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This is the Accepted version of the following publication

Sedayu, Bakti B, Cran, Marlene and Bigger, Stephen W (2018)
Characterization of Semi-refined Carrageenan-Based Film for Primary Food Packaging Purposes. *Journal of Polymers and the Environment*, 26 (9). 3754 - 3761. ISSN 1566-2543

The publisher's official version can be found at
<https://link.springer.com/article/10.1007/s10924-018-1255-y>
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Characterization of Semi-Refined Carrageenan-Based Film for Primary Food Packaging Purposes

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ABSTRACT

Carrageenan-based films demonstrate good performance and the raw materials for their production are abundant in nature and can be sustainably sourced. Similar to other naturally-derived biopolymers, however, carrageenans are relatively expensive to purify and form into useful materials. In order to potentially lower the production costs compared to pure carrageenan, semi-refined carrageenan (SRC) plasticized with 0–50% (w/w) glycerol was investigated using a solution casting method. The film color and opacity increased along with the moisture content, whereas the water vapor permeability decreased with increasing levels of glycerol. The tensile properties of the SRC films improved significantly, particularly at glycerol additions greater than 30% (w/w). Moreover, the addition of glycerol improved the thermal stability and altered the surface morphology of the films. In general, the properties of the SRC films were comparable with refined carrageenan films suggesting that SRC has potential to be further developed into more cost effective primary food packaging materials.

Keywords: semi-refined carrageenan; packaging film; physico-mechanical properties; thermal properties; glycerol.

INTRODUCTION

There has been a significant growth in the use of biodegradable plastic materials for food packaging purposes in recent decades. This may be due to increasing concerns over the adverse impacts to the environment resulting from the use of synthetic plastics [1]. Approximately eight million tons of plastic waste accumulates in the marine environment annually, and it is predicted that this amount will double by 2030 and double again by 2050 [2]. Moreover, it has been estimated that around 31% of plastic wastes in the ocean originates from single-use food and beverage packaging materials [2].

Alternatives to synthetic plastics derived from renewable sources have been developed including vegetable starch, vegetable protein, cellulose, and chitosan among many others, with some of these now available commercially [3]. However, one of the critical barriers to their wider utilization and commercial production for global markets is the economic competitiveness of existing synthetic plastics which are generally produced at very low cost [4]. For this reason, seaweed-derived carrageenan is considered to be one of the more potentially promising materials due to its abundance in nature, relatively low cost, and ease of processing [5].

Carrageenans are hydrophilic linear sulfated galactans found specifically in the cell walls of red marine algae (*Rhodophyceae*) [6]. The number and position of the sulfate groups on the disaccharide repeating unit determines the classification of carrageenan into one of three major types: lambda (λ), kappa (κ), and iota (ι) [7]. These three types have sulfate contents of 41, 33, and 20% (w/w), respectively, resulting from one, two, and three sulfate ester groups per dimeric unit [8]. Among these different forms of carrageenans, κ -carrageenan is the one most commonly used in industrial applications. It has both a double-helix conformation and linear helical portions that form a three-dimensional gel in the presence of suitable cations [9]. Pure κ -carrageenan is

commonly obtained through the extraction of tropical red seaweed, *Kappaphycus alvarezii*, which is more commonly known as *Eucheima cottonii* [10].

The majority of previous studies on the development of carrageenan-based film have utilized a refined carrageenan (RC) [11-14], with very few reports on the use of semi-refined carrageenan (SRC) for film packaging applications. Compared with RC, SRC can be produced at a significantly lower price due the fewer number of processing steps required in its production. The refinement of SRC to produce RC requires alcohol precipitation and filtration of impurities, followed by gel freezing *via* refrigeration [14]. By omitting these steps, SRC thus contains a cellulose residue that produces a less transparent gel product compared with the refined material [15]. However, opaque or colored packaging is widely used in food containers, trays, cups, wraps, and other packaging designed to preserve light- or UV-sensitive products. For such applications, SRC may serve as a potential alternative material with a subsequent reduced cost of production.

A further limitation of pure carrageenan films is that they are inherently brittle [12], which consequently limits their potential use for food packaging applications. Plasticizers are therefore typically added to the formulations in to improve their flexibility [16] and glycerol, a non-volatile polyol, is one of the more common plasticizers incorporated into carrageenan films to enhance their flexibility and processability [11,13,14]. The amount of plasticizer added to carrageenan films must be carefully optimized in order to obtain the best overall film properties.

In view of the future requirements to manufacture low-cost primary food packaging materials that are derived from renewable resources, this paper explores the physico-mechanical and chemical properties of SRC as a suitable candidate for this purpose.

2. MATERIALS AND METHODS

2.1 Materials

Semi-refined carrageenan extracted from the seaweed *Eucheuma cottonii* was purchased from W-Hydrocolloids, Inc. (The Philippines) and used without further refinement. Glycerol (molecular weight of 92.09 g mol^{-1} , Sigma-Aldrich, Australia) was used as plasticizer, and Milli-Q water was used as the solvent in the film preparations.

2.2 Film Preparation

Semi-refined carrageenan films were prepared using a casting technique [17], whereby 2% (w/w) of SRC was dissolved in 150 mL water under high-speed stirring at room temperature for 15 min. During stirring, various amounts of glycerol (0, 10, 20, 30, 40, and 50% (w/w) of SRC) were added into the SRC solution which was heated to 90°C and stirred for 30 min. To remove air bubbles, the cooled solution was allowed to stand for 10 min under ambient conditions prior to the casting process. The solution was spread evenly onto a rectangular casting tray ($38 \times 18 \times 2.5 \text{ cm}$), and allowed to dry at room temperature for 36 h.

2.3 Film Properties

2.3.1 Thickness

The thickness of the SRC film was measured at three random positions in triplicate using a digital micrometer (Schut IP54, The Netherlands) with a precision of 0.001 mm. The average thickness values were used for in the measurement of the tensile properties and water vapor permeability (WVP).

2.3.2 Color and Opacity

Film color was measured using a Chroma Meter (Konica Minolta CR-400, Japan) by measuring the L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) values. Three different locations of the film surfaces were tested in triplicate, and the average value was calculated in each case. A standard white plate ($L^* = 97.39$, $a^* = 0.03$ and $b^* = 1.77$) was used for calibration.

The opacity of the SRC films was determined by measuring the light absorption at $\lambda = 550$ nm using a UV-visible spectrophotometer (Biochrom Libra S12). Three rectangular samples (14 mm \times 12.5 mm) were taken from different locations on each of the SRC films, and assessed according to the method described by Gómez-Estaca *et al.* [18] with slight modification. To measure the light absorbance of the SRC film, the rectangular film samples were directly placed into the test cell of spectrophotometer with an empty cell being used as the reference. Measurements were performed in triplicate and the opacity (Op) was calculated in accordance with the following equation:

$$Op = A_{550}/x$$

where A_{550} is absorbance at $\lambda = 550$ nm, and x is the thickness of the film sample (mm). The units of opacity are presented as absorbance units (AU) mm^{-1} .

2.3.3 Moisture Content

The moisture content of the film samples was measured according to the method described by Farhan and Hani [14]. Rectangular sample specimens (2 cm x 2 cm) at three random positions were cut and dried in an oven at 105°C until a constant dry weight was obtained.

2.3.4 Water Vapor Permeability

The WVP of the films was determined according to a method by Sobral *et al.* [19]. A circular film sample was sealed on top of a glass permeation cup containing silica gel to obtain 0% RH inside the cup. Prior to sealing, an inert sealing grease (Vaseline™) was evenly spread between the top surface of the glass cup and its lid to ensure an adequate seal. The cups were then placed in a desiccator containing water to maintain 100% RH and were then stored at 22°C in an incubator. The weight gain of each of the sealed sample cups was recorded at 24 h intervals for 7 days with three replicates having been determined in each case. The WVP was calculated using the following equation:

$$WVP = \left(\frac{w}{tA}\right) \times \left(\frac{x}{\Delta P}\right)$$

where A is the exposed area of the film (cm^2), x is the film thickness (mm), ΔP is the water vapor partial pressure difference across the film (Pa), and the ratio w/t was calculated from the slope of the weight gain *versus* time plot.

2.3.5 Thermal Properties

Thermal transitions of the film samples were measured by differential scanning calorimetry (DSC) using a Mettler-Toledo DSC-1 thermal analyzer (Mettler-Toledo, Schwarzenbach, Switzerland) equipped with nitrogen purge gas and an intracooler-based cooling system. Approximately 5-10 mg of sample was loaded in an aluminium crucible which was sealed and an empty, sealed crucible was used as a reference. The sample was heated over the temperature range 40-280°C at a heating rate of 10°C min^{-1} , with a nitrogen flow rate of 20 mL min^{-1} .

2.3.6 Mechanical Properties

The mechanical properties of the SRC-based film were determined in accordance with ASTM Method D 882-12. At least eight specimens of film were cut into strips (120 × 20 mm) and were tested using an Instron Universal Testing Machine (Model 4301) with a 5 kN load cell at a cross-head speed of 5 mm min⁻¹. Tensile strength, percentage elongation at break, and Young's modulus values were identified from the stress-strain curves, which were assessed and processed using Instron BlueHill Series IX software.

2.3.7 Structural Properties

Infrared spectra of the SRC films, glycerol, and SRC were recorded using a Perkin-Elmer Frontier FT-IR spectrometer (PerkinElmer, Inc., USA) equipped with a horizontal attenuated total reflectance (ATR) accessory with a diamond crystal. The spectral transmittance was measured over the range 4000-600 cm⁻¹ using an average of 64 scans, at 4 cm⁻¹ resolution. Data processing was performed using Perkin-Elmer Spectrum 10TM software.

2.3.8 Surface Imaging

The microstructures of the film surfaces were imaged using a Benchtop JCM-6000 scanning electron microscope (JEOL, Tokyo, Japan) at accelerating voltage of 15 kV. Prior to imaging, the film samples were mounted on specimen stubs and then coated with gold in order to make the samples conductive. The SEM images were taken at 500× magnification.

2.4 Statistical Analysis

Statistical analyses of the experimental data were processed using IBM-SPSS Statistics 24 software. One-way analysis of variance (ANOVA) was performed, and the significance among the mean values of sample properties was determined with the Duncan test at a 5% significance level (i.e. $p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Film Color, Opacity and Thickness

Visually, the SRC films were translucent and yellowish in color with an apparent increase in flexibility as the plasticizer concentration was increased. In general, the incorporation of glycerol in the formulation increased the clarity of the film compared to the control SRC film containing no plasticizer and at levels of 30% (w/w) glycerol or greater, the yellowness decreased as shown by the decreasing b^* values as shown in Table 1. In addition, a significant increase in the transparency of the films also resulted from the addition of glycerol as shown by the decreasing opacity (see Table 1). Similar transparency results have been reported in previous studies [14,20,21] with suggestions that the increased transparency results from the increased intermolecular spacing of glycerol within the polymer matrix which enables a greater amount of light to pass through the film [22]. In comparison, RC films have been reported to be more transparent, whiter films with minimal yellowness [17,23]. This may be due to the absence of cellulose and other residual particles such as glucan, minerals, and insoluble aromatic compounds that are removed from SRC to form RC [24], which would otherwise obstruct the penetration of light through the film.

The thickness of the films also increased with the incorporation of glycerol as shown in Table 1. This is not unexpected since plasticizers may restructure the intermolecular polymer chains by increasing the free volume of the film matrix [20]. The increasing film thickness may also be explained by the increase in the interstitial spacing between the polymer chains within the film matrix [22].

Table 1. Values of surface color, opacity and thickness of the SRC films.

Glycerol content/ % (w/w)	L^*	a^*	b^*	Opacity/AU mm^{-1}	Thickness/ μm
0	$86.23 \pm 0.42^{\text{ab}}$	$-0.79 \pm 0.04^{\text{a}}$	$10.33 \pm 0.79^{\text{c}}$	$16.57 \pm 0.40^{\text{a}}$	67.4 ± 1.8
10	$86.30 \pm 0.27^{\text{ab}}$	$-0.86 \pm 0.04^{\text{b}}$	$10.94 \pm 0.60^{\text{c}}$	$15.61 \pm 0.78^{\text{b}}$	68.3 ± 0.6
20	$85.95 \pm 0.56^{\text{a}}$	$-0.87 \pm 0.04^{\text{b}}$	$10.98 \pm 1.08^{\text{c}}$	$14.24 \pm 0.69^{\text{c}}$	72.2 ± 3.3
30	$86.50 \pm 0.23^{\text{bc}}$	$-0.93 \pm 0.02^{\text{c}}$	$9.55 \pm 0.42^{\text{b}}$	$11.74 \pm 0.50^{\text{d}}$	80.7 ± 1.2
40	$86.71 \pm 0.38^{\text{c}}$	$-1.01 \pm 0.05^{\text{d}}$	$8.87 \pm 0.59^{\text{b}}$	$10.56 \pm 0.22^{\text{e}}$	83.6 ± 0.4
50	$87.14 \pm 0.40^{\text{d}}$	$-1.07 \pm 0.03^{\text{e}}$	$8.07 \pm 0.77^{\text{a}}$	$10.22 \pm 0.34^{\text{e}}$	88.9 ± 1.1

Values are given as mean with one standard deviation. Any two means in the same column followed by the same letter are not significantly different ($p > 0.05$) by a Duncan's test.

3.2 Film Moisture Content and Water Vapor Permeability

As shown in Fig. 1(a), glycerol addition plays an important role in the moisture content of the SRC films whereby it increases the moisture significantly ($p < 0.05$) at levels of 20% (w/w) glycerol and above. It has been suggested that glycerol in a polymer matrix enhances the hydrophilicity of the film thereby increasing its water sorption, and that the hydroxyl groups of this plasticizer within a polymer matrix interact with water molecules through hydrogen bonding [21,25]. Similar results have also been reported for previous carrageenan-based films as well as in the production of other water-soluble polymer films [23,26]. Moreover, Karbowski *et al.* [26] reported that the water sorption of carrageenan-based films plasticized with glycerol was significantly more sensitive at water activity (a_w) above 0.7, but insignificant at lower a_w values. Similar trends are evident between the thickness and moisture content of the SRC films with both parameters increasing relatively linearly with increasing glycerol concentrations as a result of the increased water binding capacity of the polymer matrix.

To maintain the quality of packaged food products during storage, particularly with regard to maintaining moisture content, the WVP of the packaging material is a very important parameter.

As shown in Fig. 1(b), the addition of glycerol to the SRC matrix increased the WVP of the film samples with levels of glycerol above 20% (w/w) increasing the WVP by more than 5% ($p < 0.05$). This increase is correlated with the increasing hydrogen bond formation in the polymer network resulting in a greater free-volume and more segmental motions with a subsequent decrease in the polymer density. This, in turn, allows the water vapor to permeate through the films more easily and this phenomenon is typical for other biopolymer film materials such chitosan [27] and gelatin [19]. Even though the low molecular weight of glycerol makes it effective to be used as plasticizer, its high hydrophilicity facilitates the solubility of water molecules which then permeate through the film subsequently reducing water barrier properties [27].

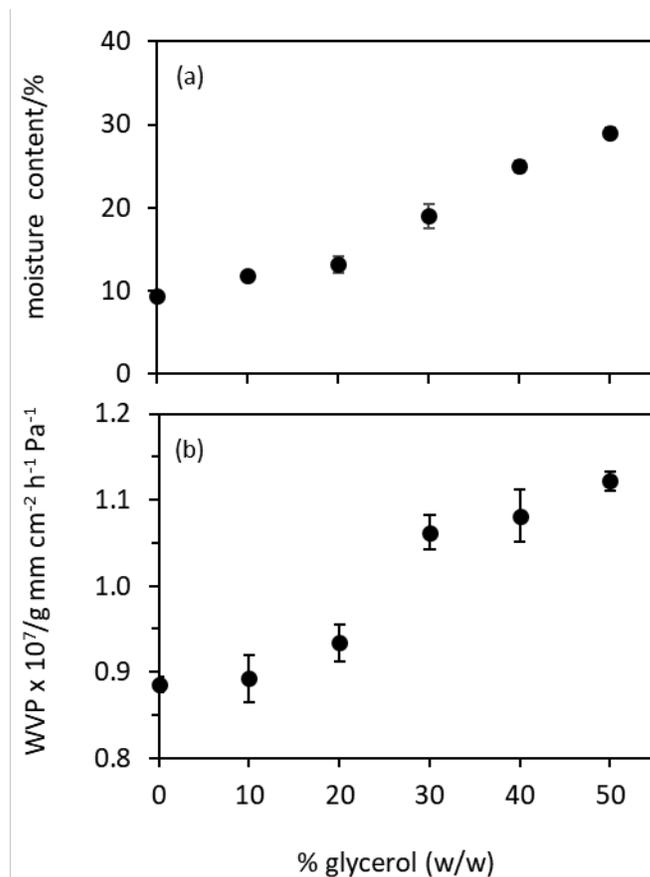


Figure 1. Effect of glycerol content on SRC film moisture content (a) and WVP (b).

3.2 Mechanical Properties

Tensile strength (TS), elongation at break (EAB) and Young's modulus (YM) are among the primary mechanical properties studied in food packaging film development. As shown in Fig. 2, the incorporation of glycerol into the SRC film formulation influenced each of these properties. Without the addition of the glycerol plasticizer, the control SRC film exhibited shrinkage and brittleness resulting in an easily torn, fragile film with an EAB of less than 2% and a TS of around 13 MPa. The TS and EAB of the films increased with the addition of the plasticizer to the polymer matrix with an almost linear increase in TS with up to 20% (w/w) glycerol addition. The highest TS was obtained at 40% (w/w) glycerol content, after which further addition of the plasticizer resulted in a decreased TS. A similar trend was observed for the EAB values with a progressive increase from *ca.* 1.3% up to 15.3% at the maximum glycerol concentration of 50% (w/w). This increase may be attributed to the formation of hydrogen bonds within the SRC polymer network that produces a more cohesive chain. Sothornvit and Krochta [25] explained that the hydrogen bonds from plasticizers interact with polymers by interrupting polymer-polymer bonding and producing longer distances between the polymer chains, which then results in a more flexible film. Similar results have also been reported for films derived from other biopolymers such as starch [28], confirming that plasticizers such as glycerol are necessary to impart favorable mechanical strength. The relatively small molecular size of glycerol enables the molecules to spread within the intermolecular space of the polymeric chains thereby decreasing the intermolecular hydrogen bonds and increasing the molecular mobility within the matrices [20].

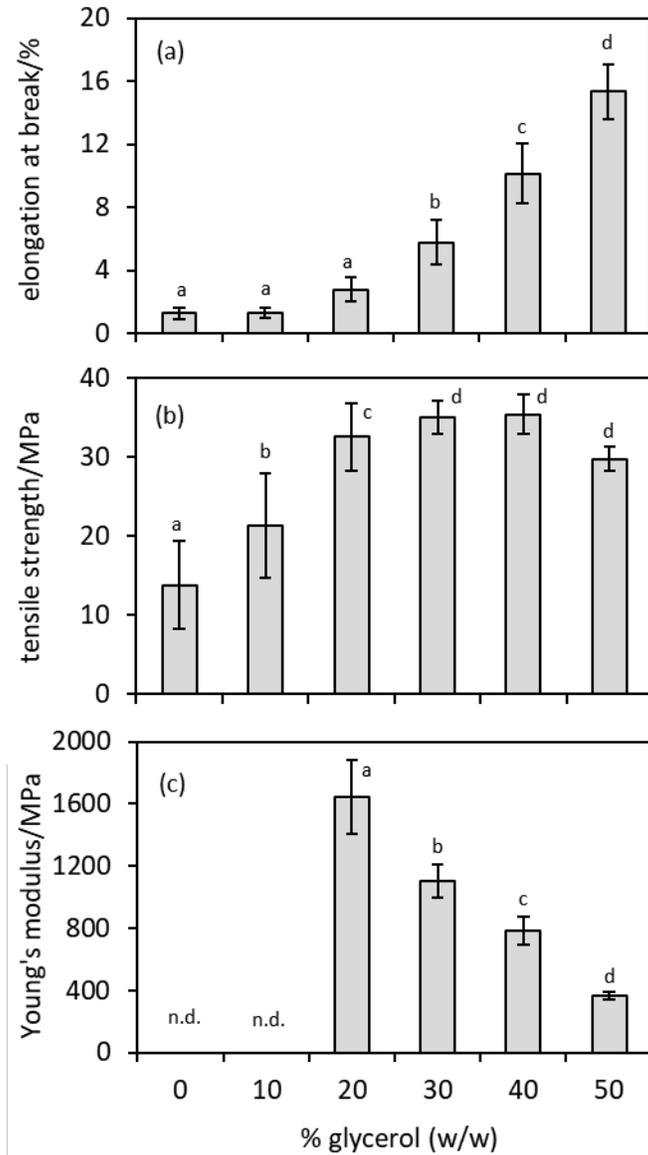


Figure 2. Effect of glycerol content on SRC film EAB (a), TS (b), and YM (c). Different letters between data bars represent significant differences, n.d. = not determined.

In addition to the TS and EAB, Fig. 2 also shows the influence of glycerol addition on the YM of the film samples (Fig. 2(c)). For zero and 10% (w/w) glycerol content, the YM was not determined but at 20% (w/w) glycerol addition, the YM was the highest measurable value which subsequently decreased with increasing glycerol content. This suggests that the incorporation of glycerol improves the flexibility of the film in accordance with the EAB results. Similar findings have been

reported for the addition of glycerol in edible wheat starch films with changes in density suggested to contribute to the improved flexibility [21]. Overall, the results of the present study indicate that the plasticized SRC films have good overall mechanical strength but with lower flexibility.

3.4 Structural Properties

The bonds and functional groups of the SRC films plasticized with glycerol were observed by comparing the FTIR spectra as shown in Fig. 3. The wide absorption bands found in the region 3600-3000 cm^{-1} are associated with the vibrational stretching of free, inter- and intra-hydroxyl group bonding [29]. These bands appear more intense with an increase in glycerol concentration as a result of the formation of hydrogen bonds involving -OH groups from the κ -carrageenan and glycerol within the polymer matrix. This is also evidenced by the H-OH bending vibration at 1644 cm^{-1} which also appears to increase with higher levels of glycerol [30]. The bands identified at wavenumbers 1218, 1035, 930 and 844 cm^{-1} correspond to the S=O bond of sulfate ester, glycosidic linkage (C-O) of 3,6-anhydro-D-galactose, C-O of the 3,6 anhydro-D-galactose, and C-O-SO₃ bonds of the D-galactose-4-sulfate respectively which represent the typical bonds found in κ -carrageenan [11,29]. The vibrational stretching at 2980-2910 cm^{-1} , which corresponds to the C-H stretch of alkanes from the aliphatic chain of glycerol, also increases in intensity following the increased glycerol concentration in the film samples [31]. Overall, the SRC film spectra, including the control film with no glycerol, showed a relatively similar pattern of bands suggesting a good miscibility of glycerol with the SRC polymer matrix.

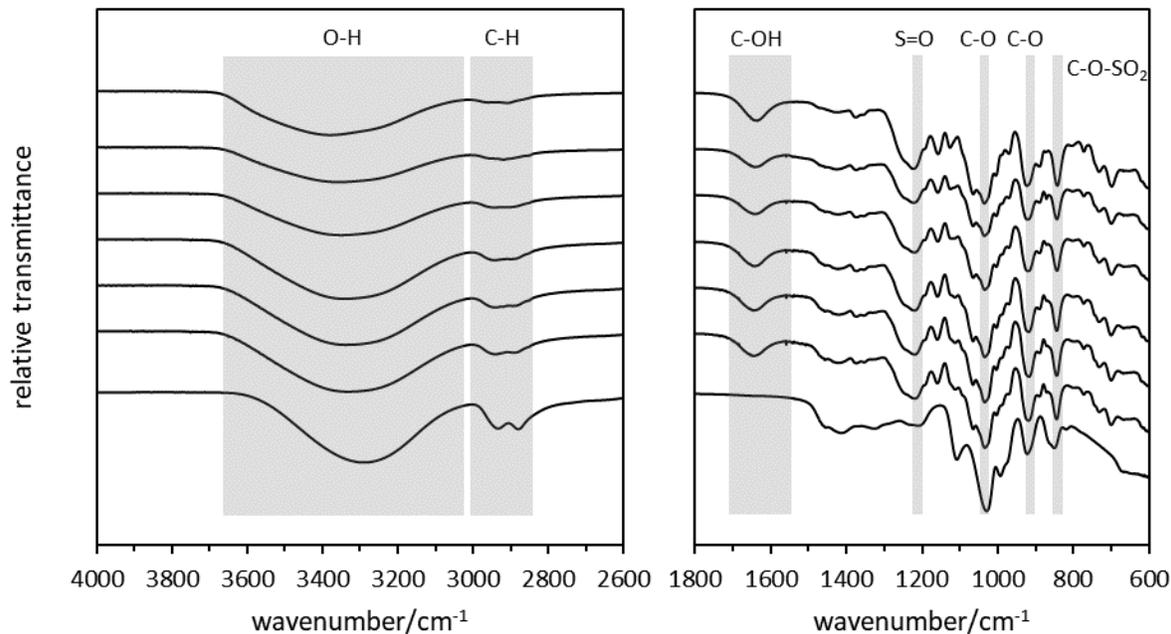


Figure 3. FT-IR spectra of SRC films with increasing glycerol content.

3.5 Thermal Properties

The thermal transitions of SRC films incorporated with different glycerol concentrations were investigated using DSC as shown in Fig. 4. The broad endothermic peaks, which are associated with the melting ranges of the film samples shifted to higher temperatures with an increase in the glycerol concentration. The SRC film without glycerol, for example, shows a minimum melting range of *ca.* 145°C which shifted up to *ca.* 177°C with the addition of 50% (w/w) glycerol. This may be attributed to the high level of interaction between SRC and glycerol molecules that creates a more stable film structure which therefore requires a higher energy of activation for the melting of crystallites in the film. This result is similar to that found in the thermal behavior of gelatin-based film and potato starch incorporated with plasticizers [32,33].

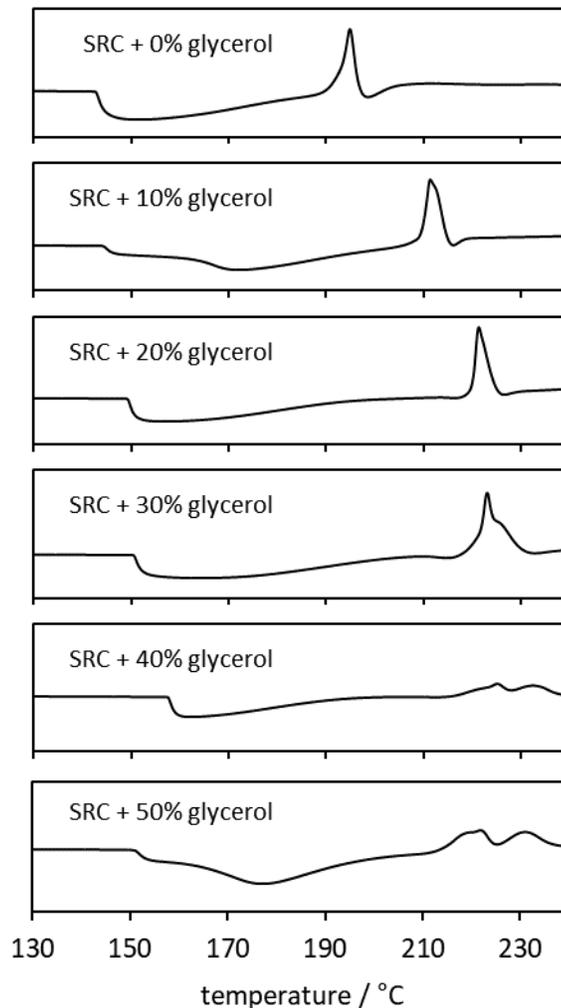


Figure 4. DSC thermograms of SRC films as a function of glycerol content.

Each DSC thermogram also revealed a highly exothermic peak at a temperature above the melting range of the SRC. These exothermic peaks appeared at temperatures ranging from *ca.* 190 to 240°C, whereby an upward shifting trend was observed with increasing concentration of glycerol in the formulation, similar to the trend observed in the melting point range. This exothermic process may be attributed to the glycosidic bond and hexatomic ring fracture of the polymer, decarbonylation, as well as dehydration processes that occur in carrageenan at higher temperatures [34]. Furthermore, interesting additional exothermic peaks at glycerol additions of

above 40% (w/w) are also apparent in the thermograms which seem to be associated with the excessive addition of glycerol to the polymer. These two peaks may be due to the fracture of SRC polymer structure followed by a volatilization of the excess glycerol, however their origin is unclear at present. Nonetheless, the overall results suggest the thermal stability of the SRC films is improved by the incorporation of glycerol. This finding is also supported by similar results obtained for cellulose- and starch-based films [35,36].

3.6 Surface Morphology

Scanning electron microscopy was used to investigate the surface morphology of the SRC films and the resulting SEM images are shown in Fig. 5. Without glycerol addition, the SRC film showed a comparatively rough surface appearance. The addition of glycerol into the polymer matrix remarkably increased the smoothness of the film surface by creating larger segregated domains over the matrix. The homogeneity and smoothness of the film may reflect the structural integrity of the polymer and demonstrate a good solubilization and homogenization of the plasticizer in aqueous medium used in the preparation of the film. This is also reflected in the optical properties of the film that are enhanced with the addition of glycerol and similar results have been reported for gelatin- and chitosan-based films [37,38]. Additionally, some platelets (*ca.* 10 μm dia.) can also be seen in the micrographs as indicated by arrows (see Fig. 5) and are dispersed randomly within the matrix. These are assumed to be residual cellulose from the SRC and the presence of these may explain in part the higher opacity of the SRC films compared to RC films since these will obstruct the light transmission through the film [29].

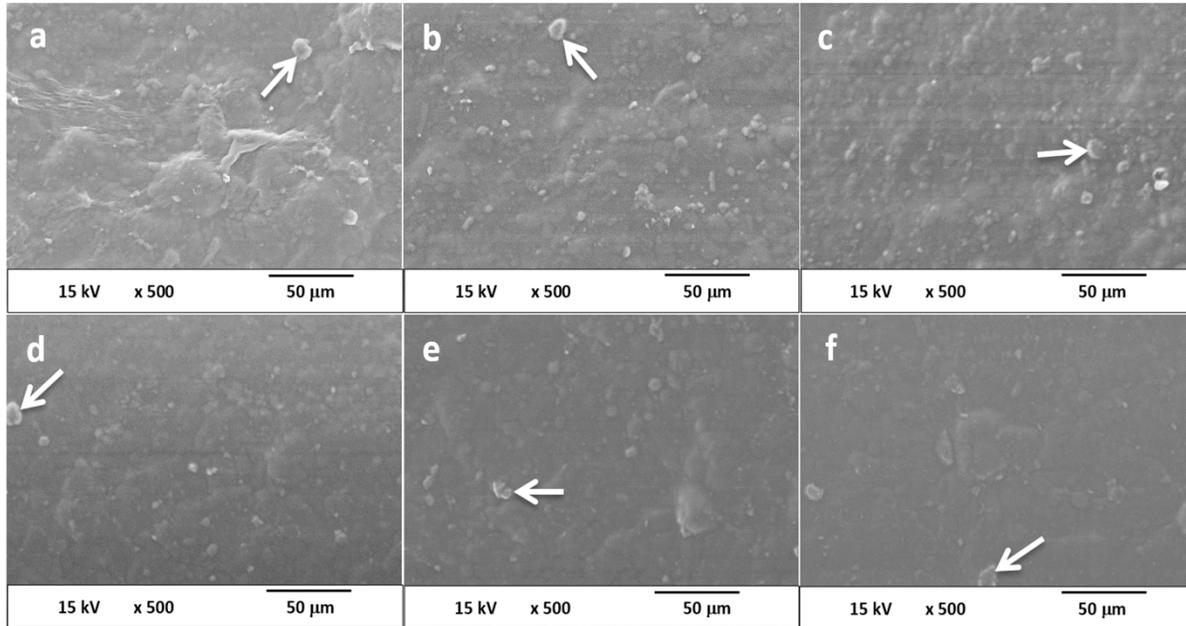


Figure 5. SEM micrographs (500× magnification) of SRC films incorporated with glycerol: (a) 0 %, (b) 10 %, (c) 20 %, (d) 30 %, (e) 40 %, (f) 50 %.

4. CONCLUSIONS

The incorporation of glycerol into SRC films enhanced the optical, mechanical, morphological, and thermal properties, but it decreased the barrier properties (WVP) of the films. Compared with RC-based films in other studies, the SRC films were more yellow in color with higher opacity as a result of the residual cellulose and other components that remain in the polymer matrix. However, the mechanical properties of the SRC films were comparable to those of RC and other biopolymer similar films, although the SRC films showed a noticeably rough surface texture. Nonetheless, this study has shown that SRC could be potentially developed for rigid and non-transparent primary food packaging applications with the potential benefit of its economic and environmental advantages.

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