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Extraction of refined carrageenan from *Kappaphycus alvarezii* and evaluation for a sheet face mask gel

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Abstract A sheet face mask is a type of facial skincare cosmetic applied to the entire face to provide hydration, a refreshing sensation and skin tightening. A paradigm shift is now underway, fuelled by consumer concerns over the long-term safety of synthetic chemicals used in these products and the broader ecological footprint of cosmetic production. Consequently, κ-carrageenan is an alternative to these synthetic chemicals due to its natural origin, being free of harmful chemicals, gelling and hydration properties. The red seaweed, *Kappaphycus alvarezii*, the only seaweed species commercially cultivated in Sri Lanka is a rich source of κ-carrageenan, however, it has not yet been used locally to extract carrageenan and test its application in skincare cosmetics. Precipitation of κ-carrageenan using KCl is a critical step in carrageenan extraction process as it imparts strength to carrageenan gels. In this study, carrageenan was extracted using three concentrations of KCl and its suitability for a sheet face mask gel was tested across three carrageenan concentrations. The results showed that comparatively higher yields of 31.89±0.05 and 33.52±2.50 were obtained at 2.50% and 3.50% KCl concentrations, respectively. All tested characteristics for a leave-on sheet face mask gel were achieved at 1.00% carrageenan concentration prepared using carrageenan extracted at 3.50% KCl concentration. Notably, prepared carrageenan gels were able to moisturise the skin without altering skin oiliness.

Keywords: *Kappaphycus alvarezii*, κ-carrageenan, KCl, moisturising effect, sheet face mask

INTRODUCTION

Carrageenan is an ionic, linear sulphated polysaccharide found in the cell wall of red seaweeds (Rupert et al., 2022). Carrageenan is a versatile ingredient in many industries such as food, pharmaceutical and cosmetic, due to its water-soluble, gelling, stabilising, nontoxicity and biodegradable properties (Wardhana et al., 2022). Carrageenan is classified into three main types such as, kappa (κ), iota (ι), and lambda (λ), depending on the number and position of sulphate groups and the presence of 3,6-anhydrogalactose (Shen and Kuo, 2017). κ-carrageenan is formed by alternating units of β-D-galactose-4-sulphate and α-D-3,6-anhydrogalactose linked by α-1,3 and β-1,4 glycosidic bonds (Cheng et al., 2022). κ-carrageenan differs from other two types as it contains one sulphate group per disaccharide unit. κ-carrageenan contains 25% sulphate ester and 34% 3,6-anhydrogalactose (Wang et al., 2018). It has the highest gelation strength compared to other types due to its constituents and helical structure (Forget et al., 2013). Its ability to form rigid gels, hydration due to strong water-binding capacity, thermal stability and antioxidant properties make κ-carrageenan a highly valuable ingredient in cosmetic applications.

The red seaweed, *Kappaphycus alvarezii* is a rich source of κ-carrageenan (Suraweera et al., 2025). This is the only seaweed species commercially cultivated in Sri Lanka along the northern and northwestern coasts. Seaweed production in Sri Lanka is rapidly expanding, increasing from 271 Mt in 2022 to 795 Mt in 2024 (Ministry of Fisheries, Aquatic and Ocean Resources, 2024). Currently, the total harvest is exported as dried raw material for the production of carrageenan or other industrial applications, and is still underutilised in Sri Lanka. The major constraint for utilising this valuable resource in Sri Lanka for the extraction of κ-carrageenan and further valorisation is the lack of simple and low-cost technologies. On the other hand, carrageenan is imported to Sri Lanka for the production of cosmetics at a high cost. This signifies the need for scientific research to test the possibility of extracting carrageenan from locally cultivated *K. alvarezii* and testing the suitability of extracted carrageenan for cosmetic products. The availability of raw material for carrageenan production is assured with the large-scale cultivation of *K. alvarezii* in seaweed farming areas in Sri Lanka.

Based on the extraction method, carrageenan is classified into two grades; semi-refined carrageenan (SRC) and refined carrageenan (RC) (Tarman et al., 2020). As the first step of RC extraction, an alkaline treatment is employed mostly using a solution of calcium hydroxide [Ca(OH)2)] or potassium hydroxide (KOH). The hydroxide component of alkali replaces sulphate component of carrageenan and increases the formation of 3,6-anhydrogalactose leading to increased gel strength (Manuhara et al., 2016). The reagent's calcium component reacts with carrageenan to form a gel (Rupert et al., 2022). At the same time, alkali-soluble salts, sugars, and proteins are removed (Heriyanto et al., 2018). Subsequent water wash removes alkali and any remaining impurities (Rupert et al., 2022). The next step is the extraction of carrageenan in a water medium followed by precipitation. The alcohol-precipitation method is used for all carrageenan types, while the potassium chloride (KCl) precipitation method is used only for κ-carrageenan (FAO, 2014). Potassium ions (K+) form a rigid gel of carrageenan with helical structures and with the increase of K+ ion concentration the helical aggregation and gel stability will increase (Shafie et al., 2022).

A sheet face mask is a type of facial skincare cosmetic product generally made of cellulose in the shape of a face and dipped in a gel. This sheet face mask is applied to the entire face except the eyebrows, eyes, and lips to provide hydration, a refreshing sensation and skin tightening. Synthetic chemicals, such as, polyethylene glycol (PEG) and carbomer are used in these products. However, PEG can sometimes irritate if applied to damaged skin and may cause skin ageing due to its dehydration effects (Mistry, 2017). Further, a paradigm shift is now underway, fuelled by consumer apprehensions over the long-term safety of synthetic chemicals and the broader ecological footprint of cosmetic production. This trend towards sustainability and perceived safety is redirecting the industry's focus toward natural bioactive compounds as substitutes for conventional synthetic ingredients (Ferreira et al., 2022; Prajaputra et al., 2024). In this scenario, κ-carrageenan is an alternative to these synthetic chemicals due to its natural origin, being free of dangerous chemicals, gelling and hydration properties.

Consequently, this study evaluated the suitability of carrageenan extracted from locally cultivated *K. alvarezii* for use in sheet face mask gels. The KCl-mediated carrageenan precipitation step was selected for optimisation due to its critical impact on gelling ability. To achieve this, carrageenan was extracted using three concentrations of KCl, and the suitability of the resulting carrageenan powders for a sheet face mask gel was tested by preparing gels at three carrageenan concentrations.

METHODS

Extraction of carrageenan

Dried red seaweed (*Kappaphycus alvarezii*) was obtained from a cultivation site in Jaffna, Sri Lanka. Seaweed was washed using running water and blotted dry. Samples of 20 g were weighed and packed in polyethylene bags and kept frozen until used for experiments. Each sample was soaked in distilled water at 1: 45 (w/v) ratio for 24 h. After soaking, seaweed was cut into 0.5-1.0 cm pieces and homogenised at 500 rpm using a homogeniser to make a pulp (Homogeniser HG-15D Daihan Scientific Co., Ltd.). The pH of this pulp was adjusted to pH 9 using a calcium hydroxide (Ca(OH)2) solution of pH 12. This solution was then heated at 90 °C while stirring continuously at 150 rpm for 2 h using a heating magnetic stirrer (VELP SCIENTIFICA). The seaweed waste (solid particles) was removed by filtration, and the filtrate was allowed to cool to room temperature. The filtrate was neutralised to pH 7 using a 1% (w/w) HCl solution, and reheated at 60 °C for 30 min. After allowing the filtrate to cool to room temperature, its volume was recorded. Carrageenan gel was precipitated from this filtrate by adding a potassium chloride (KCl) solution at three concentrations based on available literature (1.50%, 2.50%, and 3.50%) at 1:1 (v/v) ratio with the filtrate, and stirring continuously at 150 rpm for 15 min. The coagulated gel was separated from the solution by filtering through a cheesecloth. This gel was then completely soaked in 95% ethanol at 2: 1 (w/v) ratio, and stirred continuously at 150 rpm for 1 h at room temperature. Then the carrageenan gel was separated by filtering through a cheesecloth and dried at 60 °C for 24 h, or to a constant weight. Finally, the dried powder was milled to obtain a fine powder and the weight was recorded. These carrageenan extraction experiments were conducted in two replicates using *K. alvarezii* obtained from two different culture cycles.

Yield of carrageenan powder

Dry matter content of seaweed used for the experiments was measured by oven-drying at 105 °C until a constant weight was achieved. The yield of carrageenan was calculated by dividing the weight of carrageenan powder by the dry weight of seaweed. Accordingly, the yield was recorded on a dry weight basis.

Preparation of carrageenan gel from extracted carrageenan powder

Carrageenan powders extracted at three KCl concentrations (1.50%, 2.50%, and 3.50%) were used to prepare carrageenan gels at three concentrations (0.50%, 0.75%, and 1.00%). For this, carrageenan powders were dissolved in distilled water at 400 rpm for 1 h using a magnetic stirrer (VELP SCIENTIFICA) at room temperature (28 °C). Accordingly, nine different types of carrageenan gels were prepared. These carrageenan gels were autoclaved at 121 °C and 15 psi for 15 min in covered glass beakers.

Characterisation of carrageenan gel to use in a sheet face mask

pH of carrageenan gels

Three measurements of pH were taken from each prepared gels using a calibrated pH meter (Thermo Scientific ORION STAR A326 pH/RDO/DO Meter). The pH of a commercial sheet face mask gel was also measured for comparison with the prepared gels.

Spreadability of carrageenan gels

For this experiment, gels were prepared in three replicates at each gel concentration. A circle of 1.0 cm diameter was marked on a flat glass plate. A sample of 0.1 g of gel was placed at the centre of this circle using a dropper and was covered with a second glass plate of similar size. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. During this time, the gel drop spread in a circular shape, and the diameter of the resulting circle was measured. This was repeated four times for each replicate. Four types of commercial sheet face mask gels were used as references and the spreadability of these gels were measured similarly. Spreadability of the prepared gels was compared with these references. This experiment was done at room temperature (28 °C – 30 °C).

Colour of carrageenan gels

The colour of gel prepared in a glass beaker was measured using a chroma meter (Chroma Meter CR-400, Konica Minolta, Japan). The instrument was calibrated using the white standard (L^*=83.86, a^*=0.72, b^*=5.91). The L^* (lightness), a^* (redness⁄ greenness) and b^* (yellowness⁄ blueness) colour coordinates were recorded in each sample in two replicates, and the total colour difference (ΔE^*) was calculated according to the following equation (Pal and Suresh 2015);

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where ΔL^*, Δa^* and Δb^* are the average differences between two replicates of a sample with the white standard.

Effect on skin moisture and skin oiliness

This test was conducted using 12 randomly selected female panellists aged 23–27 years. The inner sides of both hands were thoroughly washed and allowed to dry for 15–20 minutes. Ten points were marked on each hand and skin moisture content (m1) and skin oil content (o1) were recorded using a skin moisture analyser (Digital moisture monitor SK-IV). Cellulose face mask sheets of 1×1 cm, which were soaked in nine prepared gels and commercial sheet face mask gel separately for 5 min, were placed on ten marked points. These sheets were removed after 10 min and any leftover gel was removed by wiping, and skin moisture content (m2) and skin oil content (o2) were recorded. The skin moisturising effect of gels was determined as the percentage change in skin moisture content, and the skin oiliness effect was determined as the percentage change in skin oil content.

Consumer preference for carrageenan gels using sensory evaluation

To avoid sensory fatigue and memory effects, only four gel types, gels prepared at 0.75% and 1.00% concentrations from carrageenan powders extracted using 2.50% and 3.50% KCl concentrations, were used for this sensory evaluation test. The 1.50% KCl concentration was excluded due to comparatively low carrageenan yield and the 0.50% carrageenan concentration was excluded due to higher spreadability. A commercial sheet face mask gel was also used. Consumer preference of the gels for sensory attributes: colour, odour, texture, moisturising ability, attractiveness and overall preference was evaluated using 12 female panellists of age 23-27. A 9-point hedonic scale (ranging from 1 = "dislike extremely" to 9 = "like extremely") was used to measure the degree of liking. Cellulose face mask sheets of 1×1 cm, which were soaked in four prepared gels and commercial sheet face mask gel separately for 5 min, were placed on five points on the inner side of the hand. Panellists were asked to indicate their preferences for five gels against the above-mentioned sensory attributes.

Statistical analysis and selection of optimum extraction conditions

Statistical analysis was performed using Minitab version 19. The differences of means of different treatments were determined using analysis of variance (ANOVA) at $P<0.05$ significance level, followed by Tukey's test. Results of the sensory evaluation data were statistically analysed using Friedman test. The optimum extraction conditions were selected based on above characteristics and statistical analysis.

Attenuated Total Reflectance–Fourier Transform Infra-Red (ATR-FTIR) spectroscopy

The ATR-FTIR spectra of carrageenan powder extracted under optimum conditions was used to characterise κ-carrageenan. The ATR-FTIR spectra were obtained using a Nicolet iS10 FTIR spectrometer (Thermo Scientific Inc., Madison, WI, USA) equipped with an ATR diamond sampler accessory. The signals were automatically collected for 32 scans over the 4000–550 cm^{-1} range, using a 4 cm^{-1} resolution. Background spectrum was collected from the clean empty crystal cell. The peak positions of the spectra were analysed for κ-carrageenan.

Results and Discussion

Yield of carrageenan powder

The yields of carrageenan powders on a dry weight basis are shown in Figure 1(A). Differences between yields were found to be not significant ($P=0.059$), depicting no effect of KCl concentration on gel precipitation. However, the yield obtained by precipitating carrageenan at 1.50% KCl concentration was comparatively low. Manuhara et al. (2016) reported carrageenan yields of 19.50%, 31.32% and 43.91% from 1.50%, 2.50% and 3.50% KCl concentrations, respectively, which aligns with the results of this study.

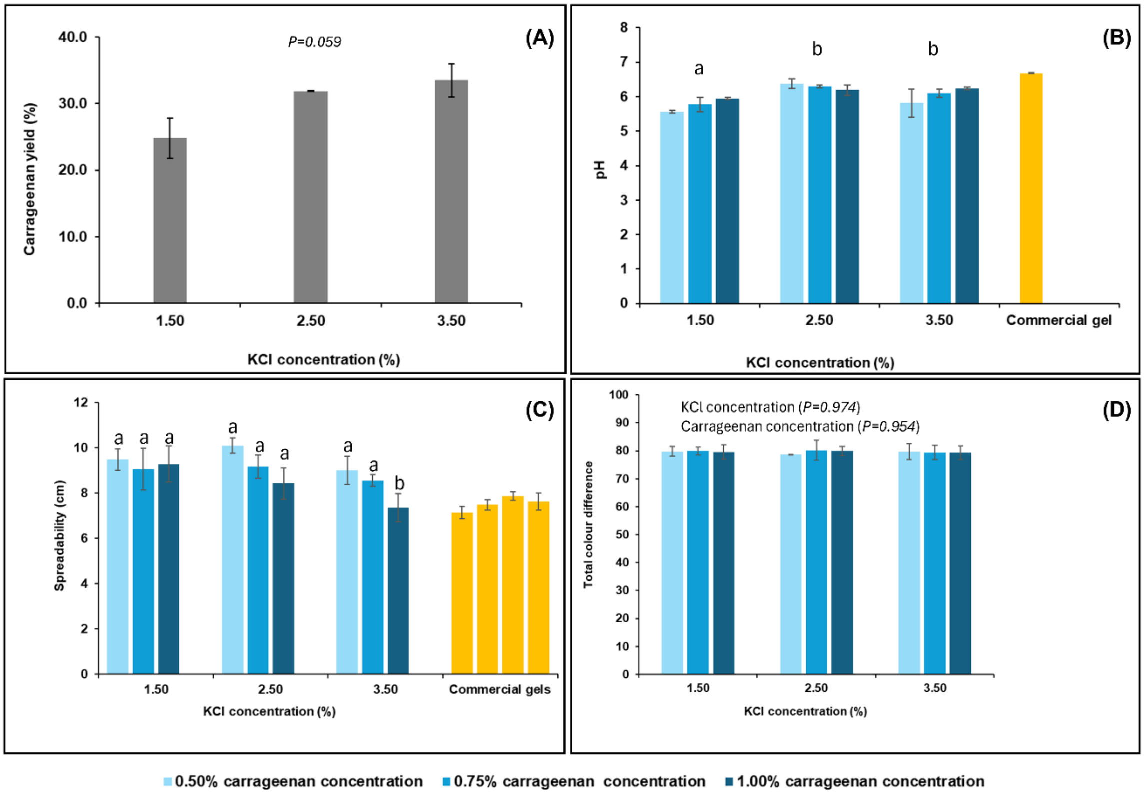

Fig. 1 (A) Yield of carrageenan powder obtained using three different concentrations of KCl. (B), (C), (D) Effect of KCl concentration and carrageenan concentration on the pH, spreadability, and total colour difference of carrageenan gels, respectively. Mean (±SD) are given.

Characteristics of carrageenan gel to use in a sheet face mask

pH of carrageenan gels

In this study, pH values of prepared carrageenan gels varied between 5.56±0.05-6.38±0.15 (Figure 1(B)). Further, the pH of carrageenan gels was found to be significantly affected by KCl concentration ($P<0.05$), but not by carrageenan concentration ($P=0.096$, Two-way ANOVA). pH of gels prepared from carrageenan extracted at 1.50% KCl concentration was significantly lower than that of the other two KCl concentrations (Tukey's pairwise comparison, $P<0.05$). pH of a facial skincare cosmetic product is a significant characteristic as it directly affects the appearance, health and barrier functions of the skin by interacting with the skin's natural acid mantle. Studies have found that the physiological pH of skin is acidic and ranges from 4.1-5.8, depending on the body part (Lukic et al., 2021). Skincare products with non-physiological pH levels can disrupt skin microbiota as these products interact with the skin microbiome and skin pH (Janssens-Böcker et al., 2025). Therefore, pH range of 5.0-7.0 is generally recommended for leave-on products and 4.5-8.0 for rinse-off products. pH values of carrageenan gels of this study fall within the acceptable pH range for leave-on products and are also comparable to the tested commercial face mask gel. Accordingly, these results confirm the suitability of these gels for use in sheet face masks.

Spreadability of carrageenan gels

Spreadability of gels has been significantly affected by the interaction effect of KCl concentration and gel concentration ($P<0.05$) (Two-way ANOVA) (Figure 1(C)). Gel prepared at the highest concentration (1.00%) using carrageenan powder extracted at 3.50% KCl concentration recorded a significantly lower ($P<0.05$) spreadability compared to other combinations. This lower spreadability may be a result of higher gel strength and rigidity caused by higher amount of K+ ions during carrageenan precipitation, and higher percentage of carrageenan powder used in gel making. The higher gel strength caused by an increased amount of K^+ ions can be explained in two main ways. According to Krempel et al. (2019), K+ ions form bridges with the d-galactose sulphate group and interact electrostatically with an anhydro-O-3,6 ring of another d-galactose ring. Furthermore, an increase in gel strength is directly proportional to 3,6-anhydrogalactose and inversely proportional to the sulphate content (Rey and Labuza, 1981). The presence of 3,6-anhydrogalactose causes orderliness in the polymer and enhances the potential for double-helix formation. Spreadability of a skin care gel is important as it determines the effective and easy application of gel on the surface of the skin, mainly the face (a vertical surface). The gel should remain on the skin without unnecessary flow. According to Wardhana et al. (2022), a good level of spreadability falls within the range of 5–7 cm. The spreadability of gel prepared at 1.00% concentration using carrageenan powder extracted at 3.50% KCl concentration was found to be the closest to 5–7 cm, among the four tested commercial face mask gels.

Colour of carrageenan gels

The colour of gels was characterised using L*a*b* colour space compared to a white colour standard. In this method, L* denotes lightness, a* denotes redness/ greenness, and b* denotes yellowness/ blueness. The total colour difference between the samples and the standard is graphically presented in Figure 1(D). The total colour difference should be closer to zero if the gel is white in colour. All samples had colour difference values close to 80, indicating a highly saturated colour. According to the two-way ANOVA there was no significant effect of KCl concentration (P=0.974) or carrageenan concentration (P=0.954) on the colour of gel which means any combination of these two treatments can produce a gel of similar colour. Values of a* range between 0.29 and 0.68 depicting a slight tendency towards redness, and values of b* range between 1.99 and 4.31 depicting a slight tendency towards yellowness. There is no specific colour for a face mask gel. It is only important in the aspect of consumer preference.

Effect on skin moisture and skin oiliness

In this study, the effect of KCl concentration and carrageenan concentration on skin moisture change after applying the prepared gels was tested using 12 female volunteer participants. Figure 2(A) shows the percentage change in skin moisture after applying carrageenan gels. There was no significant effect of KCl concentration (P=0.655) or carrageenan concentration (P=0.306) on the percentage change in skin moisture (Two-way ANOVA). Figure 2(B) shows the percentage change in skin oiliness after applying carrageenan gels. Skin oiliness change remained around zero for all prepared gels showing no effect on skin oiliness by carrageenan, compared to an increase in skin oiliness by the commercial gel. Further, there was no significant effect of KCl concentration (P=0.760) or carrageenan concentration (P=0.418) on the percentage change in skin oiliness (Two-way ANOVA). Carrageenan is widely used in skincare cosmetic gels due to its moisturising effect to enhance the overall skin-conditioning performance (Mingu et al., 2024). Carrageenan's ability to bind and hold water is important in two ways in skincare cosmetic products: as a humectant to reduce dryness of the cosmetic product during storage at room temperature, and to moisturise the skin resulting in a smoother skin surface (Shafie et al., 2022). Results of this study show the potential of using carrageenan gel alone or as an ingredient in skincare cosmetics as a skin moisturiser without altering the skin oiliness.

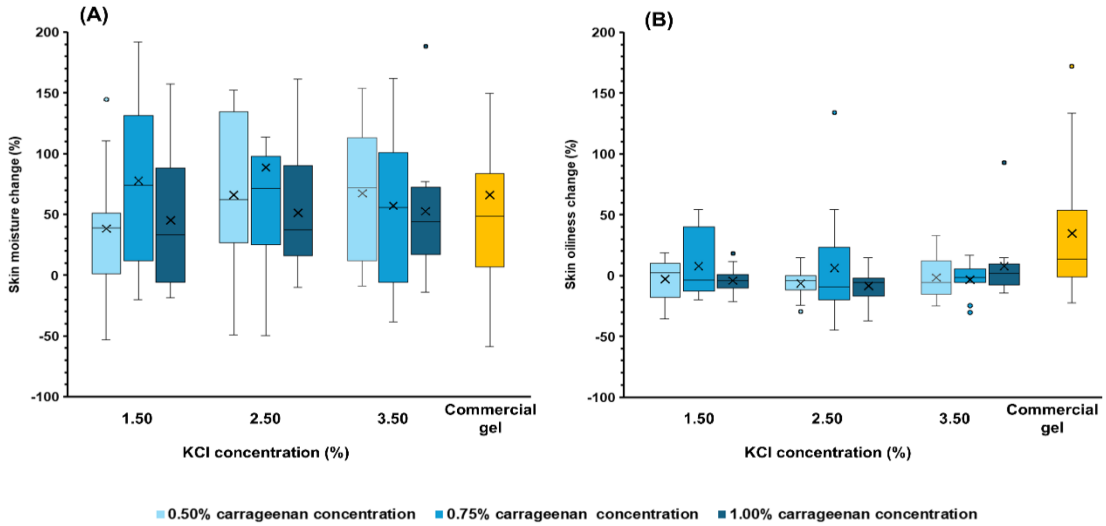

Fig. 2 (A) Box plot with horizontal lines that denote the upper quartile, lower quartile, and median values, and a cross that denote the mean value for skin moisture change (%) (n = 12 for each treatment). (B) Similar box plot for skin oiliness change (%) (n = 12 for each treatment).

Consumer preference for carrageenan gels

To avoid sensory fatigue, cognitive overload and reduced data reliability, only four carrageenan gel samples and a commercial gel were used to test the consumer preference. Among the six tested sensory attributes, colour, odour, and attractiveness of five types of gels were significantly different (P<0.05), while other attributes, texture, moisturising effect, and overall preference remained non-significant (P>0.05) (Friedman test). Only the moisturising ability of carrageenan gel prepared at 0.75% carrageenan concentration using carrageenan powder extracted at 2.50% KCl concentration was preferred over the commercial face mask gel. These results show the potential of using these gels in sheet face mask gels with improved sensory characteristics. Most importantly, these results emphasise the carrageenan's effective role in cosmetic formulations beyond a hydrating or texturising ingredient owing to the spreadability, pH, and consumer preference given only by carrageenan in the prepared gels without any other ingredient. This finding further proves the positive attributes that resonate with recent market trends of natural-origin and environmental consciousness.

Fig. 3 Sensory radar chart showing colour, odour, texture, moisturising effect, attractiveness, and overall preference of four types of carrageenan gels and commercial face mask gel.

ATR-FTIR spectroscopy

KCl concentration of 3.50% and gel concentration of 1.00% was selected as the optimum conditions for preparation of leave-on sheet face mask gel considering the required characteristics, especially the spreadability. Therefore, the chemical composition of carrageenan powder extracted at 3.50% KCl was characterised using ATR-FTIR spectroscopy. Figure 4 shows very strong absorption peaks in the 1210-1260 cm–1 (Peak A) and 1010-1080 cm–1 (Peak B) regions, respectively due to S = O of sulfate esters, and glycosidic linkages characteristic of all carrageenan types. Absorption peaks were also observed in the 925-935 cm–1 (Peak C), 840-850 cm–1 (Peak D), and 803-805 cm–1 (Peak B) regions due to 3,6-anhydro-D-galactose, D-galactose-4-sulfate, and 3,6-anhydrogalactose-2-sulfate, respectively, confirming the extracted carrageenan as κ- type (Webber et al., 2012).

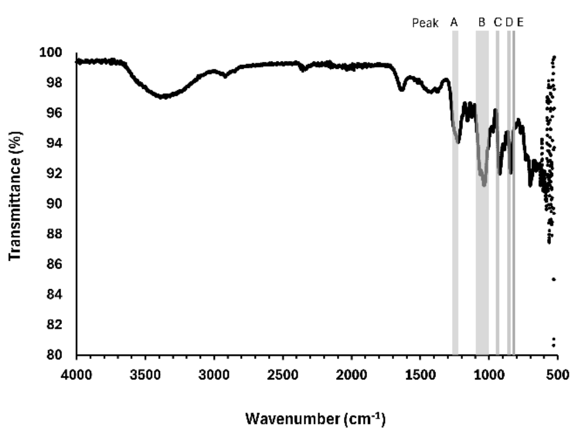

Fig. 4 FTIR analysis of refined carrageenan extracted at 3.50% KCl concentration

CONCLUSIONS

A KCl concentration of 2.50–3.50% is recommended to achieve a higher carrageenan yield. A gel prepared at 1.00% concentration using carrageenan extracted at 3.50% KCl concentration can be recommended for a leave-on sheet face mask gel considering the required characteristics, especially the spreadability. The moisturising effect of prepared carrageenan gels without altering the skin oiliness was found to be a significant finding. Further experiments are recommended to improve sensory characteristics, especially odour.

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