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Effects of different concentrations of ammonium phosphate on the yield and quality of carrageenan, *Kappaphycus striatus* (Schmitz) Doty ex Silva

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ABSTRACT

This study determined the effect of different concentrations of ammonium phosphate on the yield and quality (gel strength, viscosity, syneresis, gelling, and melting temperature) of carrageenan. The study was conducted at the Seaweeds Post-Harvest Laboratory, College of Fisheries, Mindanao State University for 33 days. The experiment involved four treatments and three replications. The four treatments were the different concentrations of ammonium phosphate (g/L) namely; Treatment 1 (T1) - 0g/L, Treatment 2 (T2) – 3 g/L, Treatment 3 (T3) – 6 g/L, Treatment 4 (T4) – 9 g/L. The results showed that the application of ammonium phosphate significantly reduced the yield and viscosity of the carrageenan by 4.81% and 1.83cPs respectively. Gel strength was enhanced by 1.53g in T2 (3/L). Control showed the best syneresis compared to the other treatment. The syneresis was significantly decreased by 5.43% with the application of ammonium phosphate. The fertilizer seemed not to have any effect on the gelling and melting temperature of the carrageenan. Ammonium phosphate is therefore not recommended for growing Kappaphycus striatus for carrageenan.

Key Words: *Ammonium phosphate, Carrageenan Kappaphycus and Seaweed*

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I. Introduction

The red alga, *Kappaphycus striatus* (Schmitz) Doty ex Silva, is one of the major commercial sources of kappa-carrageenan for the world seaweed industry (Mendonza et al., 2006). Kappa-carrageenan is used as raw materials of pharmaceutical, cosmetic, food, and other industries (Vairappan, 2006) and mostly applied in food products (Prasetyowati et al., 2008). Total production of k-carrageenan is 95% from Southeast Asia, 55% from Philippine, 38% from Indonesia, and 2.5% from Malaysia (Duduku et al., 2008; Campo et al., 2009). The Philippines is recognized as one of the global growers of seaweeds in commercial quantities. Seaweed is leading marine-based products and falls within the top three

export commodities; therefore, it contributes a lot to the national GDP (DA-BFAR, 1999). The cultivation of seaweed in the Philippines started in 1969 to 1970 with *Kappaphycus alvarezii* and *Eucheuma denticulatum* (Hung et al., 2009; Kreckhoff et al., 2015). *K. alvarezii*, is the most common variety of Kappaphycus, and it's an important source of kappa carrageenan (Montalou et al., 2008). It is widely used in the industry as a gelling and thickening agent (Nuguit et al., 2009).

Seaweed mariculture is now recognized as a very productive alternative source of livelihood and employment especially in developing countries in tropical Asia where a large portion of the shallow coastal fishery resources have been or are depleted (DA-BFAR, 1988). Some seaweed farms in the southern part of the Philippines like Tawi-Tawi, which used to be excellent productive areas are now producing only moderate seaweed yields. Due to the deteriorating quality of planting material or overstocking of seaweed which has caused nutrient depletion (Vairappan, 2006). To increase the demand for phycocolloids (carrageenan) and increase the income of seaweed farmers, there is the need to adapt intervention in farming practice like enrichment in production (Luhan et al., 2015). The most essential macronutrients that enhance the growth of seaweed are C: N: P. For great results application concentrations have to be balanced (Msuya, 2009;Harrison and Hurd 2001). Phosphorus is recognized as a nutrient that when it's applied with nitrogen, has an important role in algal growth and carrageenan content (Neish, 2008; Pickering et al., 2007; Chopin et al., 1990).

The quality of carrageenan is determined based on its purity, gel strength, and viscosity (Kreckhoff et al., 2015). The properties of carrageenan could differ based on the harvest time (Hurtado et al., 2008), region, growth conditions (salinity, deepness, nutrients), growth season, the extraction process, and parameters (Hayashi et al., 2007a; Montolalu et al., 2008). It is important to determine carrageenan because it used in different industries, and the quality must meet the quality standard of FAO (Kreckhoff et al., 2015). Though seaweed production has been successful in the Philippines, the expected yield has not been realized yet; many factors could account for this. (deGóes and Reis, 2012; Hayashi et al., 2007b; Reis et al., 2007). In this present study, the researcher seeks to determine the effect of different concentrations of ammonium phosphate on the yield and quality of carrageenan and make a recommendation for yield and quality enhancement.

II. Materials and Methods

Place and time of the study: The study was conducted at the Seaweeds Post-Harvest Laboratory, College of Fisheries, Mindanao State University -Tawi-Tawi College of Technology and Oceanography, Philippines. The study duration was February 24 – March 30, 2019.

Research design and samples: The experiment was laid out in a Completely Randomized Design with four treatments and 3 replications per treatment. The four treatments were briefly immersed (30 seconds) in different concentrations of ammonium phosphate (g/L): Treatment 1 (T1, the Control) - 0g/L, Treatment 2 (T2) – 3 g/L, Treatment 3 (T3) – 6 g/L, Treatment 4 (T4) – 9 g/L, removed and partly covered overnight and planted in the farm. The seaweeds were bought from the seaweed farmers at Pasiagan, Bongao, Tawi-Tawi, transported and placed in a rectangular fiberglass tank at the Multi-Species Hatchery, College of Fisheries, Mindanao State University–Tawi-Tawi College of Technology and Oceanography, Sanga-Sanga, Bongao, Tawi-Tawi. Flowthrough of fresh sand filtered seawater (34-35ppt) was used and airstone was attached for aeration.50grams of seaweeds were weighed per bunch; 30 bunches were tied using 7 inches "soft-tie" on a PE rope (#7) 25cm apart from each bunch. The PE rope was tied to both ends of the stakes and 0.3m from the bottom of the lines. Lines per treatment were randomly arranged. Each treatment was triplicated (3 lines or 30 bunches). Farming was done at 6 AM using the fixed-off bottom method. A 21-day old farmed samples were collected randomly from Sowangkagang, Bongao, Tawi-Tawi with 600g/treatment sun dried for 2 days, then oven dried at 60°C for 6 hours before extraction of carrageenan.

Extraction procedures: The native carrageenan extraction method was used in this study, 15 g of the clean anhydrous apical and basal samples were placed in stainless steel containers with 300ml of distilled water in a boiling water bath for approximately 1 hr. Afterward, the samples were ostracized and dicalite added as a filter aid. Homogenized algal material was pressure filtered. The product

carrageenan solution was frozen overnight and thawed in the morning, sundry for 5 days. The carrageenan yield (% dry weight) was determined according to the formula of Hung et al. (2009):

$$Yield = \frac{wc}{wm}\ x\ 100$$

Where: wc = dry carrageenan weight, wm= dry algal weight.

Rheological analyses: A 1.5% carrageenan solution, fortified with 0.2% KCl, was prepared to measure the following:

1. Gelling temperature (measured by laboratory thermometer**) –** the temperature at which the introduced glass beads (diameter of 2.85 mm; weights 30 mg) fail to sink to the bottom of the test tube at an interval of 0.5°C).
2. Viscosity was measured using Brookfield Viscometer (Model LVF) at 75°C with spindle 1 at 30 rpm in a 110.3=46 mm electrolytic beaker.
3. Gel strength(in grams) – the force required to rupture the gel matrix in the immediate vicinity of the applied load. It was measured from the carrageenan solution and allowed to gel for 15 h at room temperature (Sulu et al., 2006). The gels were prepared in a 50 ml beaker and characterized using a Stable Micro Systems texture analyzer model TA-TX2i (Stable Microsystems, Godalming, UK) equipped with a 0.5-inch diameter plunger and operating at 0.5 mm s^{-1} descent rate.
4. Melting temperature (measured by laboratory thermometer)- the temperature at which a lead shot (diameter: 4.30 mm; weight: 430 mg) gradually sinks to the bottom of the test tube at an interval of 0.5°C.
5. Syneresis index- the percent of water released from the cylindrical gels (2.2=3.5 cm) in the filter paper based on weight loss after 2 h (Romero et al., 2000).

Statistical analysis: The significant differences in carrageenan yield and quality (measured as to: gel strength, viscosity, syneresis, gelling and melting temperatures, syneresis index) among the different concentrations of ammonium phosphate of the *Kappaphycus striatus* were determined using one-way ANOVA (ρ <0.05) in SPSS software (SPSS version 20) and subjected to Duncan's Multiple Range Test for significant difference among the treatments.

III. Results and Discussion

Carrageenan yield

Table 01 provides the mean and standard error mean (SEM) of the carrageenan yield. The highest carrageenan yield (34.49 ±0.65) was obtained from Treatment 1 (0g/L), followed by Treatment 2 (3g/L) with a yield of 32.95±0.65, then Treatment 3 (9g/L) with a yield of 30.18±2.55, and the lowest was in Treatment 4 (6g/L) with a yield of 25.89±16.75. It was observed that though T1 had the highest yield, it did not statistically differ from the yield of T2. However, T1 and T2 were statistically different from T3 and T4. No statistical differences were noticed in the yield of T3 and T4. In the study of Rui et al. (1990) *Kappaphycus alvarezii* (kappa-carrageenophyte) was grown in nutrient-enriched water. The researchers revealed that carrageenan content was lower compared to plants grown in natural seawater without ammonia additions. Contrarily, the findings of Luhan et al. (2015) reported that carrageenan yield was significantly higher in enriched Kappaphycus (42%) than the control (33%); the study of Orbita (2013) affirms that a maximum carrageenan yield was obtained during the period of highest nutrient treatment. According to Naguit et al. (2009) plants of higher growth rate have a higher carrageenan content compared to slow-growing plants.

Table 01. Mean and SEM (standard error mean) of the carrageenan yield

Treatment (concentration)	Carrageenan Yield (%)
T1 (0g/L)	34.49 ±0.65[a]
T2 (3g/L)	32.95±0.65[a]
T3 (6g/L)	25.89±16.75[b]
T4 (9g/L)	30.18±2.55[ab]

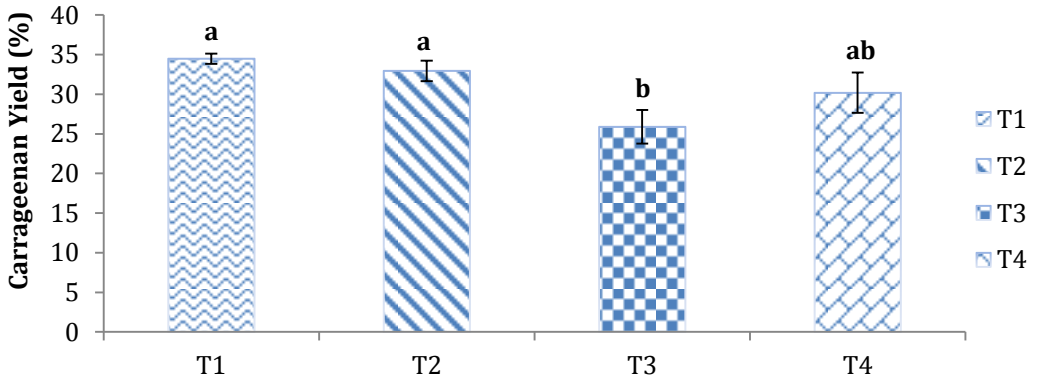

Figure 01. Carrageenan yield percentage of extracted *K. striatus* in different concentrations of ammonium phosphate.

Carrageenan viscosity

The results of Carrageenan Viscosity are shown in (Table 02). Treatment 1 (0g/L) gave the highest viscosity (6.83±0.17 cPs). This was followed by Treatment 2 (3g/L) with a viscosity of (5±0.00). Treatment 3 (9g/L) had a viscosity of (4.83±1.67) and the lowest viscosity (3.5±0.00) was recorded in Treatment 4 (6g/L). The viscosity in Treatment 1 was significantly different from the viscosities in Treatment 2, 3, and 4. Treatments 2 and 4 were not significantly different, however, both were significantly different for Treatment 3. It seemed that the application of Ammonium Phosphate rather decreased the viscosity instead of increasing. Kreckhoff et al. (2015) explain that viscosity was influenced, and salinity and viscosity decrease with maturation (Lechat et al., 1997). Suryaningrum (1988) and Syamsuar (2006) add that increased harvest period could as well reduce the carrageenan viscosity because of decreased sulphate content. This finding seems to oppose the results of this study. The application of ammonium phosphate could not have a significant effect on the viscosity of the carrageenan.

Table 02. Mean and SEM (standard error mean) of the carrageenan viscosity

Treatment (concentration)	Viscosity (cPs)
T1 (0g/L)	6.83±0.17[a]
T2 (3g/L)	5±0.00[b]
T3 (6g/L)	3.5±0.00[c]
T4 (9g/L)	4.83±1.67[b]

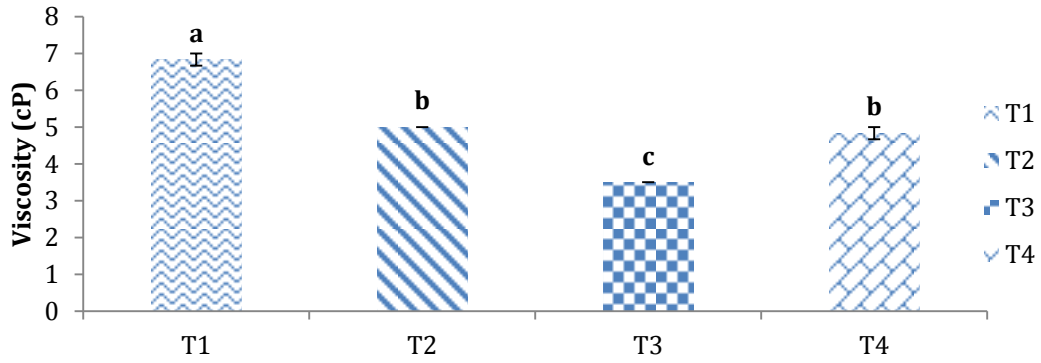

Figure 02. Viscosity of carrageenan of the extracted *K. striatus* in different concentrations of ammoniums phosphate.

Carrageenan gel strength

The Gel Strength results are displayed in (Table 03). It appeared that Treatment 2 (3g/L) had the highest gel strength (48.2±0.53g). It was followed by Treatment 1 (0g/L) with gel strength of (46.67±0.4), then Treatment 4 (9g/L) with gel strength of (38.93±1.23). The least gel strength was recorded for Treatment 3 (6g/L) with gel strength of (31.77±0.50g). The analysis showed no

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significant difference between the means of Treatment 2 and the control. However, both treatments were significantly different from Treatments 3 and 4. This means that the effect of a higher concentration of ammonium phosphate (6g/L and 9g/L) decreased the gel strength of the carrageenan. This finding supports earlier studies. It was highlighted that nitrogen enrichment increases yield but may not affect the gel strength of carrageenan. Besides, Kreckhoff et al. (2015) buttress that gel strength is affected by nitrate concentration.

Table 03. Mean and SEM (standard error mean) of the carrageenan gel strength

Treatment (concentration)	Gel strength (g)
T1 (0g/L)	46.67±0.4[a]
T2 (3g/L)	48.2±0.53[a]
T3 (6g/L)	31.77±0.50[c]
T4 (9g/L)	38.93±1.23[b]

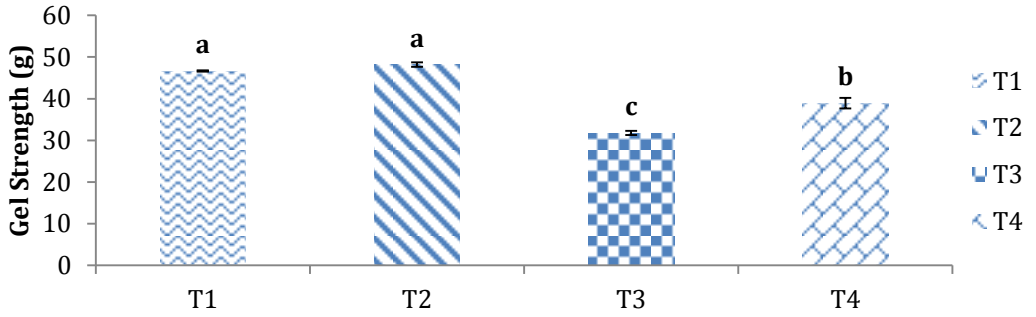

Figure 03. Gel strength of carrageenan of the extracted *K. striatus* in different concentrations of ammonium phosphate.

Carrageenan syneresis

Syneresis index refers to the amount of water exuded from the given amount of gel. The detailed results of syneresis are portrayed in (Table 04) below. Table 04 shows that Treatment (6g/L) had the highest syneresis (20.12±18); it was followed by Treatment 2 (3g/L) with syneresis of (12.61±0.14), then Treatment 4 (9g/L) with syneresis of (11.03±0.73). The least syneresis was observed in Treatment 1 (0g/L) (9.16±0.27). This implies that the application of Ammonium Phosphate can drastically reduce the syneresis in Carrageenan. The analysis indicated a significant difference between the means of the Control and the other treatments with Ammonium Phosphate. Many differences were not observed in the syneresis of Treatments 2, 3, and 4. In explaining the reason for this syneresis, Mendonza et al. (2006) documented that the higher syneresis index percentage of carrageenan could be due to the presence of alkali. According to the researcher, alkali modified carrageenan has higher syneresis. When there is a low amount of ester sulfate available for water interaction, the young apical segments exhibited better rheological and gelling properties than mature parts of the alga. The Control (0g/L) of ammonium phosphate in this study showed lesser water exudation and gave better quality to carrageenan. Besides, carrageenan gels, regardless of source (whether apical or basal), released more water than the native carrageenan gels, as shown by their higher syneresis index.

Table 04. Mean and SEM (standard error mean) of the carrageenan syneresis

Treatment (concentration)	Syneresis (%)
T1 (0g/L)	9.16±0.27[c]
T2 (3g/L)	12.61±0.14[b]
T3 (6g/L)	20.12±18[a]
T4 (9g/L)	11.03±0.73[ab]

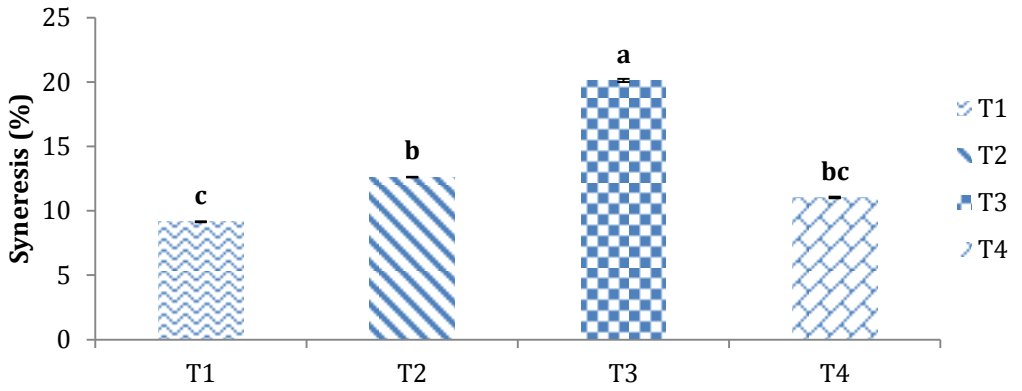

Figure 04. Syneresis index percentage of the carrageenan of extracted *K. striatus* in different concentrations of ammonium phosphate.

Carrageenan gelling and melting temperature

Shown in (Tables 05 and 06) the results on the Melting and Gelling Temperatures. No significant differences were shown for all treatments. This implies that the ammonium phosphate concentrations did not have any effect whatsoever on the carrageenan in terms of gelling and melting temperatures. This is supported by Mendonza et al. (2006) who reported that the melting temperature of carrageenan is not affected by nutrient concentrations or growth environment.

Table 05. Mean and SEM (standard error mean) of the carrageenan gelling temperature

Treatment(concentration)	Gelling Temperature (°C)
T1 (0g/L)	35±0.58[a]
T2 (3g/L)	36±0.58[a]
T3 (6g/L)	35.33±0.33[a]
T4 (9g/L)	37±0.58[a]

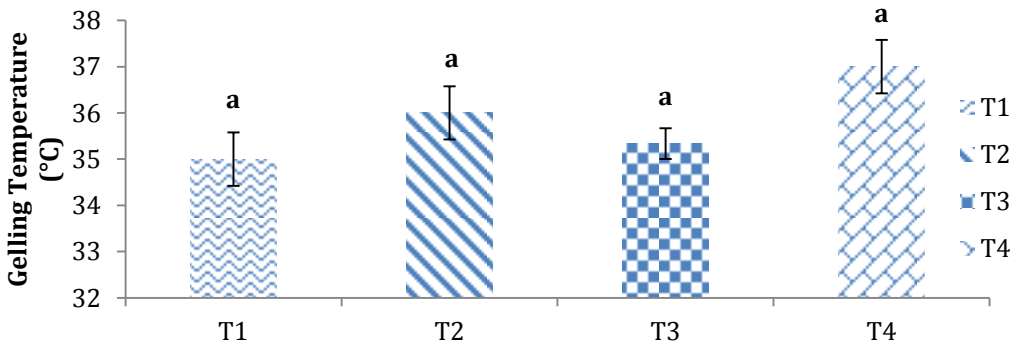

Figure 05. Gelling temperature of the carrageenan of extracted *K. striatus* in different concentration of ammonium phosphate.

Table 06. Mean and SEM (standard error mean) of the carrageenan melting temperature

Treatment (concentration)	Melting Temperature (°C)
T1 (0g/L)	47±1.00[a]
T2 (3g/L)	45.67±4.84[a]
T3 (6g/L)	35.67±3.38[a]
T4 (9g/L)	41.33.3.18[a]

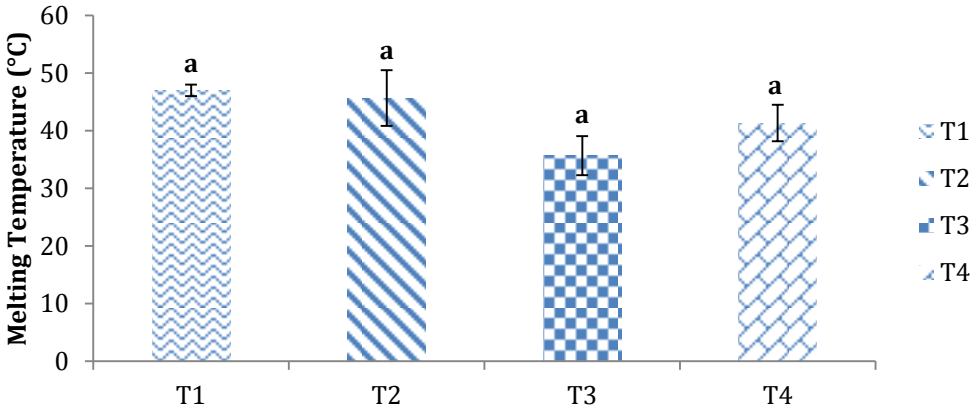

Figure 06. Melting temperature of the carrageenan of extracted *K. striatus* in different concentrations of ammonium phosphate.

IV. Conclusion

Based on the results of this study, it can be seen that the different concentrations of the ammonium phosphate did not significantly affect the carrageenan yield and quantity of carrageenan. Therefore, it is not recommended for the carrageenan yield and quality enhancement. For enhanced yield and quality, factors such as environmental parameters and nutrients in the water that could be considered during the cultivation.

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