

EVALUATION OF CARRAGEENAN GEL PROPERTIES AT DIFFERENT CONCENTRATIONS OF CALCIUM CHLORIDE DIHYDRATE SALT

(Penilaian Gel Carrageenan Beraroma Pada Variasi Kepekatan Garam Kalsium Klorida Dihidrat)

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Abstract

Aromatherapy is an alternative therapy for Complementary and Alternative Medicine (CAM) to relieve mild depression in individuals. This study aimed to evaluate the effect of incorporating calcium chloride dihydrate in carrageenan gel to increase stability property. The gel formulation was produced from a mixture of semi-refined carrageenan, sodium benzoate, alginate acid, polyethylene glycol (PEG) and sorbitan monolaurate (Span 20) followed by the inclusion of calcium chloride dehydrate (CCD) at 0%, 0.5%, 1%, 1.5%, and 2% concentrations. The effects of CCD concentration on rheology, gel Bloom strength, morphology, and thermal properties were investigated. The viscosity and shear stress decreased on incorporating of CCD in the carrageenan gel formulation. The highest viscosity of the control gel was 597.84 mPa.s. The intersection of G' and G'' at various concentrations of CCD salts in the gel formulation demonstrated that the increment of salt concentration resulted in a faster gelation process in the range 56.6 to 50.43 °C. The gel Bloom strength increased from 830.04 g at 0.5% CCD salt to 1331.39 g at 2% CCD. The highest gel strength of 1324.59 g was achieved with 2% CCD and a melting temperature of 194.4 °C. The weight loss rate for the control gel was 28.88%, the lowest weight loss rate for 2% CCD gel was 11.82%. The residual gel weight was also highest for 2% CCD: 16.74% after 21 days. A high residual gel weight reflects the stability of the carrageenan gel formulation to retain the product and last as aromatherapy gel product. The morphology images showed a highly porous gel structure on addition of CCD salts. In conclusion, CCD salt is capable of forming a strong interaction with the gel matrix to produce a stable gel.

Keywords: aromatherapy, biocomposite, depression, bloom, porosity

Abstrak

Aromaterapi adalah terapi alternatif untuk Perubatan Pelengkap dan Alternatif (CAM) untuk melegakan kemurungan ringan pada individu. Kajian ini bertujuan untuk menilai kesan penggabungan kalsium klorida dihidrat dalam gel karagenan untuk meningkatkan sifat kestabilan. Formulasi gel dihasilkan daripada campuran karagenan separa halus, natrium benzoat, asid alginik, polietilena glikol (PEG) dan sorbitan monolaurat (Span 20) diikuti dengan kemasukan kalsium klorida dehidrat (CCD) pada 0%, 0.5%, 1%, 1.5%, dan 2% kepekatan. Kesan kepekatan CCD pada reologi, kekuatan Bloom gel, morfologi, dan sifat terma telah disiasat. Kelikatan dan tegasan ricih berkurangan apabila pengambilan CCD dalam formulasi gel karagenan. Kelikatan tertinggi gel kawalan ialah 597.84 mPa.s. Persilangan G' dan G'' pada pelbagai kepekatan garam CCD dalam rumusan gel menunjukkan

bahawa penambahan kepekatan garam menghasilkan proses pemerinkatan yang lebih cepat dalam julat 56.6 hingga 50.43 °C. Kekuatan Bloom gel meningkat daripada 830.04 g pada 0.5 % garam CCF kepada 1331.39 g pada 2% CCD. Kekuatan gel tertinggi sebanyak 1324.59 g dicapai dengan 2% CCD dan suhu lebur 194.4 ° C. Kadar penurunan berat badan untuk gel kawalan ialah 28.88%, kadar penurunan berat badan terendah untuk 2% gel CCD ialah 11.82%. Berat sisa gel juga tertinggi untuk 2% CCD: 16.74% selepas 21 hari. Berat sisa gel yang tinggi mencerminkan kestabilan formulasi gel karagenan untuk mengekalkan produk dan kekal sebagai produk gel aromaterapi. Imej morfologi menunjukkan struktur gel yang sangat berliang pada penambahan garam CCD. Kesimpulannya, garam CCD mampu membentuk interaksi yang kuat dengan matriks gel untuk menghasilkan gel yang stabil

Kata kunci: aromaterapi, biokomposit, kemurungan, mekar, keliangan

Introduction

Aromatherapy is one of the complementary and alternative medicine (CAM) treatments for patients with depressive symptoms. Aromatherapy is defined as the therapeutic application of plant-derived concentrated essences extracted through distillation. It is a low-cost, non-invasive CAM used to improve psychological and mental wellbeing. Ali et al. concluded that aromatherapy is a natural and noninvasive gift of nature because the whole of the human body is rejuvenated on using aroma from essential oil [1]. Essential oils which contain volatile organic compounds that have a pharmacological effect by penetrating the human body through oral, dermal or olfactory administration are commonly used [2]. An electroencephalograph study concluded that fragrances significantly modulate the activity of different brain waves and are responsible for physiological effects of mood, stress, and working capacity [3]. Several marker compounds represent the characteristics of a fragrance, such as patchoulol as the major compound in patchouli oil scent [4]. Olfactory stimulation related to aromatherapy may result in an immediate reduction in pain as well as changes in physiological parameters such as pulse, blood pressure, skin temperature, and brain activity [5]. Application of essential oil aromatherapy gels is particularly used in the home, office, room, and car. This is because most people nowadays spend their time in a room to rest, in an office to work, or in a car to travel. A gel air freshener made from natural polymer gelatin with lavender essential oil and natural banana aroma has been shown to be tolerable for application compared to commercial ones [6].

Most fragrance-scented gel applications use gelatin or gellan gum as the primary material. Both materials are expensive. Gelatin, for example, is derived from animals and would be a source of concern for Muslim consumers

if they used the product. Therefore, this research provides an insight into the potential of using carrageenan in the gelling material for scented gel products. Adam et al. developed a carrageenan–gum Arabic and carrageenan–cellulose for application in hard capsules, demonstrating the compatibility of the components [7]. Carrageenan, by itself, can allow the active volatile ingredient of aromatherapy essential oil to evaporate. The objective of this study is the development of carrageenan gel product made from carrageenan. The incorporation of carboxymethyl cellulose (CMC) and microcrystalline cellulose (MCC) can toughen the bioplastic film and harden capsules made from carrageenan [8]. The effect of alginate gel and CaCl₂ (calcium chloride) solution made through the external gelation method may contribute to an increase in gel strength with an increment of CaCl₂ [9]. The incorporation of cellulose nanofibers (CNF) with α -tocopherol improves the properties of bioplastic film in active packaging [10]. Carrageenan gel produced via crosslinking with calcium ions followed by incorporation of different concentrations of lavender essential oil shows promising mechanical and thermal properties to be applied in the pharmaceutical industry [11]. Pectin, carrageenan, and gelatin are economically valuable hydrophilic polysaccharides that have broad usage in the food, pharmaceutical, cosmetic, and biotechnology industries as gelling, thickening, stabilizing, and emulsifying agents. These polysaccharides have promising potential in many areas and accelerate global technological breakthroughs in this line of work [12]. The sol–gel process, which has been used to create silica-based materials doped with flavor and fragrance, is an efficient alternative to previous microencapsulation methods because it prevents the degradation of the delicate compounds included and has the possibility to provide odorant and

flavored components with new and innovative beneficial properties [13]. The purpose of this study was to evaluate the effect of different concentrations of calcium chloride dehydrate salt on carrageenan gel to make a stable gel for potential of aromatherapy carrageenan gel.

Materials and Methods

Chemicals and materials

Carrageenan was purchased from TACARA, Sabah, Malaysia. Sodium benzoate, calcium chloride dehydrates (CCD), sorbitan monolaurate (Span 20), and polyethylene glycol (PEG) were purchased from Merck, Germany.

Preparation of carrageenan gel solution

In 100 mL of deionized water, 3 g of semi-refined carrageenan and 0.5 g of sodium benzoate were mixed for 1 hour at 75 °C. The solution was stirred until homogeneous. After that, 1.5 g of Span 20, 2 ml of PEG, and 0.02 g alginic acid were added. All materials were mixed in a glass beaker and stirred for 2 hours on a hot plate magnetic stirrer at 75°C. After that, the semi-solid gel was molded and left at room temperature. The same method was repeated but with the concentration of calcium chloride dihydrate manipulated at (0, 0.5, 1, 1.5, and 2 wt.%).

Rheology analysis

The viscosity of carrageenan formulation solutions was measured using a cup and bob rotational measuring block Rheometer (Rheo 3000, Brookfield, USA)

$$\text{Total Liquid Evaporation Percent} = \frac{M_n - M_{n-1}}{M_0} \times 100 \quad (\text{eq. 1})$$

$$\text{Residual Gel Weight Percent} = \frac{M_n}{M_0} \times 100\% \quad (\text{eq. 2})$$

where M_n is the weight of gel at the time of weighing; M_{n-1} is the weight of the gel in the previous week; and M_0 is the weight of gel at the initial time [14].

Gel bloom strength test

Mechanical and Bloom strength test were conducted using a CT3 Texture Analyzer (Rheo 3000, Brookfield, USA). The texture analyzer was loaded with a 50 kg

equipped with LCT 25 4000010 geometry. Approximately 16.5 mL of solution was programmed at speed of 300 rpm, with 100 MPoints at a constant temperature of 40 °C in duplicate. The dynamic rheology measurement was determined using Kinexus Lab+ Rheometer with a controlled stress rheometer using a parallel-plate geometry (gap 1 mm, diameter 40 mm). Within the linear viscoelastic regime, the values of the strain amplitude were set as 4% for all measurements. Before the heating process, the temperature of the loading platform was raised to 60 °C. The prepared fresh samples at 85 °C were put into the loading platform, and then the temperature of the loading platform was reduced to 20 °C and balanced for 1 h. The temperature ramp tests of mixed gels were conducted during heating from 20 to 95 °C and cooling from 95 to 20 °C. Both processes were carried out at a rate of 5 °C/min.

Weight variation analysis

The evaporation of liquid from the formulated gels was tested by weighing the gel each week for 4 weeks. Carrageenan gel weight loss for each week and a total weight reduction after 4 weeks of storage were calculated. Weight reduction of carrageenan gels was obtained by calculating the difference in weight of the gel at the time of weighing (M_n), with weight of gel at initial time (M_0). The difference in weight is the amount of liquid that evaporates. The percentage of total liquid evaporated and residual gel weight were measured by gravimetry and calculated by the formula:

load cell. An average of at least three measurements was taken for each formulation. A flat-end probe (TA-10) with a diameter of 12.7 mm and a Brookfield bloom jar were used for the analysis. Carrageenan gel was placed on the texture analyzer platform and positioned at the center of the probe. The analyzer was programmed to use the 'compression' testing mode and the 'distance' target option was approximately 4.0 mm. At a speed of

1.0 mm/s, the probe was pressed onto the gel and breaking force was recorded.

Thermal properties

Thermogravimetric analysis (TGA) is a precise method for studying the weight loss, decomposition pattern and thermal characterization of carrageenan gel. A TA Instruments Q 500 series instrument with a TGA heating rate of 10 °C/min for a temperature range of up to 800 °C was used. Nitrogen gas was used as the purge gas at a rate of 10 ml/min. Measurements were taken in triplets, with average values recorded.

The thermal properties of the carrageenan gel formulation solution were analyzed by differential scanning calorimetry, DSC, using a TA-Instrument Q1000 calorimeter. Samples of 8 mg were placed on an aluminum pan and nitrogen gas was purged at a flow rate of 10 mL/min and heating rate of 10 °C/min. The heating range was set between 0 to 350 °C.

Morphology analysis

A scanning electron microscope (SEM; TM3030 PLUS, Hitachi, Japan) was used to examine the surface morphology of carrageenan gel. SEM was used to examine the surface of the biocomposite obtained. The carrageenan gel was cut into 1 cm × 1 cm rectangles and applied to the specimen stub. The sample was examined and photographed at a magnification of 300×.

Statistical analysis

The confidence level p-value was calculated using ANOVA with the testing null analysis (H_0) and alternative analysis (H_i) for solution viscosity, shear stress, and gel bloom strength of the gel formulation at different CCD concentrations. For the comparison of rejection values, the level of significance was set at $\alpha = 0.05$.

Results and Discussion

Rheology analysis

Sol–gel transitions are common during the process of aqueous gelling systems, resulting in overly complex rheological behaviour. Several factors such as time, temperature, and concentration frequently cause dramatic changes in viscoelastic behaviour [15]. The viscosity of carrageenan gels with different concentrations of CCD is shown in Figure 1. Viscosity and shear stress decreased on increasing the concentration of CCD from 0 to 2 wt.%. ANOVA test results for the viscosity and shear stress have p-values of 0.00012 and 0.000262 at $\alpha = 0.05$, respectively. In previous studies, a solution of carrageenan blended with *Chlorella vulgaris* lipid had a viscosity value of 245.29 mPa.s [16] and that of carrageenan–gum Arabic solution increased to 1058 mPa.s at a 67% w/w ratio of semi-refined carrageenan to gum Arabic [7].

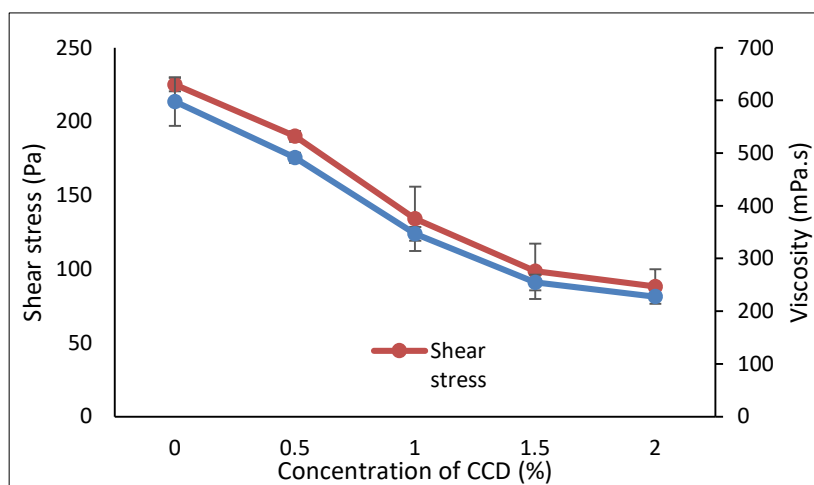


Figure 1. Viscosity and shear stress at different concentrations of CCD

Figure 2 shows the effect of temperature on the sol–gel transition and gel–sol transition for the gel formulations; the analysis is divided into cooling and heating processes. Both G' and G'' values decreased sharply as temperature increased. During the cooling process, G' values were always greater than G'' values, and the samples displayed elastic behavior. However, as the temperature increased during the heating process, G' and G'' values both increased slightly, and G'' gradually surpassed G' , indicating viscous behavior. During the heating process, melting temperature (T_m) was defined as the temperature at which the G' and G'' values crossed, and the gelling temperature (T_g) was defined likewise for the cooling process. At 0% CCD salt concentration, T_g was 56.66 °C and gradually decreased as the concentration of CCD salt increased, to 50.43 °C at 2% CCD salt. The T_m ranged from 62.17 to 43.88 °C on increasing the concentration of CCD salt. The highest T_m and T_g , 56.66 and 62.17 °C, respectively, were obtained for gels made without 0% CCD salts.

Figure 3 shows the melting and gelling temperature from G' and G'' crossover. The storage modulus of a polymer determines its solid-like properties. The higher the storage modulus, the more difficult it is to degrade the polymer. Loss modulus, which is the inverse of storage modulus, describes a polymer's liquid-like nature. When the storage modulus is large, the loss modulus is small, and vice versa [17]. During the cooling stage, the loss modulus is greater than the storage modulus during the initial cure period. This means that during this stage, the gel's liquid-like (viscous) behavior takes precedence over its solid-like

(elastic) character. When $G' = G''$, a crossover point is reached, implying that a crosslinking reaction is initiated. This is referred to as the gel point. G' was much greater than G'' in the final cure stage, meaning that the gel had mostly solidified into an elastic solid. The results for the gelling point, the intersection of G' and G'' , for various concentrations of CCD salts added to the gel formulation demonstrate that increasing the salt concentration resulted in faster gelation (shorter gel point), as shown in Table 1. In a previous study by Cui et al., kappa carrageenan/konjac gum solution with the addition of gellan gum and Ca^{2+} , which remained a gel in the solid state under heating treatment at 85 °C, demonstrated good thermal stability [18], higher than that found in the current study. Here, the highest T_m was 62.17 °C without the addition of CCD salts; this difference is probably due to the addition of gellan gum to the formulation by Cui et al. Gellan gum is insoluble in cold water and must be heated to 90 °C or higher to be completely dissolved [19]. According to Tako et al., in their formulation that consisted of iota-carrageenan and CCD salt, gelation up to 80 °C was observed in the presence of an intermolecular Ca^{2+} bridge with intra- and intermolecular hydrogen bonding and van Der Waals' forces [20]. The higher crossover temperature of iota-carrageenan solution induced with sodium chloride salt indicates a stronger interaction between iota-carrageenan helices and salt ions [21]. The addition of calcium chloride to gelatin extracted from skins of tropical fishes can reduce the G' and G'' values of the gelatin solution compared to calcium sulphate and magnesium sulphate [22].

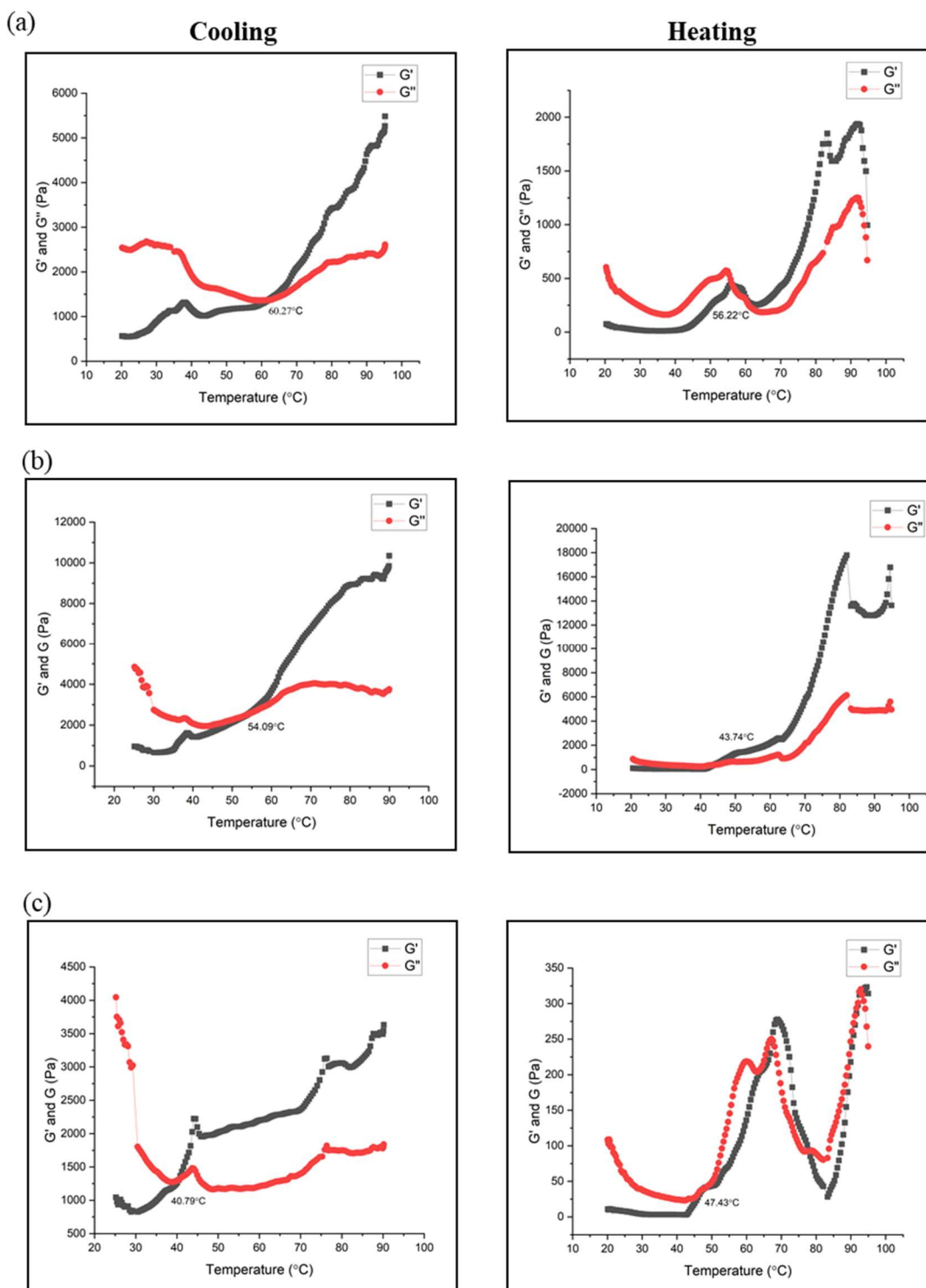


Figure 2. Changes in modulus (G' and G'') of gels with different concentrations of CCD salts: (a) 0%, (b) 1%, and (c) 2% during heating and cooling

Table 1. Gelling point and melting point of gels with different concentration of CCD salts

| Sample (%) | T _{melting} (°C) | T _{gelling} (°C) |
|------------|---------------------------|---------------------------|
| 0 | 62.17 ± 1.94 | 56.66 ± 1.48 |
| 0.5 | 50.53 ± 1.62 | 56.17 ± 1.75 |
| 1 | 43.90 ± 1.11 | 56.14 ± 0.54 |
| 1.5 | 48.94 ± 0.57 | 52.73 ± 2.61 |
| 2 | 43.88 ± 0.14 | 50.43 ± 5.45 |

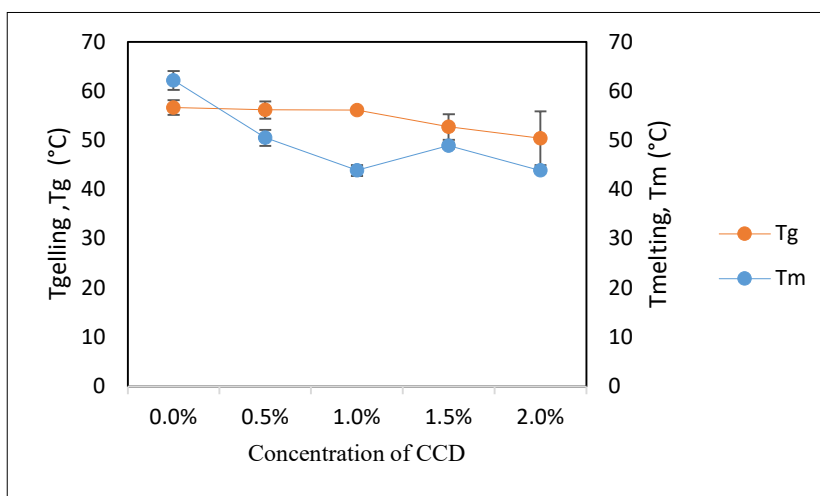


Figure 3. Melting and gelling temperature from G' and G'' crossover

Weight variation analysis

Figure 4 (a) and (b) show the weight variation and residual gel weight of the gel air freshener after 4 weeks. The highest residual gel weight percentage was observed for 2% CCD salt concentration, indicating that the formulation solution's ability to prevent evaporation is better for the higher CCD concentration in comparison to lower concentrations. According to Hutagaol, gels made with 3% carrageenan show the ability to maintain the substance to better prevent

evaporation [14]. In this study, gels were formulated with 3% carrageenan and different concentrations of CCD salts. Carrageenan gel at 2% shows the ability to maintain its structure at higher concentrations of CCD salts. Figure 4 shows the weight and residual gel weight (%) of the carrageenan gel solution for 45 days. The results obtained in this study support the hypothesis that the greater the resistance of carrageenan gel, the less weight is lost or the greater the weight remaining, meaning less volatile oil and water are evaporated [14].

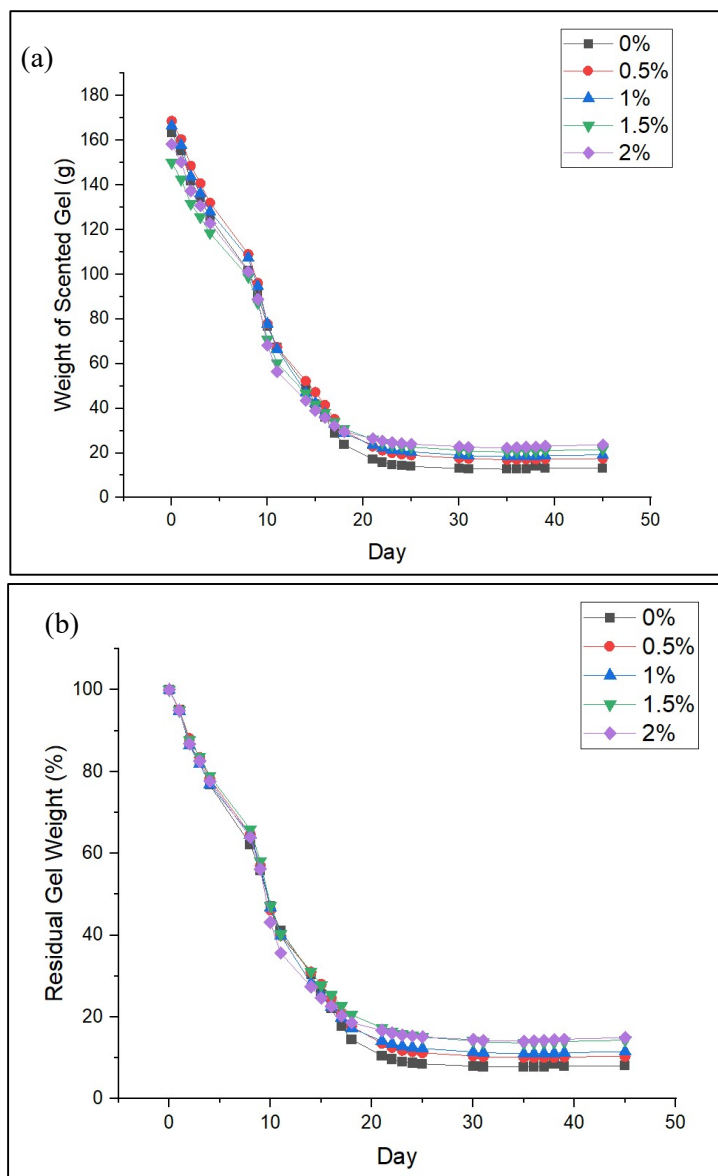


Figure 4. (a) Weight variation (b) residual gel weight

Gel bloom strength test

As shown in Figure 5, the analysis of gel Bloom strength, a commercially important parameter, showed varying values for different samples. The gel formation process is extremely sensitive to the structural and compositional properties of semi-refined carrageenan (SRC). The highest gel Bloom strength, 1331.39 g, was obtained for the carrageenan gel formulation with 2% CCD salt at the lowest viscosity (probability of rejection p -value = 0.00017). The gel Bloom strength has an

inverse relationship with viscosity: the higher the gel strength, the lower the viscosity. Formulation solutions containing gellan gum as their gelling agent with the inclusion of calcium ions show decreasing gel strength because of the excessively high concentration of CCD salts. This is because gellan gum molecule relation developed the coagulation state, preventing the stable three-dimensional network structure from being retained [19]. In this study, gel Bloom strength increased with the addition of CCD salts. A better gel strength could also

be attributed to improved carrageenan gelation, as it is believed that chloride salts can improve carrageenan gel strength [23]. In a previous study, the addition of hydroxypropyl methyl cellulose to carrageenan solution increased the viscosity from 172 to 616.5 mPa.s due to the intermolecular interaction between composite chains in the solution which may cause higher mechanical properties [24]. According to Sarbon et al., addition of CCD salts to gelatin solution decreases the gel strength of gelatin but calcium sulphate and magnesium sulphate increase it [22]. This is because of the formulation of a gelatin network in which salt acts as elastic masses for

the gelatin. In contrast, in this study, the gel strength increased at higher concentrations of CCD salt. This is probably due to other effects of chemicals such as sodium benzoate and sorbitan monolaurate in the formulation, the effect of which on the gel strength needs to be investigated. Iota-carrageenan hydrogels with various weight percentages of calcium chloride have been successfully developed [25]. Using nanosilica as the carrageenan reinforcement agent increases the mechanical properties of the bioplastic film at a melting temperature of 68 °C [26].

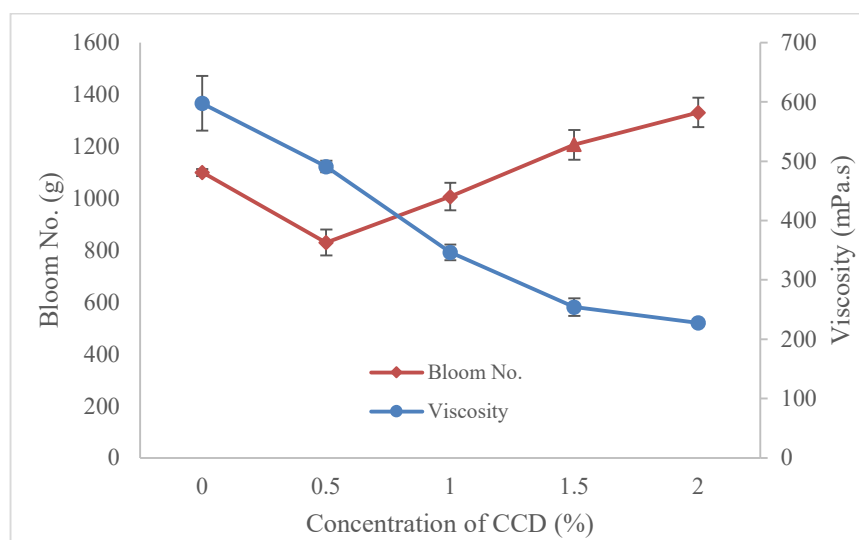


Figure 5. Gel strength test using Bloom gel analysis of carrageenan gel

Thermal properties

TGA was performed to assess the thermal properties of the carrageenan gel formulation. Figure 6(a) and (b) present the relative weight loss from thermogravimetric curves and the weight loss first derivative thermogravimetric (DTG) curves, as functions of temperature in a nitrogen atmosphere, of the carrageenan gels made with various CCD salt concentrations. The results for weight loss obtained from TGA analysis provide an intercorrelation between the variation of CCD salt concentration and weight loss.

Figure 6 (b) shows that the control gel sample had a four-stage weight loss. In the first stage, up to 50 °C,

approximately 11.66 % of the weight was lost. This could be attributed to the vaporization of water (absorbed and bound water). The second stage of weight loss began at about 263.4 °C and continued up to 800 °C. At this stage, faster thermal degradation and a weight loss of more than 25% were demonstrated. In the formulation with 2% CCD salts, fewer residues remained at 800 °C and the total weight loss at about 210.2 °C was 11.82%. Carrageenan–poly (vinyl alcohol) composite film heated at 800 °C has shown an increased trend of weight loss with the addition of inorganic ions. This can be explained by the presence of mineral salt produced from the ash [27].

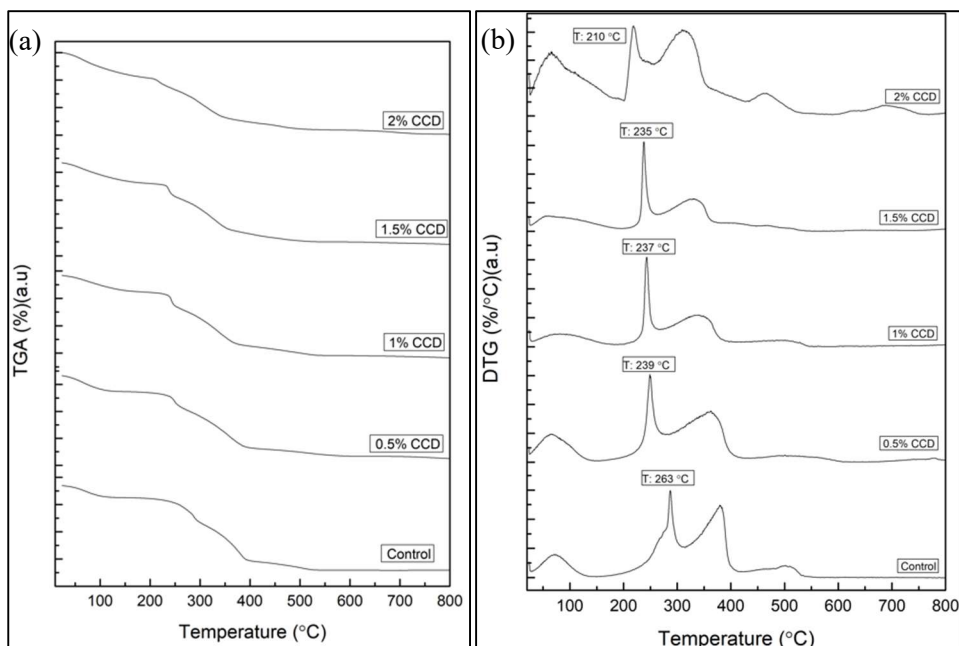


Figure 6. (a) TGA and (b) DTG thermogram for carrageenan gels

Figure 7 and Table 2 shows the DSC analysis on carrageenan gel at different concentrations of CCD salts. The DSC run curve of the control carrageenan gel exhibited an exothermic peak centered at about 185.04 °C corresponding to 323.45 mW of heating. A peak position situated at a higher temperature indicates a stronger interaction with water. Thus, these results suggest that there is an interaction between semi-refined carrageenan and water that may be responsible for the formation of a more stable gel under this condition. According to Figure 7, the DSC results show the enthalpy change during high-temperature heating of samples. In the DSC thermograms, there only one major intense peak was recorded, which was an endothermic transition at temperatures around 185~194 °C. The endothermic transition resulted from the heat absorption for dehydration of water and sample decomposition [28]. Melting temperature decreases with the addition of calcium chloride in bovine gelatin [22].

Table 2. DSC analysis on carrageenan gel at different concentrations of CCD salts

| Sample | Sample Mass (mg) | T _m /°C | Heat flow (mW) |
|----------|------------------|--------------------|----------------|
| 0% CCD | 7.17 | 185.04 | 323.45 |
| 0.5% CCD | 8.68 | 184.85 | 504.06 |
| 1% CCD | 8.73 | 188.86 | 145.78 |
| 1.5% CCD | 8.39 | 193.9 | 154.92 |
| 2% CCD | 8.89 | 194.36 | 415.81 |

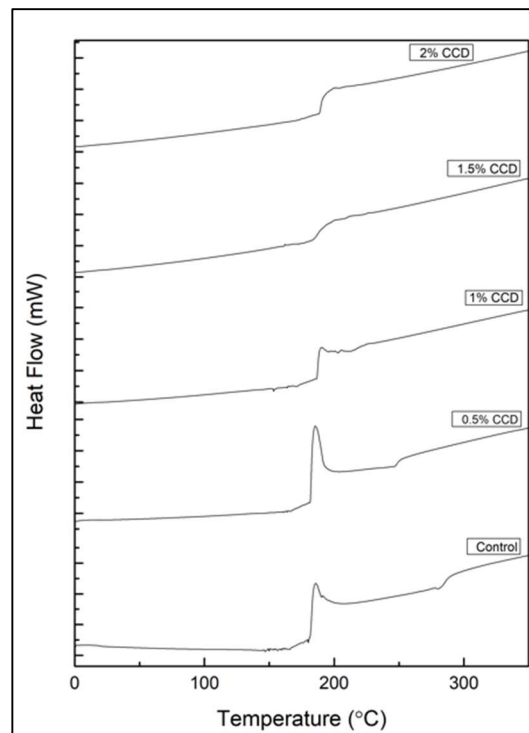


Figure 7. DSC thermograms for carrageenan gels

Morphology analysis

Figure 8 (a) and (b) show SEM images for carrageenan gels from the control sample and 2% CCD salt sample, respectively. Both samples display sponge-like particles with a prominent macroporous structure. In this case, the diameter of the pores seemed to increase at higher calcium chloride concentrations. The images show that the carrageenan gel without salt has a network of smaller and shallow pores. The average diameter of pores at 0% CCD salt was 416.4 μm while at 2% CCD it was 514.1 μm . However, in contrast, the gel produced with the presence of salt displayed larger pores and a higher degree of porosity. The networks became denser by

adding CCD salt. This finding is supported by Pan et al., who found that the surface morphology of porcine-myosin- κ -carrageenan mixtures demonstrated large aggregates and cavities at higher calcium chloride concentrations [23]. Adding gellan gum and calcium chloride to surimi gel results in a finer and denser structure compared to surimi gel without the addition of gellan gum and calcium chloride that shows coarser and larger voids due to low breaking force and water-holding capacity [29]. The structure of calcium- ι -carrageenan blended gel has pores that are denser and fragmented; and with addition of glutamic acid, a sheet structure is observed, leading to a weak and open structure [30].

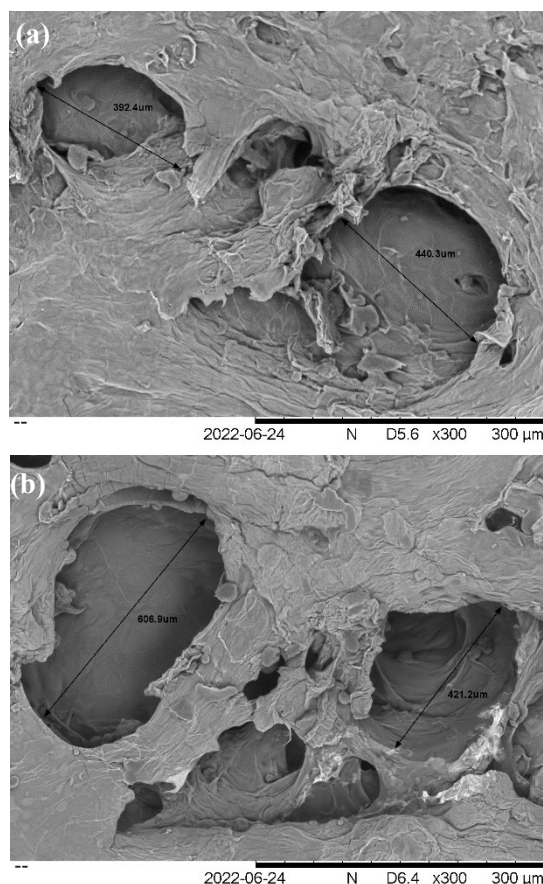


Figure 8. SEM images of surface morphology of carrageenan gel: (a) 0% at 300× and (b) 2% at 300×

Conclusion

In conclusion, the findings in this work provide an important insight into the role of CCD salt to form a strong interaction between gel matrix chemicals to produce stable gel properties. The addition of 2% CCD to the gel formulation manages to increase the residual gel weight from 8% to 15% and improve the mechanical and thermal activities of the gel prepared. This finding suggests the role of CCD salt can be fully utilized in the formulation of a base for aromatherapy scented gel, offering the opportunity to produce a better-quality gel. The mechanical and thermal properties show that the 2% concentration of CCD salts can be used as a base for aromatherapy scented gel, which offers a high gel strength and is expected to enhance the formulation solution of carrageenan scented gel due to the ionic compound of calcium ions.

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