrs treatments and the lowest was at 24 hrs treatment. After the treatments, agar yields were less than native agar under intact condition; conversely, under squeezed condition, the yield slightly fluctuated among the treatments and no big difference was observed between native and alkali-treated thalli (Fig. 1).

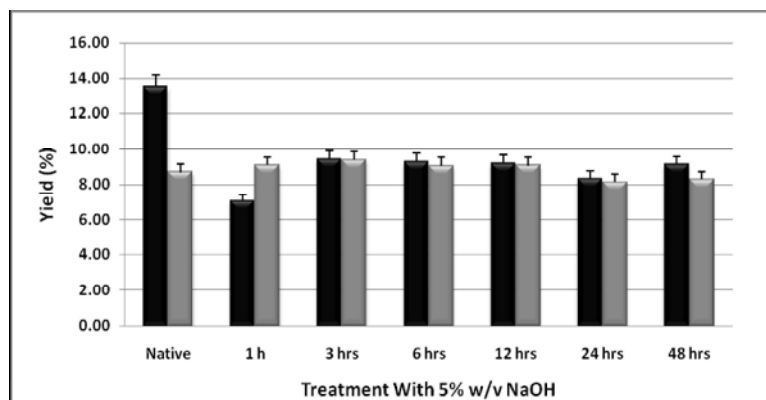


Fig. 1: Agar yield of *G. manilaensis* from intact thalli (black bar) and squeezed thalli (grey bar) for native extraction (without NaOH) and alkali-treated thalli (5% NaOH) at a different lengths of times (1 hr, 3 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs) (n=3).

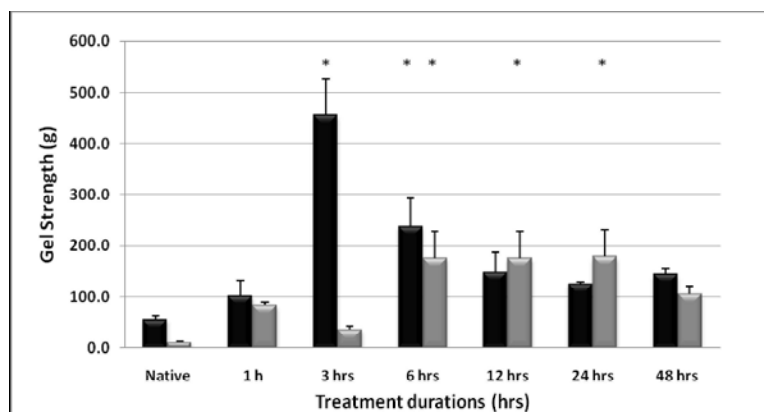


Fig. 2: Agar gel strength of *G. manilaensis* from intact thalli (black bar) and squeezed thalli (grey bar) for native extraction (without NaOH) and alkali-treated thalli (5% NaOH) at a different range of times (1 hr, 3 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs). Vertical lines represent the standard deviation. Asterisk (*) indicates significant difference ($p < 0.05$) between alkali-treated and native extraction (non-treated) (n=3).

Rheological Analysis of Gel Strength: The mean gel strengths of native extraction and alkali-treated thalli from *G. manilaensis* are shown in Fig. 2. Agar gel strengths were varied among native and alkali-treated agar. The gel strength was very low (< 60 g) for native agar in both conditions of thalli (intact and squeezed), but after treating with alkali, the gel strengths among the different lengths of time showed higher values than their native extraction. The gel strength of agar extracted under intact condition was highest at 3 hrs alkali treatment (453.7g) and lowest at 1 hr treatment (84.5g), while under squeezed condition, agar gel strength was highest at 24 hrs treatment (178.3g) and lowest at 3 hrs treatment (36.5g) (Fig. 2).

Analysis of Tukey's honest significant difference (HSD) test for agar samples showed that native extraction and alkali-treated thalli could be classified in groups under intact and squeezed conditions and no significant difference ($p > 0.05$) within group, but was significantly different ($p < 0.05$) between groups (Table 1). Analysis of variance for the gel strength for intact and squeezed samples also showed significant difference ($p < 0.05$) between groups in intact and squeezed thalli of *G. manilaensis* (Table 2).

For intact condition, native extraction (non-treated) was significantly different ($p < 0.05$) between 3 hrs and 6 hrs alkali treatment. Treatment at 6 hrs, 12 hrs and 24 hrs of squeezed condition showed significant difference ($p < 0.05$) with their native agar (Fig. 2, Table 2).

Table 1: Results of a Tukey HSD to determine the classification of native extraction and alkali-treated thalli in groups under intact and squeezed conditions

Parameter	Order
Intact	Native < 1 hr < 24 hrs < 48 hrs < 12 hrs < 6 hrs < 3 hrs
Squeezed	Native < 3 hrs < 1 hr < 48 hrs < 12 hrs < 6 hrs < 24 hrs

The cases underlined with a common line indicated mean values not significantly different (Tukey HSD test, ($p > 0.05$))

Table 2: One-way ANOVA calculated for gel strength of native extraction (without NaOH) and alkali-treated thalli (5% NaOH) at different lengths of times (1 hr, 3 hrs, 6 hrs, 12 hrs, 24 hrs and 48 hrs) for *G. manilaensis*

Source of variation	DF	SS	MS	F	p
Intact Thalli					
Between Groups	6	313670.286	52278.381	34.674	.000**
Within Groups	14	21108.167	1507.726		
Total	20	334778.452			
Squeezed Thalli					
Between Groups	6	87467.405	14577.901	11.717	.000**
Within Groups	14	17418.667	1244.190		
Total	20	104886.071			

^a DF, degrees of freedom; SS, sum of square; MS, mean square; F, ratio of variances; ** significant ($p < 0.05$)

DISCUSSION

Agar Content: It is well known that agar yield of *Gracilaria* species are varied from each other [1]. Agar content of *G. manilaensis* in both intact and squeezed thalli were varied between native extraction (non-treated) and alkali-treated thalli. The native extraction of intact thalli produced higher agar content than the alkali-treated thalli. This may be due to the degradation of polysaccharides during treatment and also lost of agar through diffusion during the extraction process, as suggested by authors [10]. Among alkali-treated thalli, 1 hr treatment produces less agar compared to other treatments. This finding suggested that alkali treatment was not suitable to be performed at 1 hr, but, the treatment must be performed at longer time between 3 to 12 hrs, which could produce more than 8% agar.

Agar contents in squeezed thalli condition were not much different among the treatments and no big difference in values was observed from native and alkali-treated. As mentioned earlier, there were no reports available on the agar content extracted from agarophyte squeezed thalli. In this study, results indicated that agar yields of squeezed thalli were not affected by alkali pre-treatment prior to extraction. From previous studies, the fine structure of the cell wall of *Gracilaria* sp. consists of a cuticle, outer and inner walls [11-12], but after extraction process, the cuticle layer ruptured [13]. The squeezing of thalli in this study may rupture the cell

wall of *G. manilaensis* before the extraction process was done, thus, the agar residues might be lost. In addition, the disruption of cell wall may also occur during the cutting of the thalli and this presumably reduces agar yield.

Rheological Analysis of Gel Strength: Gel strength is the indication of agar quality. The higher the gel strength, the higher the agar quality. The gel strength of agar varied with season, location, species and also extraction process [4, 14-16]. In this study, the gel strength of native extraction in both intact and squeezed thalli was very low. These results indicated that native extraction in both conditions yielded high content of sulfate and low content of 3,6-anhydrogalactose, as reported by author [1]. To improved quality of agar gel strengths, samples of *G. manilaensis* were subjected to alkali treatment. Alkaline treatment reduced sulfate content and increased 3,6-anhydrogalactose content [1, 17]. Therefore, alkali treatment plays an important role to increase agar quality (gel strength, total sulfate and 3,6-anhydrogalactose content) [10]. This is in agreement with results found in this study, i.e: the gel strength increased in alkali-treated thalli (intact and squeezed) compared to native extraction.

Among the alkali-treated thalli under intact condition, the 3 hrs treatment produced highest gel strength and agar content. Analysis of variance and analysis of Tukey's HSD test of gel strength showed significant difference ($p < 0.05$) between native extraction and 3 hrs

treatment of intact thalli. Hence, these results showed that under intact condition 3 hrs treatment could be considered as the best treatment to obtain higher agar content and agar gel strength. However, squeezed thalli showed different results compared to intact thalli, with the lowest gel strength at 3 hrs alkali treatments and the highest at 24 hrs alkali treatment. As noted before, there was no report about the agar quality from squeezed thalli, but, from this study, results revealed that perhaps alkali treatment did affect agar gel strength of squeezed thalli. After alkaline pre-extraction, the gel strengths of alkali-treated thalli were higher than their native extraction. From analysis of variance and Tukey's HSD test, under squeezed condition, 6 hrs, 12 hrs and 24 hrs alkali treatments showed a significant difference ($p < 0.05$) with native agar extraction. Alkali treatments of squeezed thalli extraction at 6 hrs, 12 hrs and 24 hrs produced high gel strength, as well as, high agar content but from the economic point of view, 6 hrs alkali treatments seemed to be the optimal time for the alkali treatment.

CONCLUSION

The study of different agar extraction processes of *G. manilaensis* collected from the earthen pond at Pantai Merdeka, Kedah revealed that agar content in native extraction and alkali-treated samples were varied among intact and squeezed thalli. Native extraction of intact thalli produced higher agar content. Both intact and squeezed thalli showed a very low gel strength, but after treatment with alkali, the gel strengths were higher compared to their native agar extraction. In this study, alkali treatment affected agar content and agar gel strength of intact thalli; whereas for squeezed condition, alkali treatment did not affect agar content but affected agar gel strength. From the study, it may be concluded that 5% NaOH treatment for 3 hrs of intact thalli and 6 hrs, 12 hrs and 24 hrs of squeezed thalli may be considered the best treatment for producing high agar yield and agar gel strength.

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