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Effect of Extractant-to-Semi Refined Carrageenan Water Ratio on the Physicochemical Characteristics of Carrageenan from Kappaphycus striatum

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ABSTRACT

Kappaphycus striatum, a red seaweed rich in sulfated polysaccharides, serves as a promising raw material for carrageenan production due to its favorable gelling and stabilizing properties. This study aimed to investigate the effect of different extractor solvent ratios on the physicochemical characteristics of refined carrageenan derived from K. striatum. Refined carrageenan was extracted from semi-refined material using hot water at solvent-to-seaweed ratios of 1:40, 1:50, and 1:60, followed by precipitation with isopropyl alcohol. The parameters assessed included moisture content, yield, gel strength, sulfate content, and viscosity. Moisture content remained relatively constant across treatments (≈10–11%) and complied with FAO/JECFA standards. In contrast, yield and gel strength increased significantly with higher solvent ratios, with the highest values observed at the 1:60 ratio (30.04% yield; 1090.23 g/cm² gel strength). Sulfate content also increased, peaking at 21.42% for the same treatment. Viscosity was highest at the 1:50 ratio (87 cP), indicating a favorable balance between molecular hydration and structural integrity. All treatments produced carrageenan within acceptable quality standards for commercial use. The results demonstrated that the extraction solvent ratio played a critical role in determining the performance characteristics of refined carrageenan. Therefore, adjusting the water-to-seaweed ratio during extraction may serve as a practical approach to optimize carrageenan properties for targeted industrial applications. Contribution to Sustainable Development Goals (SDGs):

SDG 1 :No poverty
SDG 9: Industry, Innovation and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 14: Life Below Water

1. INTRODUCTION

1.1. Research Background

Carrageenan, a group of sulfated polysaccharides, constitutes a significant food additive primarily obtained through the extraction of specific red seaweeds. This extraction process typically employs hot water or alkaline solutions [1]. The utility of carrageenan extends broadly beyond the food industry, encompassing diverse non-food applications. In the food sector, carrageenan is highly valued for its favorable physical properties, functioning effectively as a thickener, stabilizer, and gelling agent [2]. Furthermore, it finds application as an ingredient in cosmetics, paints, textiles, and is extensively utilized within the pharmaceutical industry [3]. The demand for carrageenan continues to grow, with an approximate annual increase of 5.5%, driven by ongoing product development in the food sector, particularly in items like ice cream and various other dairy products [4].

Commercially, carrageenan is generally traded in two primary forms: refined carrageenan and semi-refined carrageenan. The fundamental distinction between these two forms lies in their cellulose content and the clarity of their solutions [5]. The production process for semi-refined carrageenan typically omits the alcohol or potassium chloride (KCl) precipitation step, resulting in a gel or solution that is notably more turbid compared to refined carrageenan [6]. In Indonesia, semi-refined carrageenan production currently predominates over refined carrageenan manufacturing. The purification of semi-refined carrageenan into its refined counterpart can be achieved by re-extracting the semi-refined product with water at elevated temperatures, typically around 80°C. At this stage, the ratio of the extractor solvent volume to the semi-refined carrageenan significantly influences the final characteristics of the resulting refined carrageenan.

Previous research by Ref. [7] investigated the extraction of refined carrageenan using various water-to-semi-refined carrageenan ratios, specifically 1:40, 1:50, and 1:60. Their study utilized semi-refined carrageenan as raw material, which had been previously extracted under specific conditions (8% KOH at 90°C for 2 hours) and employed celite as a filter aid. Their findings indicated that the 1:50 ratio yielded the most favorable results, producing a carrageenan with a yield of 23%, a gel strength of 2,058 g/cm², and a viscosity of 122.5 cP. Further supporting the critical role of extraction parameters, a recent study by [8] revealed that the water ratio significantly influenced the yield, sulfate content, and gel strength of carrageenan, although it did not have a significant effect on water content. This research highlighted that optimal carrageenan characteristics were achieved with a 1:60 treatment, yielding a water content of 11.26 ± 0.52%, a yield of 28.00 ± 0.23%, a sulfate content of 18.87 ± 0.57%, a gel strength of 1370.73 ± 23.57 g/cm², and a peak viscosity of 70 cP.

While studies such as [9] have reported significant carrageenan yields, averaging 40-50%, from Kappaphycus striatum and Kappaphycus alvarezii cultivated in nine locations within West Nusa Tenggara Province, the detailed characteristics of refined carrageenan extracted from their semi-refined counterparts remain less extensively documented. Given this gap, the primary objective of the present study was to comprehensively investigate the effect of varying extractor water-to-carrageenan volume ratios on the characteristic properties of the refined carrageenan produced.

1.2. Literature Review

Carrageenan is a hydrocolloid compound composed of esterified forms of potassium, sodium, magnesium, and ammonium sulfate with galactose and 3,6-anhydrogalactose. It is a linear-chain polysaccharide with a molecular weight exceeding 100 kDa. The polysaccharide backbone consists of alternating galactose and 3,6-anhydrogalactose units, which may be sulfated and are linked through α-(1→3) and β-(1→4) glycosidic bonds [10], [11]. Carrageenan is primarily derived from red seaweeds (Rhodophyceae), which have diverse thallus morphologies and pigmentation. These seaweeds contain photosynthetic pigments such as carotene, xanthophyll, phycobilins (mainly phycoerythrin), chlorophyll a, and b. The cell wall structure is rich in cellulose and produces hydrocolloids like carrageenan, agar, furcellaran, and porphyran [9], [11], [12]

1.2.1. Carrageenan extraction

Carrageenan is a hydrocolloid compound composed of esterified forms of potassium, sodium, magnesium, and ammonium sulfate with galactose and 3,6-anhydrogalactose. It is a linear-chain polysaccharide with a molecular weight exceeding 100 kDa. The polysaccharide backbone consists of alternating galactose and 3,6-anhydrogalactose units, which may be sulfated and are linked through α-(1→3) and β-(1→4) glycosidic bonds [10], [11]. Carrageenan is primarily derived from red seaweeds (Rhodophyceae), which have diverse thallus morphologies and pigmentation. These seaweeds contain photosynthetic pigments such as carotene, xanthophyll, phycobilins (mainly phycoerythrin), chlorophyll a, and b. The cell wall structure is rich in cellulose and produces hydrocolloids like carrageenan, agar, furcellaran, and porphyran [9], [11], [12]

1.2.2. Carrageenan extraction

Carrageenan extraction typically involves hot water or hot alkaline solutions to create an alkaline environment for seaweed biomass. This condition is achieved by adding alkaline agents such as NaOH, $Ca(OH)_2$, or KOH to raise the pH to 8–10. Alkaline treatment facilitates the removal of hydrophilic 6-sulfate groups from carrageenan monomers and promotes the formation of 3,6-anhydrogalactose, a hydrophobic compound that enhances gel strength. Additionally, this treatment increases the melting point of carrageenan above cooking temperatures and improves its whiteness and gel strength [13], [14]

1.3. Research Objective

The study aimed to comprehensively investigate the effect of varying extractor water-to-carrageenan volume ratios on the characteristic properties of the refined carrageenan produced.

2. MATERIALS AND METHODS

The materials used in this study included Kappaphycus striatum seaweed sourced from seaweed farmers in Kertasari, Sumbawa; potassium hydroxide (KOH, Merck, Germany); isopropyl alcohol; sulfuric acid (H_2SO_4, Merck); potassium sulfate (K_2SO_4, Merck); dinitrosalicylic acid; barium sulfate ($BaSO_4$, Merck); hydrochloric acid (HCl, Merck); hydrogen peroxide (H_2O_2, Merck); potassium bromide (KBr); barium chloride ($BaCl_2$, Merck); and distilled water. The equipment employed comprised standard laboratory glassware, a Rapid Visco Analyzer (RVA-4, Tecmaster N13713, Newport Scientific Pty Techmaster, Ltd., Warriewood, Australia), and a Texture Analyzer (TA-XT Plus; Stable Micro Systems Ltd., UK). Additional supporting equipment included a hotplate, magnetic stirrer, pH meter, thermometer, refrigerator, tray, blender, oven, Soxhlet apparatus, Kjeldahl flask, Whatman No. 42 filter paper, mortar, and analytical balance.

2.1. Semi-Refined Carrageenan Extraction

The semi-refined carrageenan was prepared using the method described by [15] Cleaned seaweed was extracted with a 6% KOH solution (Merck, Germany) for 60 minutes at a temperature of 70°C, using a solvent-to-seaweed ratio of 40:1 (based on dry weight). The extracted sample was then filtered and rinsed under running water until the rinse water reached a pH of 8–9. The pH was periodically measured by collecting rinse water in a container and testing it with pH paper. The sample was then cut into smaller pieces (±3 cm) and dried in an oven at 50°C for 18 hours. The dried material was milled and sieved using a 60-mesh screen.

2.2. Refined Carrageenan Extraction

Refined carrageenan was produced following the method outlined by [8]. The extraction was conducted by boiling the semi-refined carrageenan in pre-heated water. The water-to-sample volume ratio was adjusted according to the treatment groups: 40, 50, and 60 times the weight of the raw material. Extraction was carried out for one hour at 80–90°C, with occasional stirring. The resulting filtrate, containing dissolved carrageenan, was poured into a container with an equal volume of isopropyl alcohol to induce precipitation. This precipitation step lasted approximately 30 minutes. The carrageenan fibers obtained were filtered and dried in an oven at 50°C for approximately 18 hours. The dried product was then ground using a blender and sieved through a 60-mesh screen.

2.3. Viscosity Analysis (Rapid Visco Analyzer)

Viscosity analysis was performed using the method of [16] with a Rapid Visco Analyzer (RVA). A 1.5% carrageenan solution was prepared and placed into an aluminum canister. Distilled water was added to the canister and the sample was stirred until homogeneously mixed. The canister was then mounted onto the RVA unit, and the sample underwent a controlled heating and cooling cycle with continuous stirring. The sample was first heated to 50°C and held at this temperature for 1 minute. The temperature was then increased to 95°C at a rate of 6°C/min and held for 6 minutes. Subsequently, the sample was cooled back to 50°C at the same rate and held for 3 minutes. The resulting data were displayed as a viscosity curve.

2.4. Gel Strength Analysis (Texture Analyzer)

The gel strength of the carrageenan samples was analyzed using a Texture Analyzer (TA-XT Plus, Stable Micro Systems Ltd., UK) based on a modified method from FMC Marine Colloid. A 3 g sample of carrageenan was dissolved in 197 g of distilled water and heated using a hotplate until the solution reached 80°C for 15 minutes. The hot solution was poured into a plastic mold (6 cm diameter, 6.5 cm height) and allowed to set at 10°C (refrigeration temperature) for approximately 16 hours. The gel's breaking force (g) and deformation were measured using a 12.7 mm diameter cylindrical probe (P/0.5 R), which was applied vertically to the gel surface at a constant speed of 1 mm/s to a depth of 15 mm. The load cell used for texture analysis had a 5 kg capacity.

2.5. Sulfate Content Analysis

Sulfate content was determined using the [17] method. One gram of the carrageenan sample was placed in an Erlenmeyer flask and mixed with 50 mL of 0.2 N HCl. The mixture was heated to a boil for 1 hour, after which 25 mL of H_2O_2 was added and the mixture was heated for an additional 5 hours until the solution became clear. The solution was then transferred to a beaker, brought to a boil, and 10 mL of 10% $BaCl_2$ solution was added. The mixture was further heated on a hot plate for 2 hours to precipitate the sulfate as $BaSO_4$. The resulting precipitate was filtered using ash-free filter paper (Whatman No. 42) and washed with distilled water until free of chloride ions. The filter paper was dried and ashed at 700°C until a white residue was obtained. The ash was cooled in a desiccator and weighed to constant mass

3. RESULT AND DISCUSSION

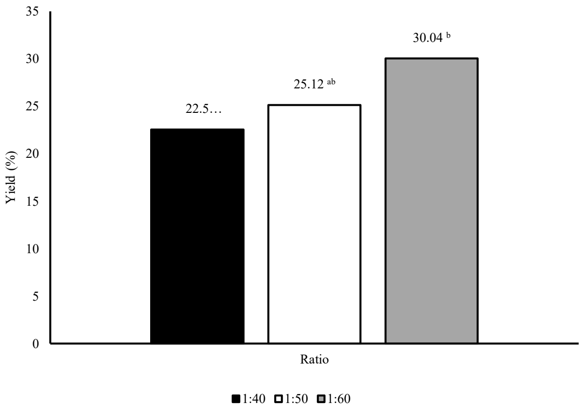

Figure 1 Carrageenan Yield

3.1. Moisture content

Figure 1 shows that refined carrageenan from K. striatum had moisture contents of about 10.84±0.43%, 10.95±0.76%, and 11.23±0.65% at solvent ratios of 1:40, 1:50, and 1:60, respectively. In other words, moisture increased only slightly (by ~0.4 percentage points) as the solvent ratio increased. This change is very small compared to the experimental error ranges, indicating no clear or statistically significant trend. In practice the moisture content is essentially constant across ratios; for example, one film-forming study reported ~11.24% moisture in κ-carrageenan, similar to our ~11%. All measured moisture values (≈10–11%) are well below the FAO/JECFA limit of 12% for commercial carrageenan. Thus, the product easily meets regulatory specifications. By comparison, [18] reported much lower moisture (on the order of 6–7%) under comparable extraction conditions. The higher moisture here could reflect differences in species, processing or post-drying conditions. In any case, our values remain within acceptable standards and are comparable to, or somewhat higher than, values reported in the literature for semi-refined carrageenan.

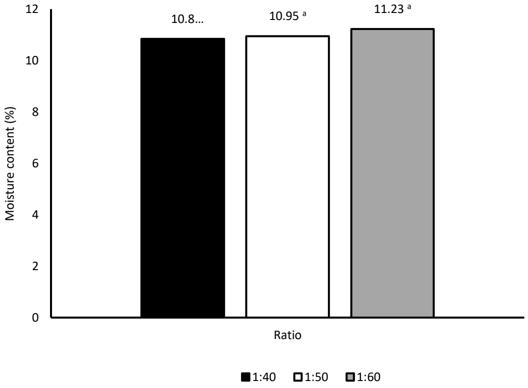

Figure 2 Moisture content of carrageenan

3.2. Moisture content

According to Ref. [18], the yield value is a crucial indicator for evaluating the effectiveness and efficiency of a carrageenan extraction process. Based on Figure 1, it can be observed that the extractor solvent ratio significantly influenced the yield of K. striatum carrageenan. A clear trend shows that higher extractor solvent ratios resulted in higher carrageenan yields. Specifically, the yield at a 1:40 ratio was 22.54 ± 0.92%, which increased to 25.12 ± 0.76% at a 1:50 ratio. The highest yield of 30.04 ± 2.69% was achieved at the 1:60 extractor solvent ratio. This indicates a statistically significant difference between the lowest (1:40) and highest (1:60) ratios.

The superior yield at the 1:60 water ratio compared to 1:40 or 1:50 can be attributed to the more dilute carrageenan solution formed at higher solvent volumes. This allows for a more complete dissolution of carrageenan from the seaweed matrix. During the subsequent precipitation phase, this leads to a smaller amount of insoluble residue or seaweed pulp, enabling a more efficient separation of the dissolved carrageenan. Conversely, at smaller solvent ratios, the carrageenan solution tends to be more viscous, causing some dissolved carrageenan to remain entrapped within the solid residue during precipitation [19].

[18] also reported the highest carrageenan yield at a 1:60 extractor solvent ratio, achieving a yield of 33% without the aid of a filter. This supports the general observation that higher solvent ratios can lead to improved yields. Furthermore, samples treated with lower extractor solvent ratios tended to form a thicker solution more rapidly during extraction compared to those with higher ratios. This rapid thickening can hinder the efficient dissolution of carrageenan, consequently affecting the final yield. During the precipitation process with isopropyl alcohol, the carrageenan yield was obtained as fibers and a turbid gel. It was observed that a smaller extractor solvent ratio tended to result in a higher proportion of the yield being in a gel-like form. The carrageenan extraction process itself can involve either boiling seaweed in hot water (for native carrageenan) or using an alkaline solution. [20] noted that the use of alkaline solutions accelerates the formation of carrageenan from its precursors and significantly enhances its gel strength.

3.3. Gel Strength

One of carrageenan's most critical functional properties, indispensable for its vast array of industrial applications, is its reversible sol-gel transition. This ability allows it to convert liquids into solid, thermo-reversible gels, underpinning its role as a thickener, stabilizer, and gelling agent. This gelling capability is attributed to 3,6-anhydrogalactose units within its polysaccharide backbone, a feature abundant in gelling types like kappa- and iota-carrageenan. The efficacy of carrageenan is profoundly influenced by its gel strength, which is sensitive to extraction parameters, as demonstrated by the Kappaphycus striatum data in Figure 3.

As shown in Figure 3, a clear and direct correlation exists between increasing extractor solvent ratios and significantly higher carrageenan gel strength for K. striatum. Gel strength substantially increased from 645.83 ± 7.03 g/cm^2 at a 1:40 ratio to 1046.83 ± 24.89 g/cm^2 at 1:50, peaking at 1090.23 ± 16.33 g/cm^2 at the 1:60 ratio. This finding unequivocally demonstrates that optimizing the extractor solvent ratio is critical for enhancing the gel strength of Kappaphycus striatum carrageenan, with the 1:60 ratio proving most effective in yielding the strongest gels. This improvement is likely due to more efficient hydration and solubilization of carrageenan polymers at higher solvent volumes, facilitating better network formation upon cooling.

Comparing these results, our K. striatum gel strengths were notably higher than those reported by [21] (504.3–683.9 g/cm^2), suggesting more effective extraction conditions in our study. Conversely, our values were generally lower than those found by[18] (1429.52–2273.32 g/cm^2). Such discrepancies can be attributed to various factors, including seaweed geographical origin, cultivation practices, post-harvest processing (e.g., alkali treatment), and precise analytical methodologies.

The ability of carrageenan to form gels is linked to 3,6-anhydrogalactose, which adopts a $^1C_4$ conformation crucial for forming helical secondary structures. These helices then associate to create a 3D gel network [22], [23]. K. striatum contains both kappa- and iota-carrageenan, whose combined presence influences the overall gel characteristics. Kappa-carrageenan forms strong, rigid gels, while iota-carrageenan yields more elastic ones. The high gel strength observed in K. striatum is likely a synergistic result of an optimized extraction ratio, effective polymer composition, and intrinsic structural features. Beyond extraction, gel strength is also influenced by carrageenan yield, concentration, temperature, and the presence of ions like potassium and calcium, which facilitate helix cross-linking [24], [25].

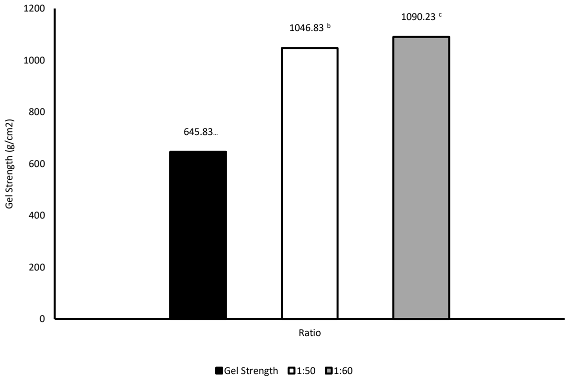

Figure 3 Carrageenan Gel Strength

3.4. Sulfate Content

The influence of the extractor solvent ratio on the sulfate content of carrageenan in this study is presented in Figure 4. Generally, a higher extractor solvent ratio was observed to result in a higher sulfate content. Based on Figure 4, it is evident that the extractor solvent ratio significantly influenced the sulfate content of Kappaphycus striatum carrageenan. While the 1:40 and 1:50 ratios yielded similar sulfate levels of 13.97 ± 0.76% and 15.01 ± 0.42% respectively, the sulfate content dramatically increased to 21.42 ± 0.67% at the 1:60 extractor ratio. This indicates a statistically significant increase in sulfate content at the highest solvent ratio. The sulfate content for K. striatum in this study ranged from 13.97 ± 0.76% to 21.42 ± 0.67%. These values were generally lower compared to the sulfate content reported by [21], which ranged from 22.4 ± 0.43% to 23.1 ± 1.96%.

[26] also noted that a higher extractor solvent ratio tends to yield higher sulfate content. The highest sulfate content in this study was indeed obtained at the 1:60 treatment for K. striatum samples. The elevated sulfate content in K. striatum carrageenan is likely attributable to the formation or higher retention of iota-carrageenan during the extraction process. K. striatum is known to contain iota-carrageenan, typically ranging between 4-10 mol% [21], [27]–[29] clarified that iota-carrageenan possesses sulfate groups at both the C2 and C4 positions of its disaccharide repeating unit, whereas kappa-carrageenan has sulfate groups only at the C4 position. The increased solubility or more complete extraction of highly sulfated fractions at higher solvent ratios could explain the observed increase in sulfate content.

The presence and concentration of sulfate groups in carrageenan significantly influence its resulting characteristics. A higher sulfate content can lead to a decrease in gel strength and, conversely, increase the viscosity of carrageenan solutions [1], [21]. This inverse relationship between sulfate content and gel strength is a crucial consideration for the application properties of extracted carrageenan.

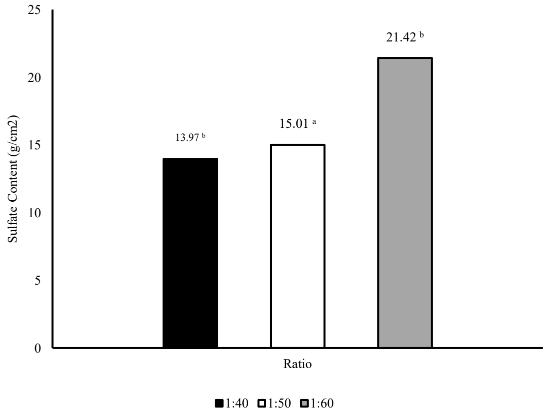

Figure 4 Sulfate content

3.5. *Viscosity*

Viscosity is a highly critical quality characteristic of carrageenan, serving as a key parameter for determining its suitability for various applications. The Food and Agriculture Organization [17] sets a standard for carrageenan viscosity at greater than 5 cP when measured at 75°C at a concentration of 1.5%. The viscosity profiles of Kappaphycus striatum carrageenan at a 1.5% concentration, obtained in this study, are presented in Figure 5. The viscosity profile of K. striatum carrageenan revealed that the 1:50 extractor solvent ratio treatment generally exhibited higher viscosity compared to the other two treatments (1:40 and 1:60). At an initial temperature of 50°C, the viscosity of K. striatum carrageenan was recorded as 97 cP for the 1:40 treatment, 103 cP for the 1:50 treatment, and 75 cP for the 1:60 treatment, respectively. Throughout the heating and cooling cycles, the carrageenan viscosity demonstrated remarkable stability, with minimal deviation from its initial values. The peak viscosity for K. striatum carrageenan across all treatments was observed at approximately 62°C, where the respective peak viscosities were 76 cP (1:40), 87 cP (1:50), and 64 cP (1:60). For quality assessment, carrageenan viscosity is typically measured at 1.5% concentration and 75°C. At this standard temperature, the lowest viscosity was obtained for the 1:60 treatment in K. striatum samples.

Generally, the viscosity values of K. striatum carrageenan were notably influenced by the extraction ratio and correlated with other physicochemical properties, particularly gel strength. The strong inverse relationship between high 3,6-anhydrogalactose content and gel strength also contributes to the viscosity profile, where higher gel strength tends to result in lower solution viscosity. Furthermore, sulfate content plays a significant role in determining carrageenan solution viscosity, with higher sulfate levels generally contributing to increased viscosity due to enhanced molecular hydration and repulsion, which can inhibit strong gel network formation but promote chain entanglement in solution [11], [30].

The viscosity values obtained in this study for K. striatum carrageenan were greater than those reported by [21], who found values ranging from 18-22 cP at 75°C for K. striatum samples. Conversely,[18] reported a broader range of carrageenan solution viscosities at 75°C, from 37.5-95 cP, which partially overlaps with our findings. Crucially, all viscosity values obtained in this study for K. striatum carrageenan consistently meet the minimum FAO standard of 5 cP at 75°C, affirming the quality of the extracted product for industrial applications.

Ref. [30] emphasized that carrageenans capable of altering solution viscosity at low concentrations are highly valued as stabilizers. At such low concentrations, carrageenan effectively prevents separation and forms a stabilizing network that can maintain particles, such as cocoa particles in suspension [12]. This highlights the functional importance of achieving optimal viscosity profiles for specific product formulations.

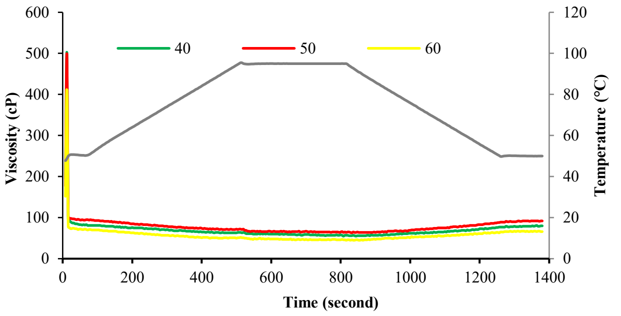

Figure 5 Carrageenan Vicoscity profile

4. CONCLUSION

This study confirmed that the extractor solvent ratio significantly affects the quality of refined carrageenan from Kappaphycus striatum. Moisture content remained stable (≈10–11%) across all treatments, well within the FAO/JECFA limit. In contrast, yield, gel strength, sulfate content, and viscosity varied notably. The highest yield (30.04%) and gel strength (1090.23 g/cm²) were achieved at the 1:60 ratio, indicating that greater solvent volumes improve carrageenan solubilization and network formation. Sulfate content also peaked at 1:60, reflecting enhanced extraction of sulfated fractions, though high sulfate may reduce gel strength. Viscosity was highest at the 1:50 ratio (87 cP at ~62°C), suggesting an optimal balance between sulfate content and polymer interaction. All viscosity values met FAO standards,

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confirming product quality. Overall, a 1:60 ratio is ideal for maximizing yield and gel strength, while 1:50 may be preferred when viscosity is a priority. These findings emphasize the importance of optimizing extraction conditions for desired carrageenan properties

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