Development of Semi-Refined κ-Carrageenan-Based Films for Food Packaging Applications

Thesis submitted for the degree of Doctor of Philosophy

by

Bakti Berlyanto Sedayu, M. Sc.

College of Engineering and Science
Institute for Sustainable Industries and Liveable Cities
Victoria University
Melbourne, Australia

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Abstract

Carrageenan-based films exhibit some desirable properties for food packaging applications, but the high cost of source materials has been a considerable constraint for commercial development. This thesis presents a series of investigations designed to develop novel composite films for food packaging purposes utilizing a more economical and renewable raw material resource, namely semi-refined carrageenan (SRC). Additionally, the development and use of SRC as a biodegradable film-forming alternative to the current and commonly utilized refined carrageenan (RC) will also bring about benefits to the environment. Reinforcing the SRC with nanoclay and/or nanocellulose, lamination with poly(caprolactone), and surface photo-crosslinking are among the approaches used to enhance the SRC film properties.

In the initial investigation, the preparation and characterization of SRC films plasticized with up to 50% (w/w) glycerol was performed using a solution casting method. The resultant film color and opacity increased with increasing levels of glycerol along with the moisture content, whereas the water vapor permeability decreased. 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 plasticized SRC films were comparable to those of refined carrageenan film counterparts.

To overcome inherently poor water sensitivity and barrier properties of the SRC, film samples were reinforced with nanocellulose fibrils (NCF) and were benchmarked against similar films made from refined carrageenan (RC) with regard to the water sensitivity, physicomechanical and thermal properties. The level of NCF was varied from 1% to 7% (w/w) and, in general, the NCF reinforcement improved the overall properties of both the SRC and RC films including the water sensitivity and moisture barrier properties. Nonetheless, NCF inclusion in SRC film was less effective with regard to the mechanical and thermal properties enhancement compared with NCF inclusion in RC film. The enhancement in properties was attributed to the greater

cohesiveness of the reinforced polymer structure and the crystalline regions formed in the structures of SRC and RC films by NCF incorporation.

Semi-refined carrageenan film was also reinforced with nanoclay (NC) in combination with surface lamination using a thin layer of poly(caprolactone) (PCL) with a view to improving the barrier properties and hydrophobicity of the film and concurrently improving the mechanical properties. The water vapor permeability, moisture uptake, and water solubility decreased by 20%, 24% and 11%, respectively and the water contact angle increased from *ca.* 72° to 95° upon inclusion of the NC in the formulation. The tensile strength and elongation at break increased by 17.9% and 2.8% respectively, and the thermal stability also increased slightly. The PCL lamination was the main contributor to the enhanced barrier and mechanical properties of the films, whereas the NC inclusion contributed more to the enhanced thermal properties.

In a further study, the surface regions of SRC film samples were photo-crosslinked with UV light of 0.46 W m^{-2} intensity using 6% (w/v) sodium benzoate solution as a photosensitizer. The surfaces were coated with the photosensitizer and exposed to the light source for 5, 10, 20 and 40 min. The effects of the surface crosslinking on the overall properties of the SRC films were investigated and related to the possible changes in the morphology of the substrate. The UV exposure and crosslinking were found to increase the crystallinity and the thermal stability of the films but with minimal colour changes. The mechanical properties were improved relative to the control sample with a *ca*. 42–55% increase in the tensile strength, *ca*. 142–144% increase in the modulus but with a concomitant *ca*. 50–52% decrease in the elongation at break. The crosslinking decreased the inherent moisture content in the films by *ca*. 50–52% and decreased the water vapour transmission rate by *ca*. 16–21% relative to the control. Changes in water sensitivity were observed with increases of *ca*. 35–44%, 18–22% and 21–22% in the water solubility, moisture uptake and water contact angle respectively. The latter increases were attributed to possible photodegradation products and the presence of residual photosensitizer that rendered the samples more hydrophilic. Under the conditions of the experiment, a UV exposure time of *ca*. 10–20 min was found to be optimal in enhancing the mechanical and water barrier properties.

These overall findings of the research have provided a most promising step towards the production of an economical food packaging material from SRC that has a minimal impact on the land environment.

Publications Arising from this Work

Papers in refereed journals:

1. A Review of Property Enhancement Techniques for Carrageenan-based Films and Coatings by Sedayu, B. B., Cran, M. J., Bigger, S. W. in *Carbohydrate Polymers*, 216, 287-302. 2019. doi: https://doi.org/10.1016/j.carbpol.2019.04.021.
2. Characterization of Semi-refined Carrageenan-Based Film for Primary Food Packaging Purposes by Sedayu, B. B., Cran, M. J., Bigger, S. W. in the *Journal of Polymers and the Environment*, 26, 3754–3761p. 2018. doi: https://doi.org/10.1007/s10924-018-1255-y.
3. Improving the Moisture Barrier and Mechanical Properties of Semi-Refined Carrageenan Films by Sedayu, B. B., Cran, M. J., Bigger, S. W. in the *Journal of Applied Polymer Science.* doi: https://doi.org/10.1002/app.49238
4. Reinforcement of Refined and Semi-Refined Carrageenan Film with Nanocellulose by Sedayu, B. B., Cran, M. J., Bigger, S. W. has been published in *Polymers*. doi: https://dx.doi.org/10.3390%2Fpolym 12051145
5. Effects of Surface Photo-Crosslinking on the Properties of Semi-Refined Carrageenan Film by Sedayu, B. B., Cran, M. J., Bigger, S. W in *Food Hydrocolloids*, 106196. doi: https://doi.org/10.1016 /j.foodhyd.2020.106196

Conference presentations:

1. Bakti B. Sedayu, Marlene J. Cran, and Stephen W. Bigger, "Semi-Refined Carrageenan-Based Film for Food Packaging Purposes", presented at the 1st International Conference on Bioinspired Materials and Membranes (IBMM), Melbourne-Australia. 2018.
2. Bakti B. Sedayu, Marlene J. Cran, and Stephen W. Bigger. "Effects of Glycerol Incorporation on Semi-Refined Carrageenan Film Properties", presented at the 9th International Conference on Biopolymers & Polymer Sciences, Bucharest-Romania. 2018.

3. Bakti B. Sedayu, Marlene J. Cran, and Stephen W. Bigger. “Enhancement in the Barrier and Mechanical Properties of Seaweed-based Films by Nanoclay Inclusion and Poly(caprolactone) Lamination”, presented at Innovation on Polymer Science and Technology 2019, Bali-Indonesia. 2019.

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Melbourne, March 2020

Bakti B. Sedayu

Declaration

“I, Bakti Berlyanto Sedayu, declare that the PhD thesis entitled **Development of semi-refined κ-carrageenan-based films for food packaging applications** is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated this thesis is my own work”

[redacted]

Bakti Berlyanto Sedayu Date: 8 March 2020

Abbreviations

AgNPs	silver nanoparticles
AM	antimicrobial
ANOVA	one-way analysis of variance
ATR	attenuated total reflectance
a_{w}	water activity
CMC	carboxymethyl cellulose
CNFs	cellulose-nanocrystals or nanowhiskers
CNFs	cellulose-nanofibrils
CuO	copper oxide
DSC	differential scanning calorimetry
EAB or EB	elongation at break
EM	elastic modulus
EM	elastic modulus
FDA	Food and Drug Administration
FTIR	fourier-transform infrared
GSE	grapefruit seed extract
LBG	locust bean gum
LDPE	low-density polyethylene
NC	nanoclay
NCF	nanocellulose fibrils
Op	opacity
PBS	poly(butylene succinate)
PCL	poly(caprolactone)
PES	Processed Eucheuma Seaweed
PHAs	poly(hydroxyalkanoates)
PLA	poly(lactic acid)
PVP	poly(vinyl pyrrolidone)
RC	refined carrageenan
SEM	Scanning electron microscopy
SRC	semi-refined carragenaan
T_{g}	glass transition temperature
TGA	thermogravimetric analysis
THF	tetrahydrofuran
T_{m}	melting temperature
TS	tensile strength
UV	ultra-violet
WCA	water contact angle
WS	water solubility
WVP	water vapour permeability
XRD	X-ray diffraction
YM	Young’s modulus
ZnO	Zinc oxide

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CHAPTER 1

INTRODUCTION

1.1 Overview

Chapter 1 presents a general overview on the development of semi-refined carrageenan (SRC) film in particularly in view of food packaging applications. Research background, carrageenan-based film characteristics and general properties enhancement approaches in bio-based polymer are generally described. The aims and research significance of this project is also presented in this chapter. The Introduction (**Sec. 1.2**) of this chapter is a part of published paper entitled "A Review of Property Enhancement Techniques for Carrageenan-based Films and Coatings" by Sedayu, B. B., Cran, M. J., Bigger, S. W in *Carbohydrate Polymers*, 216, 287-302. 2019. doi: https://doi.org/10.1016/j.carbpol.2019.04.021.

1.2 Introduction

The extensive use of plastic materials over the years has indisputably contributed to increasing environmental pollution (Thompson et al., 2009; Woodall et al., 2014). For example, in 2018, there were 359 million tons of plastics used worldwide with more than 99% of these derived from petrochemicals (Plastic Europe, 2019). These conventional plastics are resistant to degradation in nature, and this is particularly evident in the oceans and on seabeds where vast quantities of plastic wastes accumulate (Andrades et al., 2016; Enders et al., 2015). As a result, such materials can lead to serious environmental problems and can be harmful not only to wildlife (Bond et al., 2013; Neves et al., 2015; Rummel et al., 2016) but also to human health, particularly in form of microplastics which can enter the food chain (Efferth & Paul, 2017; The Lancet Planetary, 2017).

In recent decades, this environmental issue has drawn considerable attention from governments, industry and researchers in order to find sustainable, biodegradable alternatives to synthetic plastics. This is particularly the case in packaging applications that represent the largest proportion of the overall plastic usage. Approximately one-third of plastics manufactured worldwide are transformed into packaging materials, with the majority converted into single-use applications such as flexible and rigid food packaging, plastic bags and film, among numerous others (Siracusa et al., 2008). Extensive research has been conducted to produce bioplastics from many different sources including plants, microbes, and animals (Pathak et al., 2014). Some are now commercially available in the global market such as poly(lactic acid) (PLA), poly(hydroxyalkanoates) (PHAs) and starch-based polymers, with new materials frequently investigated for various uses (Accinelli et al., 2012). Even though these materials can be produced from many renewable sources, there are still challenges to be addressed due to their limited biomass-availability and also their cultivation or synthesis. Seaweed is one such material that can offer a source of raw materials from which packaging materials can be produced.

Compared with terrestrial plant sources, seaweed offers some considerable advantages including its low cost and abundance in nature. Moreover, as a non-terrestrial plant material, it is unnecessary to clear valuable land to plant crops or compete with food production. Additionally, seaweeds are purported to lessen impacts on the food chain and are generally safe from exposure to chemicals or fertilizers (Rajendran, Puppala, Sneha Raj, et al., 2012). Amongst several polysaccharides extracted from seaweeds, carrageenan is one of the most promising phycocolloids that demonstrates excellent film-forming ability (Blanco-Pascual et al., 2014; Paula et al., 2015; Siah et al., 2015).

Carrageenan, obtained from red seaweeds of the class Rhodophyceae, is a sulfated polygalactan with 15–40% ester sulfate content and with an average molecular weight above 100 kDa. Its polymer chains are formed by alternate units of D-galactose and 3,6-anhydrogalactose joined by α–1,3– and β–1,4–glycosidic linkages (Necas & Bartosikova, 2013). Most carrageenan used in the global market is the κ-carrageenan (Lopez-Pena & McClements, 2014), which is commonly derived from tropical seaweed *Euchema cottonii* (Campo et al., 2009) (See **Fig. 1-1**). This marine algae species has been intensively farmed in Indonesian waters and waters of The Philippines (Bono et al., 2014). *Eucheuma* species are hardy, multibranched bushy plants. They are non-rooting algae that are naturally growing and are attached to dead coral. *Eucheuma* species reproduce by sporulation and are vegetative, so they can be sectioned anywhere and re-growth will occur in all sections. This feature makes *Eucheuma* species adaptable to different environments in various forms (Parker, 1974) (see **Fig.1-1**).

Figure 1-1. Seaweed *Eucheuma cottonii* harvested from Indonesian water

Recent studies have reported the transformation of carrageenan into packaging films for a range of different applications (Kanmani & Rhim, 2014a; Martins, Cerqueira, et al., 2012; Setha et al., 2016; Shojaee-Aliabadi, Mohammadifar, et al., 2014). However, these polymer films still exhibit limitations with particular regard to their water vapour permeability (WVP) and water resistance since they are naturally hydrophilic (Alves et al., 2011), as well as being brittle (Larotonda, 2007; Shojaee-Aliabadi, Hosseini, et al., 2014). As a consequence, such inherent weaknesses may limit the widespread packaging applications of these materials. Regarding these shortcomings, it is required to make investigations to improve the properties of carrageenan-based films for the purpose of obtaining a functional biodegradable film which can be applied in real food packaging production.

The performance of carrageenan films can be modified by combining these with other polymers like polysaccharides, proteins, lipids, or synthetic polymers (Fabra et al., 2008; Martins, Cerqueira, et al., 2012; Shojaee-Aliabadi, Mohammadifar, et al., 2014). The resulting physicomechanical and barrier properties depend on the functionality of each constituent, as well as their compatibility in the mixture (Bourtoom, 2008; Galus & Lenart, 2013). To enhance water vapor permeability, lipids or other hydrophobic materials are frequently mixed into hydrocolloid-based films (Hambleton et al., 2009). Several attempts have also been made to enhance the barrier properties of hydrophilic polymers, such as blending with hydrophobic materials (Machmud et al., 2013), blending with other natural or synthetic polymers (Fouda et al., 2015; Park et al., 2001), and crosslinking (Rodrigues et al., 2012). Moreover, natural antimicrobial compounds like plant essential oils can also be incorporated in hydrophilic films to enhance their mechanical and barrier characteristics and also enhance their antimicrobial functionality towards spoilage and pathogenic microorganisms (Shojaee-Aliabadi, Hosseini, et al., 2014; Soni et al., 2016a)

Other considerations that should be taken into account when developing biodegradable packaging materials include the cost efficiency and the "eco-friendliness" of their production process. These considerations are important in order to produce materials that are able to commercially compete with existing conventional plastics that are relatively inexpensive.

An extensive search of the literature reveals that almost all carrageenan-based films/composites are produced from refined or pure carrageenan rendering these materials relatively expensive compared with their synthetic commercial counterparts. Thus, in the current project, semi-refined carrageenan (SRC) has been investigated to be transformed into a novel food-packaging grade material that will reduce the production costs and minimize the environmental impact associated with the production, use, and disposal of the material.

1.3 Research Significance and Contribution to Knowledge

Almost all previous research studies on carrageenan-based film fabrication have utilized a refined carrageenan; very little is known about the use of semi-refined carrageenan (SRC) as a substitute. Compared with refined carrageenan, SRC has a significantly lower price due to a fewer number of processing steps required in its production. During SRC production, alcohol precipitation and the filtration of impurities steps are not required, and there is also no need for a refrigeration process for freezing the gel as in the case of refined-carrageenan production (Dewi et al., 2012). In the current project, some modifications of previously known methods of carrageenan film production are explored to obtain an optimum processing method that produces SRC film and thick-section plastic with desirable properties at minimum cost.

A novel nanotechnological approach is also explored by reinforcing nanocellulose and nanoclay materials during film preparation to enhance the physicomechanical properties of the bioplastic-films. The nanocellulose for reinforcement material can be obtained from the waste (residual cellulose) that remains after carrageenan processing. This innovation may thus create a closed-cycle production that should minimize waste during carrageenan bioplastic production. Furthermore, a hydrophobic polymer namely poly(caprolactone) has been incorporated through a layering technique in the films' preparation to overcome certain limitations of carrageenan-based films i.e. water vapour permeability and water resistance. Another approach to overcome the main properties constraint of this polymer film is the modification of the film surface through a UV photo-crosslinking. Each of these techniques have been investigated in other hydrophilic polymers such as starch-based and poly(lactic acid) polymers and have shown a desirable improvement, but these have not yet been applied in the case of carrageenan-based polymers.

Overall, the findings of this project thus contribute knowledge in the area of eco-friendly bioplastic production by minimizing chemical use and reducing energy consumption associated with the production process, by using semi-refined carrageenan as the raw material instead of pure carrageenan. Ultimately, the project findings can be used for the further development of packaging materials with outcomes that have the potential to contribute to the suppression of environmental degradation caused by plastic wastes.

1.4 Research Objectives

In view of producing a novel biodegradable film that can be applied for food packaging application made from a relatively cheap and sustainable material, semi-refined carrageenan, the specific aims of this research project are:

- To prepare and characterize SRC-based film using a solution solvent casting method with incorporation of glycerol as a plasticizer.
- To enhance the mechanical and barrier properties of the SRC film by reinforcement with nanocellulose fibrils.
- To enhance the water sensitivity, mechanical properties, and thermal stability of the SRC film by using a combination of nanoclay reinforcement and poly(caprolactone) lamination.

- To improve the mechanical properties of SRC by modification of the film surface through UV photo-crosslinking. Such modification may also be of particular use in rigid-food packaging applications.

1.5 Thesis Outline

This thesis is comprised of the following chapters:

- Chapter 1 describes a general overview of the background of the current study, the potential of SRC utilization for alternative biodegradable plastic materials, as well as the aims and significance of this research work.
- Chapter 2 presents a literature review on the development of carrageenan-based films including their applications in broad range of areas and encompasses the various techniques to enhance the properties of the films.
- Chapters 3–6 investigate respectively the incorporation of glycerol, nanocellulose and nanoclay, lamination of SRC films with poly(caprolactone) as well as surface photo-crosslinking in SRC film preparation, with particular regard to the effects of these on the film properties.
- Chapter 7 summarizes the overall experimental work and makes recommendations for future work.

The chapters dealing with experimental work (Chapter 3–6) are presented according to a scientific journal format comprised of the introduction, materials and methods, results and discussion, and the conclusion.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This chapter presents a literature review of the current status of carrageenan-based film development including the material sources and characteristics, and the strategies to obtain desirable film properties with particular regard to food packaging applications. The economical perspective for carrageenan-based film development and its potential application are also described. This chapter has been published in a manuscript entitled "A Review of Property Enhancement Techniques for Carrageenan-based Films and Coatings", Sedayu, B. B., Cran, M. J., Bigger, S. W., *Carbohydrate Polymers*, **216**, 287-302, 2019. doi: https://doi.org/10.1016/j.carbpol.2019.04.021.

2.2 Introduction

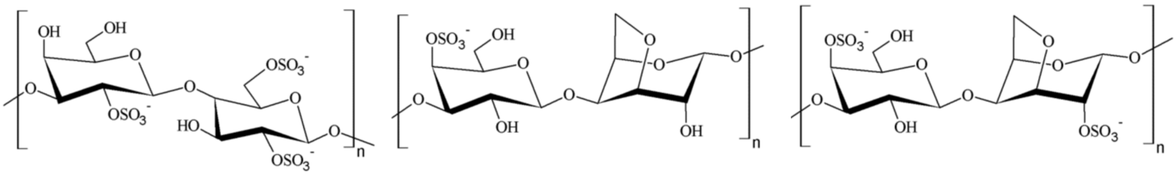
The development of carrageenan-based films for packaging purposes is a relatively new and emerging research area. Consequently, there are a limited number of articles in the literature that review the current status of this topic although some previous reviews have described the potential applications and some general preparations of seaweed polysaccharides-based composites (Abdul Khalil, Saurabh, et al., 2017; Tavassoli-Kafrani et al., 2016). However, these reviews have not provided insight on the development of specific carrageenan-based film composites and, in particular, the techniques that are available to improve the physicomechanical properties of such materials. This review therefore considers the fundamental knowledge and current state of research into strategies to enhance the properties of carrageenan-based films with a focus on food packaging applications.

2.3 Carrageenan

2.3.1 Chemical Structures and Characteristics

Carrageenan is a type of hydrophilic linear sulfated galactan found specifically in the cell walls of red marine algae (*Rhodophyceae*), which is primarily extracted from the genus *Chondrus*, *Eucheuma*, *Gigartina* and *Iridaea* (Jouanneau et al., 2010; Nanaki et al., 2010). Carrageenan is of typically high molecular weight with an average molecular mass of around 100 to 1000 kDa. In addition to the ester sulfate and galactose groups as the main backbone in its structure, other polysaccharide residues and substituents are also present including glucose, xylose, uronic acids, methyl ethers, and pyruvate groups (Aldalbahi, 2012).

The sulfate groups on the disaccharide repeating unit determine the classification into one of three major carrageenan types: lambda (λ), kappa (κ), and iota (ι) (Al-Alawi et al., 2011) as depicted in **Fig. 2-1**. In general, κ-carrageenan containing about 25-30% of ester sulfate groups with 28-35% of 3,6-AG exhibits the strongest gelling ability followed by ι-carrageenan (28-30% of ester sulfate groups and 25-30% of 3,6-AG), and λ-carrageenan (32-39% of ester sulfate groups and no 3,6-AG content) (Barbeyron et al., 2000).



λ-carrageenan κ-carrageenan ι-carrageenan

Figure 2-1. Chemical structures of carrageenans (Cunha & Grenha, 2016)

In addition to the specific types of carrageenan described above, there are also some hybrid types. In nature, seaweeds generally do not produce pure carrageenan but more likely mixtures of different sulfated polysaccharides in their structures including hybrid carrageenans (van de Velde, 2008). The composition of carrageenans in a given seaweed is determined by several factors, such as its biological stage (Gómez-Ordóñez & Rupérez, 2011), gender of the gametophyte, environmental condition (Hilliou, 2014), as well as the extraction method used in carrageenan recovery (Larotonda et al., 2016; Souza et al., 2011). Hilliou (2014) reported that the vegetative phase of the *Gigartinales* seaweed order produces highly sulfated forms of μ-carrageenan, which is a non-gelling type, whereas in the reproductive life stage, κ- and ι- types of gelling carrageenan together with ν- and η-carrageenans are produced. Furthermore, in *Kappaphycus striatum* seaweed the young-age seaweed contains less-gelling carrageenan types than the matured seaweed (Hilliou, 2014).

2.3.2 Carrageenan Sources

The name carrageenan is derived from the Gaelic word, *carraigín* or “little rock” that has been used as early as 400 A.D. in Ireland. The first types of carrageenans were obtained from the "Irish moss" seaweed (*Chondus crispus*) which was used for animal feed and medicinal purposes (Loureiro, Cornish, et al., 2017; Loureiro, Hurtado, et al., 2017). During World War II, the world's demand for carrageenan noticeably increased particularly in relation to the food processing industries, with *Chondus crispus* and *Gigartina* seaweeds harvested from natural stock being the major sources. *Chondus crispus* grows naturally in Canada, Ireland, France, Spain and Portugal, and *Gigartina* beds are found in Southern Europe and South America (Valderrama et al., 2013). In the 1940's, Canada with its abundant natural beds of *Chondus crispus* had become the world's largest carrageenan-seaweed supplier which continued for almost three decades. However, the discovery of alternative carrageenan-seaweed species, *Eucheuma sp.* in the Philippines, followed by the success of its cultivation for large-scale farming production, has made this species a major source for carrageenan with the Philippines displacing Canada as the largest supplier of carrageenans. Indonesia soon followed and adopted successful seaweed farming from its neighbouring country such that in the last few decades it has surpassed the Philippines' carrageenan-seaweed production to become the leading global supplier of carrageenan, predominantly derived from the *Kappaphycus* and *Eucheuma* seaweeds (Hurtado et al., 2015; Valderrama et al., 2013). To date, around 90% of world's carrageenan is derived from these two species and their farming is now spreading worldwide including regions of South-east Asia, Pacific Islands, mainland of East Africa, South America, and the Central Americas (Hurtado et al., 2015). The success of carrageenan seaweed farming worldwide may also denote a sustainable resource of raw material stocks for future industrial production of carrageenan-based plastics.

Similar to land-based crops, however, carrageenan seaweed farming is also subjected to the challenges of nature *i.e.* herbivorous fish predation, stormy weather, parasite epiphyte, and outbreaks of the "ice-ice" disease. This disease, which presents as a white bleaching of the seaweed's thalli, is one of the biggest problems associated carrageenan seaweed farming. Outbreaks have been reported since the early years of *Kappaphycus* farming development in the Philippines and since then, the disease has been reported in almost all carrageenan seaweed farms worldwide (Hafting et al., 2015). This outbreak is recognized as the seaweed's physiological stress in responding to its surrounding environmental changes such as temperature and salinity fluctuations. However, it is commonly followed by pathogenic microbial infestations which cause complete necrosis and fragmentation of the infected thalli (Loureiro, Hurtado, et al., 2017). This, in turn, can cause a tremendous drop in seaweed production, as well as the quality of the carrageenan (Mendoza et al., 2002). To respond to this challenge, there is an urgent need to develop seaweed strains that are tolerant to fluctuations in marine environmental conditions, resistant to disease and produce greater carrageenan yields (Hayashi et al., 2017; Kim et al., 2017).

2.3.3 Production of Carrageenan

The extraction and processing of the three main types of carrageenan is very similar in principle. It invariably involves the removal of impurities, separation of the carrageenan from the aqueous extraction medium and other constituents, followed by drying. For the production of κ-carrageenan, raw material can be obtained from seaweed species including *Eucheuma*, *Chondrus*, *Hypnea*, *Iridaea* and *Furcellaria* (McHugh, 2003; Prajapati et al., 2014). For these species, the dried mass of seaweed is washed to remove all the impurities and other particulates attached to the surface of its thalli followed by a hot alkali treatment using solutions of NaOH or KOH to remove any remaining impurities. This treatment also increases the 3,6-AG content which subsequently increases the gel strength (Haug, 1967). The carrageenan is then boiled in water until it forms a slurry, after which the volume of the solution is reduced by evaporation before it is cooled and allowed to gel. Next, the gelled carrageenan is pressed to remove most of the water before it is further dried and milled into carrageenan powder.

Additional steps are performed for the *Eucheuma* and *Furcellaria* seaweed species, whereby precipitation using KCl solution is applied (Stanley, 1987). Following the hot water extraction step, the carrageenan slurry is extruded through spinnerets and collected in a container of cold KCl solution to form a gel before it is dehydrated, dried and powdered (Minghou, 1990). For the production of λ-carrageenan, *Gigartina* and *Chondrus* (Irish moss) seaweeds are commonly used (van de Velde & De Ruiter, 2002) and since λ-carrageenan does not characteristically form a gel, the dehydration process is usually performed using a drum dryer and/or alcohol precipitation with propanol or ethanol. However, thermal processing using a drum dryer may adversely impact the carrageenan quality, so alcohol precipitation is the more common industrial practice (Prajapati et al., 2014).

As the gelling strength of ι-carrageenan is not as high as κ-carrageenan, the filtrate of ι-carrageenan is not suitable for dehydration through the gel-pressing technique, thus precipitation in alcohol solution has been shown to be the best practice for its production (Prajapati et al., 2014). After hot extraction, the liquour extract is filtered through a fine filter, evaporated, and precipitated with isopropanol to get a fibrous carrageenan coagulum. This material is then pressed to remove the alcohol solvent before it is washed with water, dried, and milled to the required particle size (McHugh, 2003; Tavassoli-Kafrani et al., 2016).

2.4 Semi-Refined Carrageenan

The aforementioned methods describe the production of refined carrageenan, but in addition to these materials, semi-processed/semi-refined carrageenans are also extensively produced for various industries including use as additives in pet foods and dairy products (Dewi et al., 2012). According to the US Food and Drug Administration (US Food and Drug Administration, 2017), semi-refined carrageenan (SRC) can be acceptably referred to as carrageenan and there is no legal distinction between these two materials. However, in the European Union (EU), these materials are recognized as different substances and are thus regulated separately. In this case, the SRC is regulated under the name "Processed Eucheuma Seaweed (PES)" with the trade "E" number of E407a, whereas pure carrageenan is registered with the number E407 (EUR Lex, 2004).

During the production of SRC using *Eucheuma cottonii*, the seaweed is subjected to alkali treatment before it is dried and powdered (Anisuzzaman et al., 2013). In addition to removing the pigments, some proteins, and other impurities during the alkali treatment, the hydroxide ions penetrate the thallus and reduce the amount of ester sulfate groups in the structure. The 3,6-AG content is also increased, and this subsequently improves the gel strength (Anisuzzaman et al., 2013; Mustapha et al., 2011).

Compared with the refined carrageenan, SRC has a significantly lower price due to the reduced number of processing steps required in its production. Fewer resources are therefore required including significantly less water in order to produce the final SRC product. In addition, the alcohol precipitation and filtration of impurities steps are not required and there is also no need for a refrigeration process to freeze the gel as in the case of refined-carrageenan production (Dewi et al., 2012; Farhan & Hani, 2017). The water-soluble component in SRC is primarily κ-carrageenan (Jurasek & Phillips, 1998) and although this chemical species forms the main part of the physical structure, an insoluble cellulose component originating from the algae cell walls is retained (Gunning et al., 1998; Phillips, 1996). The cellulosic content together with other residual plant debris constitutes around 20-30% of the SRC, and this can impart undesirable optical properties to any films formed using this material (Ghosh et al., 2006).

2.5 Economic Perspectives

It is evident that seaweed-based film can offer many advantages that demonstrate its potential to be developed further into high-end commodities (Rajendran, Puppala, Sneha, et al., 2012). However, the pathway of carrageenan film development for commercial applications is still in its infancy with issues such as quality and economic feasibility of the end-products compared with conventional plastics or other available bio-based plastics in the market the main barriers to wider development and acceptance.

In general, cost effectiveness is the main hurdle to be overcome for the development of bio-based plastic materials through to the commercialization stage (Mekonnen et al., 2013) since production costs are generally more expensive than those of petroleum-based plastics (Changwichan et al., 2018; Song et al., 2009). Nonetheless, some unique properties of bio-based plastic materials may allow for future cost reductions, particularly in the end-of-life phase where bio-based materials can be more cost effective with regard to waste disposal management (van den Oever et al., 2017). There are also examples of other bio-polymer resins such as PLA and PHAs that are becoming more competitive in price compared to their petroleum-based counterparts in the global market. Additionally, the bio-based plastic price is relatively more stable than fossil-based plastics because biomass prices typically fluctuate much less than the oil price (van den Oever et al., 2017).

In comparison with other sources of bio-based plastic materials such as those derived from corn, cassava, sugarcane, and other terrestrial crops, seaweed may be a less expensive material since it does not require the use of arable lands, fresh water, and fertilizer or chemicals (Konda et al., 2015). However, the extraction process of carrageenan does require a large amount of chemicals and energy (Abdul Khalil, Tye, et al., 2017) which is the key drawback that must be overcome to achieve economic feasibility for industrial implementation. Nonetheless, it has been previously observed that the price of bio-based plastics becomes more competitive with its synthetic counterparts whenever the economics of large-scale production, conversion into products, and logistics become more favorable (van den Oever et al., 2017). For example, various single use-plastic products such as bottles, food tray and lid containers, overwraps, cups, and some flexible films made from PLA are now widely available in the market with competitive prices to those made from conventional plastics. This is because PLA and its end-products are currently manufactured on a large industrial scale with similar product processing technologies that are utilized for synthetic plastics (Muller et al., 2017). Similarly, the price of bio-poly(butylene succinate) (bio-PBS) has dropped from 4.00 €/kg to *ca*. 2.50 €/kg due to the production of succinic acid from biomass becoming more efficient with larger production volumes (van den Oever et al., 2017).

To achieve successful commercial production of carrageenan films in a way similar to PLA, bio-PBS, and other commercial bio-based polymers, significant innovative breakthroughs are needed across the whole production chain, starting from seaweed cultivation and harvesting, transport, carrageenan extraction, through to the manufacture and marketing of products. Another important consideration that compensates in part for the higher production costs is that carrageenan-based plastics can offer consumers significant overall environmental benefits compared with some other materials and such perspectives have previously been associated with growing market demands (DeGruson, 2016). End-products derived from carrageenan may also be developed for niche markets where slightly higher production costs may be more acceptable. Moreover, utilization of less expensive semi-refined carrageenan may also reduce production costs of films for various uses (Sedayu et al., 2018).

2.6 Carrageenan-Based Films

2.6.1 Gel Formation

In an aqueous solution, κ- and ι-carrageenan demonstrate a thermo-reversible sol-gel transition, whereas no gel state occurs in λ-carrageenan solutions (Yuguchi et al., 2002). Gelation of carrageenan has been described as a conformational transition of random coils into helical structures which aggregate during low temperature and/or by the presence of cations in the solution (Piculell, 1995; Rees et al., 1970). In the sol-phase, carrageenan conformation adopts a random coil formation whereby the anhydro-galactose sequences twist into a double helix at low temperature. Further aggregation also occurs among the twisted double helical parts, and these subsequently form a cross-linked domain creating an infinite network structure to accomplish gelation (Yuguchi et al., 2002). Moreover, since the repeating units of the ester sulfate groups are electrically charged, the cations have been found to contribute in the gelation process by mediating between the double helices (Morris et al., 1980). During this process, the transition kinetics are driven by the amount and molecular weight of the galactan, the types of cations involved, and the temperature of the solution (Piculell, 1995). In an aqueous solution, the transition of molecular conformation from random coils to double helices of κ- and ι-carrageenan occurs at 38°C and 45°C, respectively, which is well known as the coil-helix transition temperature (Nanaki et al., 2010; van de Velde & De Ruiter, 2005).

To generate a helical structure in κ- or ι-carrageenan solutions, a minimum degree of polymerization of around one hundred is required (Hjerde et al., 1998). Partially de-sulfated κ-carrageenan shows similar structural behavior to fully sulfated κ-carrageenan, except that its coil helix transition temperature is higher than that of the latter (Zhang et al., 1991; Zhang et al., 1994). By selecting the appropriate type and concentrations of salts in solution, various carrageenan helical behaviors can be manipulated, ranging from liquid-nematic crystals to double-helical rods and super-helical rods in gels (Piculell et al., 1997).

Iota-carrageenan is unable to form a liquid crystal structure which again reflects the difference in molecular behavior among the carrageenans (Lahaye, 2001). In general, κ- and ι-carrageenan are comprised of alternating 1,3 linked β-D-galactopyranose-4-sulfate and 1,4-linked 3,6-anhydro-D-galactopyranose units with different amounts of ester sulfate groups. The sulfate groups are positioned at the outside of the helix chains during conformational changes and the subsequent stabilization of this helix conformation depends on the hydrogen bonds formed between the chains (Makino et al., 2001). A hydrogel is then formed when surrounding water molecules penetrate the matrix (Nanaki et al., 2010).

Hybrid carrageenan structures possess properties that are distinct from those of the single polymeric counterparts (Souza et al., 2011). However, because of the profound gelling ability among the hybrid groups, most studies have focused on the κ/ι-hybrid carrageenans (Larotonda et al., 2016; Sánchez-García et al., 2010; Souza et al., 2011; van de Velde, 2008). Kappa/iota-hybrid carrageenans are sulfated polysaccharide structures consisting of blocks of κ- or ι-carrageenan disaccharide units distributed in their molecular chain (van de Velde, 2008; van de Velde & De Ruiter, 2005), and are usually found together with small amounts of non-gelling disaccharide units, such as μ- and/or ν-carrageenans (Souza et al., 2011). The structure and composition of the disaccharide units determines the conformational transition from the random coil state to the helical state of the polymer during gel formation as well as the functional polymer properties (Larotonda et al., 2016; van de Velde, 2008). It has been reported that κ/ι-hybrid carrageenans extracted from *Martocarpus stellatus* seaweed exhibit a gelling property which is intermediate between those of single κ- and ι-carrageenans gels. In contrast, λ-carrageenan produces only viscous solutions, without gel formation (de Araújo et al., 2011; Gu et al., 2005).

2.6.2 Film Formation and Characteristics

Compared to other bio-based films, the development of carrageenan-based films is still in its infancy. As such, the only reported film fabrication method to date is solvent casting which involves casting carrageenan gel then evaporating the solvent (water) to form a solid film. Typically, ι-carrageenan forms a clear, elastic, soft gel with no syneresis in the presence of calcium salts, whereas κ-carrageenan forms a strong, hard, brittle gel (Farhan & Hani, 2017; McHugh, 2003). The differences in gelling properties contribute to the properties of films or composites produced using these different types of carrageenan. For example, the three-dimensional structure of ι-carrageenan exhibits a dense, compact, and organized film structure (Hambleton et al., 2012). Edible films made of ι-carrageenan demonstrated good mechanical properties with the carrageenan stabilizing the emulsion in the polymer matrix (Hambleton et al., 2012). In packaging applications, such films may also exhibit decreased gas permeability to potentially impede surface dehydration and maintain the taste attributes of certain products such as fruits and cheeses.

Similarly, as in the case of ι-carrageenan, κ-carrageenan polymers exhibit a compact film structure (El-Fawal, 2014; Nanaki et al., 2010), and the high gelling ability of κ-carrageenan imparts excellent film-forming properties (Wu & Imai, 2012) with higher mechanical and water barrier properties, as well as greater transparency (Park, 1996; Paula et al., 2015). **Table 2-1** lists various functional properties of carrageenan-based films in comparison with other bio-based and synthetic films used in food packaging applications. These basic properties are amongst the most important properties that are commonly considered when developing materials for industrial applications and include the physico-mechanical properties of tensile strength (TS) and elongation at break (EAB), along with the functional properties of water vapor permeability (WVP) and gas permeability. Clearly, the development of novel packaging films requires the optimization of these properties to ensure the material can adequately protect the product quality and safety during storage until it is delivered to consumers (Lagaron, 2011).

Table 2-1. Comparison of carrageenan-based films physico-mechanical and functional properties with other bio-based and synthetic films

Film	**TS** /MPa	**EAB** /%	**WVP** /10^{11} g m^{-1} Pa^{-1} s^{-1}	**OP** /10^{12} cm^{3} m^{-1} Pa^{-1} s^{-1}	**References**
κ-carrageenan	42.5	3.9	7.5	5.2	Larotonda et al. (2016)
ι-carrageenan	2.5	1.04	36	-	Paula et al. (2015)
κ/ι-hybrid carrageenan	55.2	3.4	6.7	3.3	Larotonda et al. (2016)
SR κ-carrageenan	35.43[a]	10.14[a]	27.8[a]	1.67[b]	[a]Sedayu et al. (2018) [b]Farhan and Hani (2017)
SR ι-carrageenan	8.9	12.05	-	-	Aji et al. (2018)
PLA	15.2[a]	40.4[a]	2.47[a]	62[b]	[a]Li et al. (2017) [b]Tee et al. (2015)
PHB	35-50	2-4	0.79-0.95	-	Arrieta et al. (2017)
Cellophane	85.8	14.4	8.4	2.9	Larotonda et al. (2016)
Chitosan	60.7	3.3	4.5	10.4	Larotonda et al. (2016)
Whey protein	2.2	20	1,380	1.7	Larotonda et al. (2016)
Corn starch	47.4	3.6	18	0.15	Larotonda et al. (2016)
LDPE	7.85-34.5[a]	100-1330[a]	0.091[b]	21.6[b]	[a]MatWeb (2019) [b]Larotonda et al. (2016)
HDPE	11.6-228[a]	160-1010[a]	0.023[b]	4.9[b]	[a]MatWeb (2019) [b]Larotonda et al. (2016)

OP: Oxygen permeability; SR: semi-refined; LDPE: Low-density polyethylene; HDPE: High-density polyethylene

The data in **Table 2-1** shows that films produced from refined κ- or κ/ι-hybrid carrageenan exhibit superior properties among the other carrageenan films with higher TS and lower WVP values. Larotonda et al. (2016) reported that hybrid κ/ι-carrageenan films are less hygroscopic with higher oxygen and UV barrier performance than films made of κ-carrageenan. Moreover, SRC films show higher stretchability than those that are made of refined carrageenan. The overall TS of carrageenan films, with the exception of the ι- type, is also comparable with other commercial bio-based films and is in fact higher than those of PLA and synthetic plastic films, LDPE and HDPE. Nonetheless, carrageenan films have lower flexibility, particularly in comparison with the synthetic films and in the case of κ-carrageenan, a brittle film structure has also been reported (Cian et al., 2014; Zarina & Ahmad, 2015). This characteristic may limit the use of these materials for wider applications such as flexible thin films although κ-carrageenan may be suitable to be developed as a rigid packaging material.

High film barrier properties are vital for many food packaging applications, particularly in order to prevent the migration of low molecular weight chemical compounds such as gases and vapors (Lagaron, 2011). Similar to almost all bio-based films, carrageenan films have poor WVP which is a result of the large number of hydroxyl and sulfate groups in their structures rendering carrageenan films highly hydrophilic (Cunha & Grenha, 2016; Distantina et al., 2013; Roh & Shin, 2006), and this consequently imparts poor moisture barrier properties and a poor water resistance (Fabra et al., 2008). In contrast, carrageenan-based films have excellent oxygen barrier properties, which are superior to those of PLA and LDPE films.

2.7 Property Enhancement of Carrageenan-Based Films

Attempts to improve or manipulate carrageenan-based film properties have been investigated encompassing physical and/or chemical processes. For food packaging purposes, barrier property enhancement of the carrageenan films is the major motivation in addition to the enhancement of other functional properties such as mechanical, optical and AM properties. Various technical approaches have been used to obtain desirable properties of carrageenan-based film including blending, plasticizing and nanoparticle incorporation as described in the following section and summarized in **Table 2-2**.

Table 2-2. Property modifications of carrageenan-based films.

Carrageenan and other components	**Property Enhancements**	**Disadvantages**	**Reference(s)**
κ-carrageenan, alginate	Increased mechanical properties, thermal stability, optical properties		Ye et al. (2017)
κ-carrageenan, alginate	Improved water resistance, thermal stability		Xu et al. (2003)
κ-carrageenan, alginate	AM activity		Cha et al. (2002)
κ-carrageenan, cassava starch, LDPE	Increased Young's modulus, hardness		Prachayawarakorn and Pomdage (2014)
Carrageenan, starch	Increased shear stress, EAB, decreased WVP	Decreased TS	Abdou and Sorour (2014)
κ-carrageenan, rice starch	Improved barrier properties, increased T_g, mechanical properties		Larotonda et al. (2005)
κ-carrageenan, agar	Increased EAB, water resistance, decreased WVP	Decreased TS	Rhim (2012)
κ-carrageenan, agar, konjac glucomanan	Agar increased barrier properties, water resistance; konjac improved EAB, hydrophobicity	Agar, konjac glucomannan decreased optical properties, TS, konjac glucomannan decreased barrier properties	Rhim and Wang (2013)
Carboxymethyl κ-carrageenan, carboxymethyl cellulose	Increased mechanical properties		Rudhziah, Rani, et al. (2015)
κ-carrageenan, pea starch	Increased EAB	Decreased TS, thermal stability	Lafargue et al. (2007)
κ-, ι-, λ-carrageenan blends			Nanaki et al. (2010)
κ-carrageenan, locust bean gum	Increased thermal stability, mechanical properties	Increased WVP	Martins, Cerqueira, et al. (2012)
κ-carrageenan, pectin, mica flakes	Increased hydrophobicity, mica flakes alone specifically increased WVP and gas barrier properties		Alves et al. (2010); Alves et al. (2011)
κ-carrageenan, chitosan	Increased EAB	Rougher surface, increased WVP, decreased TS, water resistance	M. Shahbazi et al. (2016)
κ-carrageenan, chitosan	Increased water resistance		
κ-carrageenan, plant essential oils (*Zataria multiflora* Boiss, *Mentha pulegium*, *Satureja hortensis*)	Decreased WVP, higher stretchability, water resistance, and EAB, AM activity	Decreased transparency, TS	Shojaee-Aliabadi et al. (2013) Shojaee-Aliabadi, Hosseini, et al. (2014) Shojaee-Aliabadi, Mohammadifar, et al. (2014)
κ-carrageenan, essential oils (oregano & thyme)	Decreased WVP, increased water resistance, EAB, AM activity	Decreased transparency, TS	Soni et al. (2016b)

Carrageenan and other components	**Property Enhancements**	**Disadvantages**	**Reference(s)**
ι-carrageenan, fat, aroma compounds	Decreased permeability of aroma compounds, increased surface hydrophobicity	Rough microstructure, morphology	Hambleton et al. (2009)
ι-carrageenan, fat, emulsifier	Increased surface hydrophobicity	Rough microstructure, morphology	Karbowiak, Debeaufort, et al. (2006)
κ-carrageenan, latex	Increased TS, energy absorption		Machmud et al. (2013)
κ-carrageenan, cinnamon oil	Decreased WVP	Decreased mechanical properties	Praseptiangga et al. (2016)
κ-carrageenan, various plant oils (corn, soybean, olive, sunflower)	Decreased WVP, increased water resistance	Increased opacity, yellowish color, decreased TS	Rekemin and Abedin (2017)
κ-carrageenan, palmitic acid	Increased water resistance	Decreased mechanical properties	Wibowo et al. (2016)
κ/ι hybrid carrageenan, zein prolamine	Reduced WVP, improved TS, stretchability	Decreased water resistance, EAB	Sanchez-Garcia et al. (2010)
κ-carrageenan, potassium sorbate	AM activity		Choi et al. (2005)
κ-carrageenan, citric acid	AM activity against *E. coli*, *P. mirabilis*, *D. chrysanthemi*, *S. aureus*, but not *P. aeruginosa*	Decreased mechanical properties, hydrophobicity	El-Fawal (2014)
κ-carrageenan, nano-silver	Fungicidal activity to *Fusarium*, *penicillium spp*. & *Aspergillus niger*, higher thermal stability, increased TS	Decreased transparency, hydrophobicity	Fouda et al. (2015)
κ-carrageenan, grapefruit seed extract	AM activity, decreased UV light transmittance, increased EAB	Decreased barrier properties, optical properties, hydrophobicity, mechanical properties	Kanmani and Rhim (2014a)
κ-carrageenan/alginate, nisin, lysozyme, EDTA, grapefruit seed extract	AM activity	Decreased TS, EAB	Cha et al. (2002)
κ-carrageenan, zinc oxide	AM activity, increased hydrophobicity, EAB	Reduced optical properties, mechanical properties	Kanmani and Rhim (2014b)
Carrageenan, nanoparticles (zinc oxide, copper oxide)	Increased EAB, thermal stability, AM activity against *E. coli, L. monocytogenes*	Decreased TS	Oun and Rhim (2017)
Carrageenan, chitin nanofibrils	AM activity against *L. monocytogenes,* increased TS, EAB, thermal stability	Increased opacity, hydrophilicity	Shankar et al. (2015)
κ-carrageenan, essential oils	AM activity, increased flexibility, improved water barrier properties	Decreased TS, optical properties	
κ-carrageenan, nanoclay, silver	Reduced WVP, increased TS, EAB, thermal stability, hydrophobicity and AM activity		Rhim and Wang (2014) Rhim and Wang (2013)

Carrageenan and other components	**Property Enhancements**	**Disadvantages**	**Reference(s)**
κ-carrageenan, nanocellulose fibers	Reduced WVP and gas barrier properties, increased TS		Savadekar et al. (2012)
κ-carrageenan, chitin nanofibrils	Increased WVP, TS, AM activity, stiffness, hydrophobicity	Decreased EAB, transparency	Shankar et al. (2015)
κ-carrageenan, cellulose nano-crystals, nanoclay	Increased TS		Zakuwan et al. (2013)
κ-carrageenan, nanoclay	Increased TS, EAB, thermal stability, AM activity against *L. monocytogenes*	Rougher microstructure	Martins, Bourbon, et al. (2012)
κ-carrageenan, nanosilica	Increased mechanical properties, hydrophobicity, decreased WVP		Rane et al. (2014)
κ-carrageenan, nanoclay	Improved mechanical properties, barrier properties, hydrophobicity		Rhim (2012)
κ/ι hybrid carrageenan, cellulose nano whiskers	Increased water barrier properties, water resistance		Sánchez-García et al. (2010)
Carrageenan, agar, poly(lactic acid)	Increased WVP, TS, water resistance	Decreased gas barrier properties, EAB	Rhim (2013)

2.7.1 Blending Carrageenans

Blending carrageenans or hydrogel polymers with other polymeric materials is among the more simple and effective methods to obtain desirable film properties (Makhijani et al., 2015). The compatibility of the component polymers is a significant factor since the final properties of the film will be influenced by the conformational arrangement of the polymer chains. An ordered conformational structure creates a compact and stable network in the blended matrix, whereas disorder results in chain interactions with a greater level of space-occupancy by the chain coils. In this regard, the compatibility of blended materials with carrageenan polymers can be determined by investigating the miscibility and thermodynamic behavior (Shahbazi et al., 2017). However, the final properties of blended films comprised of carrageenan and other materials will vary depending on the types of materials added, the ratio of the polymers, and the degree of miscibility. Carrageenan polymers have been blended with a range of different polymers including starch, lipids, proteins, and other materials to obtain desired film properties (Fabra et al., 2008; Prachayawarakorn & Pomdage, 2014; Sanchez-Garcia et al., 2010).

Polysaccharides

Polysaccharides have been extensively explored for various film applications, and these are considered to be the most widely used biomaterials for both food and non-food purposes. A wide range of film properties can be obtained by combining different types of polysaccharides since these can demonstrate various intrinsic properties (Nisperos-Carriedo, 1994). Among the biopolymers, starch is the most popular resource used as a filler in polymer blending since it is less expensive than many other materials and is abundant in nature (Fakhouri et al., 2013). Starch consists of two main components: amylose and amylopectin, whereby the first structure is primarily linear and is crystalline with an average molecular weight around 500,000 Dalton, whereas the second is highly branched with very high molecular weight (Chandra & Rustgi, 1998; Hyang Aee et al., 1998; Rosa et al., 2005).

The properties of carrageenan-starch films are inherently influenced by the origin and specific properties of the starch such as the source material from which the starch was derived (rice, pea, cassava, etc.). Combining carrageenan with starch may improve the moisture sensitivity of the film and increase certain mechanical properties such as the elongation at break (EAB) and the shear stress, however, these increases only occur within a certain range of mixing ratios (Abdou & Sorour, 2014; Lafargue et al., 2007). The amount of starch in the mixture plays an important role in determining the mechanical properties since its native crystalline structure is disrupted and irreversibly swells to a remarkably larger size when it undergoes gelatinization (Ali Akbari Ghavimi et al., 2015). This phenomenon affects the film texture rendering it more brittle and may consequently decrease the tensile strength of the film (Abdou & Sorour, 2014; Prachayawarakorn & Pomdage, 2014).

In addition to starch, combining carrageenan with various sulfated polysaccharide polymers such as agar and alginate, or combining the different types of carrageenan (κ, λ and ι) have also been investigated considering these polymers are very similar in their chemical structure and also demonstrate a good degree of miscibility (Nanaki et al., 2010). Combining carrageenan with agar has been reported to exhibit an improvement in its moisture barrier properties and water resistance, but it lowered the optical and tensile properties (Rhim, 2012; Rhim & Wang, 2013). In another study, blending carrageenan with alginate resulted in an increase in its flexural properties and transparency, and also increased its thermal stability (Ye et al., 2017). The level of hydrophilicity from each of these polymer groups appears to impact the moisture transfer rate, water resistance as well as the mechanical properties of the products, and it is associated with the amount of polar sulfate groups in their molecular structures (Roh & Shin, 2006; Xu et al., 2003).

The blending of carrageenan with other polysaccharide-based polymers including carboxymethyl cellulose (CMC), konjac glucomanan, pectin, locust bean gum (LBG), and chitosan, has also been investigated in the production of various films (see **Table 2-2**). It is reported that the addition of LBG or CMC can significantly increase the mechanical strength of carrageenan films (Martins, Bourbon, et al., 2012; Rudhziah, Rani, et al., 2015), whereas pectin and konjac glucomanan can be added in the formulation to improve the water sensitivity and elongation properties of the films (Alves et al., 2006; Rhim & Wang, 2013).

In blends of carrageenan with chitosan, organic acids are usually involved in film preparation since chitosan can only be dissolved in organic or acidified solutions (Kim et al., 2006). As the interaction of carrageenan-chitosan in the polymer matrix is driven by electrostatic attraction forces between negatively charged carrageenan and the positively charged chitosan molecules, the final properties of carrageenan-chitosan films is affected by the types and concentrations of the organic solvents that are used (Park et al., 2001). The level of acidity of the solvents influences the electrostatic bonds since the protons from the acidic solvent protonate the sulfonate anionic groups of carrageenan, which can subsequently reduce the electrostatic interactions between the carrageenan and chitosan (Park et al., 2001).

Lipids

Hydrophobic polymers such as lipids have been frequently used in hydrogel films to typically improve barrier properties and water resistance (Hambleton et al., 2009). In food packaging applications, barrier properties play an important role as the mass transfer of various components such as water vapor, gas and flavor compounds can permeate through a membrane between the food and its surrounding environment, which leads to physico-chemical changes and ultimately to food deterioration (Karel & Lund, 2003). In the case of lipid-blended carrageenan films, the properties are strongly affected by their structure (bilayer formation) and the distribution of fat molecules within the matrix (Fabra et al., 2008). Among the groups of lipids used in carrageenan film production, plant oils appear to be the most popular compounds that are incorporated into the polymer matrix.

Microstructure morphological studies using scanning electron microscopy (SEM) have shown that pure carrageenan films form a smooth and compact surface area (Shojaee-Aliabadi et al., 2013), and that the moisture diffusion through a carrageenan film is mainly governed by the hydrophilic layer of the matrix. When lipids are added to the carrageenan matrix, lipid layers form within the polymer structure that can significantly decrease the water transfer (Karbowiak et al., 2011). Furthermore, Shojaee-Aliabadi et al. (2013) suggest that the hydrophobic dispersed phase in hydrocolloid matrices form a tortuous conformation and this obstructs the water vapor transmission through the film. A similar phenomenon was also found in the transfer rates of flavour compounds, whereby carrageenan films incorporated with fats exhibited a less uniform matrix, which appeared as a solid emulsion in which the hydrocolloid polymer matrix surrounded the lipid phase dispersion of fat globules in various dimensions (Karbowiak et al., 2011). The globule particles result in fewer open spaces in the matrix that would facilitate the diffusion of flavour compounds and this subsequently limits the permeability of the latter (Hambleton et al., 2009).

An increase in water resistance has also been reported after blending carrageenan with lipids (Karbowiak, Debeaufort, et al., 2006) and this may be due to a decrease in the hydrophilicity of the carrageenan matrix. The hydroxyl groups within the carrageenan may interact with the oil component of the lipid through non-covalent bonds, thus reducing the availability of the hydroxyl groups to interact with water molecules resulting in a more water-resistant film (Shojaee-Aliabadi et al., 2013; Torrieri et al., 2015). Conversely, incorporating lipids into the carrageenan matrix can also impair the mechanical properties of the carrageenan whereby the lipid structure may partially substitute the stronger hydrocolloid polymer network with weaker hydrocolloid-lipid interactions within the matrix. This may lead to a weakening of the mechanical properties of the film such as the TS (Rekemin & Abedin, 2017), although this is often accompanied by an increase in the EAB. This is possibly caused by the lipid generating a plasticizing effect in the polymer matrix by altering the strong interaction forces of the hydrocolloid polymer network with a lessening of polymer cohesion, which then enables chain mobility during film stretching (Atarés et al., 2010). In addition, the carrageenan-lipid polymer structure also influences the optical properties of the film which will generally increase with the addition of lipids in the formulation as a result of the lipid droplets in the polymer matrix contributing to a greater extent of light scattering. The extent of scattering also depends on the size of the droplets and the components in the dispersed phase of the polymer matrix (Sánchez-González et al., 2009; Shojaee-Aliabadi, Hosseini, et al., 2014)

Proteins

Although carrageenan blends with protein-based polymers are less common in the scientific literature, most investigations are aimed at improving the mechanical properties of the films. This ultimately results in poor water barrier and water resistance properties, since both components are highly hydrophilic (Fabra et al., 2008). Interestingly, the addition of zein into carrageenan film formulations studied by Sanchez-Garcia et al. (2010) not only exhibited improved mechanical properties but also profoundly decreased the WVP. Zein is a biopolymer from the prolamine-group that can be found in corn endosperm and it has a special feature of an unusually high resistance to water. A carrageenan-zein film plasticized with glycerol was found to exhibit strong adhesion between the two components however a phase separation within its polymer matrix was still identified suggesting that both components although not entirely miscible, are relatively compatible (Sanchez-Garcia et al., 2010). It was reported that the TS of the carrageenan-zein film was up to 72% greater than that of a pure carrageenan film, with 17% lower WVP, and the film was notably more water resistant than the plasticized pure carrageenan (Sanchez-Garcia et al., 2010).

Other Materials

Some other materials have been investigated in mixtures with carrageenan including poly(vinyl pyrrolidone) (PVP) (Fouda et al., 2015), mica flakes (Alves et al., 2010), and latex (Machmud et al., 2013; Norhazariah et al., 2016). Blended carrageenan-PVP polymer film plasticized with polyethylene glycol (PEG) showed a high transparency with a homogenous polymer matrix and good flexibility (Fouda et al., 2015). It has been suggested that blending PVP into hydrocolloid polymer systems can increase the hydrophobicity of the hydrocolloid (Çaykara et al., 2006). It has also been suggested that blending a crude carrageenan with latex derived from *Calostropis gigantean* enhances the tensile properties with a demonstrated higher TS compared with those of starch-based plastics (Machmud et al., 2013). Furthermore, the inclusion of mica flakes in carrageenan has been shown to enhance its barrier properties including gas and WVP by creating a tortuous structure (Alves et al., 2011).

2.7.2 Incorporation of Plasticizers

Although carrageenan is well known for its film forming ability with excellent optical properties, its native film exhibits brittleness that restricts its functionality for food packaging applications. The addition of plasticizers is therefore an essential requirement in the formulation to improve film flexibility and workability. Plasticizers are generally additive substances with low molecular weights that intercalate and disperse within the polymer network, disrupting hydrogen bonding, and spreading the polymer chains apart, which then increases the flexibility of the film (McHugh & Krochta, 1994). Plasticizers incorporated into the matrix should be compatible with the polymer structure and have similar solubility in the solvent used to optimize the intermolecular reactions. Moreover, to maintain the physio-mechanical properties of the film it is also important that the plasticizer does not evaporate from the film matrix (Wittaya, 2012b). Plasticizers derived from polyol groups including glycerol, sorbitol and poly(ethylene glycol) are the most commonly used in the production of hydrogel films. The strong molecular interaction between the polymer chains and plasticizer *via* hydrogen bonding is responsible for the mechanical strength improvement of the film (Khazaei et al., 2014; Sothornvit & Krochta, 2000). It has been reported that the addition of glycerol and sorbitol into carrageenan-based films enhances the flexibility, TS and EAB of the material (Farhan & Hani, 2017; Harumarani et al., 2016). However, other reports have suggested the opposite; that the incorporation of glycerol can result in a decrease of the TS of the final carrageenan film (Wibowo et al., 2016). This suggests that the type and amount of plasticizer critically influences the mechanical properties of the final product (Cao et al., 2009; Rahman & Brazel, 2004).

The selection of a plasticizer to modify the properties of a specific polymer system is usually based on its compatibility with the polymer system, the amount of it required in the formulation, the processing method used, the desired physical, thermal, and electrical properties of the final film products, and also cost considerations (Cao et al., 2009; Cheng et al., 2006). In addition to increasing the mechanical properties, incorporating plasticizer in the carrageenan film matrix may also enhance the optical properties. This is ascribed to the intermolecular spacing of the plasticizer within the polymer conformation, which enables more light to pass through the film (Jongjareonrak et al., 2005). This is also supported by several investigations reporting that higher amounts of plasticizer incorporated in a hydrocolloid polymer result in a smoother surface texture of the film, which subsequently benefits the optical properties (Farhan & Hani, 2017).

Conversely, the addition of plasticizers to a carrageenan polymer matrix may also increase its moisture sensitivity and WVP. This is due to the reorganization that occurs in the hydrocolloid polymer structure that increases the free volume and segmental motions of the polymer network, hence facilitating water molecules to permeate more easily through the membrane (Khazaei et al., 2014). In addition, the moisture transfer is also affected by the hydrophilic character of the added plasticizer whereby the more hydrophilic the plasticizer, the higher the rate of moisture transfer through the film (Wittaya, 2012b).

2.7.3 Incorporation of Antimicrobial Agents

The incorporation of AM compounds into polymeric packaging materials has become one of the most popular concepts in developing active packaging. The purpose of this approach is to protect food products from microbial growth, particularly from surface spoilage, thus prolonging the shelf-life, and maintaining the safety and quality of the foodstuff contained in the package (Choi et al., 2005; Hotchkiss, 1997). Such is the case with the development of carrageenan-based films where several AM agents have been incorporated into the matrix including organic acids (El-Fawal, 2014; Wibowo et al., 2016), essential oils (Shojaee-Aliabadi et al., 2013), natural extract AM compounds (Kanmani & Rhim, 2014a), and metallic oxides (Oun & Rhim, 2017).

Organic acids incorporated into carrageenan films have been shown to effectively inhibit microbial spoilage in packaged foods. The incorporation of citric, succinic or lactic acid in hydrocolloidal packaging film has demonstrated a bacteriocidal and bactiostatic action against *E. coli*, *P. mirablis*, *S. aureus* and some other pathogenic strains (El-Fawal, 2014; Rocha et al., 2014; Schreiber, 2012). Due to the non-volatility of most organic acids under food-storage conditions, the transition of the active compound from the film packaging to the food product can only take place by direct contact between the film and the food surface (Hauser et al., 2016). In such cases, edible films or coatings may be the best concept for this type of AM packaging. However, the incorporation of organic acids such as citric acid into the carrageenan polymer structure appears to lessen its mechanical properties along with its water resistance (El-Fawal, 2014; Fouda et al., 2015). Hauser et al. (2016) suggested that to obtain desirable properties of the films that incorporate organic acids, the compatibility of the organic acids with the polymer and its constituents, the thickness of the film, and the storage conditions all have to be taken into consideration. Potassium sorbate is another commonly used AM agent in carrageenan film packaging formulations (Cha & Chinnan, 2004; Flores et al., 2007), and an investigation by Choi et al. (2005) found that such films exhibit a similar potential for inhibiting selective bacteria, yeast and mould to the use of potassium sorbate alone.

Recent studies have reported an increasing interest in the use of natural AM compounds for use in active packaging such as plant extracts and essential oils (Kuorwel et al., 2011; Suppakul et al., 2003). Kanmani and Rhim (2014a) and Cha et al. (2002) investigated the addition of grapefruit seed extract (GSE) that has a high content of polyphenolic compounds into a κ-carrageenan film matrix and found the film to have an effective antibacterial effect against food-borne pathogens. The phenolic compounds in the GSE, however, impacted the optical properties of the film in that it imparted a yellowish colour to it. Nonetheless, it was reported that the colour change did not significantly impact the film transparency, and to the contrary, it notably increased the ultraviolet barrier property of the film. Other disadvantages were also found in relation to the mechanical properties and sensitivity of the film to moisture.

Various essential oils known to impart AM activity have also been incorporated into carrageenan-based films such as oregano, thyme, *Zataria multiflora Boiss*, *Mentha pulegium, Satureja hortensis*, and cinnamon oil (Praseptiangga et al., 2016; Shojaee-Aliabadi, Hosseini, et al., 2014; Shojaee-Aliabadi et al., 2013; Soni et al., 2016b), with these oils resulting in similar effects as GSE incorporation. However, the hydrophobicity of these compounds profoundly enhances the water resistance and moisture transfer properties of the films. Some AM peptides such as nisin and lysozyme have also been used in carrageenan film formulations, but each of these compounds appears to effectively inhibit only specific bacterial growth and imparts less antibacterial activity in comparison with other AM agents such as ethylenediaminetetraacetic acid (EDTA) and GSE (Cha et al., 2002). Furthermore, the AM activity of lysozyme is sensitive to high temperatures (Carrillo et al., 2014) and this impacts its performance in film packaging applications.

In addition to the wide range of organic AM compounds, the incorporation of inorganic materials in carrageenan films has been investigated due to their high performance in inhibiting food deterioration as well as their stability during high temperature treatment. Zinc oxide (ZnO), copper oxide (CuO), and silver nanoparticles (AgNPs) are among the inorganic compounds that have been used in AM carrageenan films. The incorporation of ZnO and CuO in carrageenan films has demonstrated a strong antibacterial activity against *E. coli* and *L. monocytogenes* and these have been notably observed to perform more effectively against the former Gram-negative bacterium (Oun & Rhim, 2017). Moreover, AgNPs were also shown to be effective in inhibiting pathogenic fungi growth including the growth of *Aspergillus* sp., *Penicillin* sp., and *Fusarium oxyforum* (Fouda et al., 2015). This antifungal activity may be attributed to the interaction between the positively charged metallic ions with the negatively-charged species of the membrane and other biomolecules (protein and DNA) of the microorganisms, which consequently alters the membrane and internal structures, and finally leads to bacterial death (Anitha et al., 2012). Similar to the incorporation of organic AM agents, these metallic compounds also diminish the mechanical and optical properties, but they can improve the thermal stability of the final carrageenan films (Fouda et al., 2015; Kanmani & Rhim, 2014b).

2.7.4 Reinforcement with Nanoparticles

The development of nanotechnologies in the field of biodegradable films has been a significant recent breakthrough. Reinforcing a biopolymer matrix with small amounts of nanomaterials can significantly enhance the physico-mechanical, thermal, and barrier properties, which can enable the development and extend the use of these materials in various applications including food packaging. Different types of nano-sized materials are frequently used in film packaging applications to improve the final properties of the packaging material. Considered below are the nanomaterials that have been used in carrageenan-based film production.

Nanoclays

Clay minerals are the major components of clay raw materials and these have a dominant platelet microstructure. The shape of individual layers depends on the type of clay which may be composed of two, three, or four tetrahedral silicates and octahedral hydroxide sheets (Ghadiri et al., 2015; Nazir et al., 2016). In carrageenan films, the main types of clays that have been investigated include mica (Sanchez-Garcia et al., 2010), and montmorillonite (Rhim, 2012; Shojaee-Aliabadi, Mohammadifar, et al., 2014) with the purpose of enhancing the functional properties of the resulting composites. Nanoclays have been widely used as nano-fillers as they are abundant, readily available, low cost, and are environmentally friendly materials (Jafarzadeh et al., 2016).

Nanoclay minerals are rigid fillers used to reinforce soft polymeric matrices and these generally increase the stiffness (Nazir et al., 2016), with agglomerated nanoclays typically decreasing the film’s flexural properties (Fu & Naguib, 2006). Carrageenan composites reinforced with nanoclays exhibit a remarkable improvement particularly in their mechanical and barrier properties compared with the base material alone (Shojaee-Aliabadi, Mohammadifar, et al., 2014). For example, the WVP of pure κ-carrageenan film decreased by around 26% after reinforcement with 5% (w/w) of nanoclay, and similar results have also been reported for the film prepared from carrageenan blended with other polysaccharides (Rhim & Wang, 2013; Shojaee-Aliabadi, Mohammadifar, et al., 2014). The enhancement of moisture barrier properties may have resulted from the dispersed clay layer subsequently increasing the tortuosity imposed by the matrix and the obstruction of the micro-paths within the polymer network (Rhim, 2011; Sorrentino et al., 2007a). In addition, reinforcing clay nanoparticles can also increase the gas barrier properties as well as the water resistance of carrageenan composites and this has been observed by contact angle and swelling analyses (Alves et al., 2010; Rane et al., 2014; Rhim, 2013). However, the latter properties are found to be highly influenced by the hydrophilicity of the materials that are used (Rane et al., 2014). If excessive amounts of nanoclays are incorporated within a polymer matrix, particle agglomeration can occur resulting in reduced barrier properties which can consequently impact the blocking mechanism (Sanchez-Garcia et al., 2010).

The inclusion of nanoclays within a carrageenan polymer matrix can significantly improve the mechanical strength of the sample films. This may be due to the strong interaction between the intercalated silicates and the polymer matrix through hydrogen or ionic bonds (Almasi et al., 2010; Lee & Kim, 2010). The distribution of intercalated clay particles with a high elastic modulus profile creates strong interfacial contact with the polymer network and enables effective stress transfer, finally resulting in a higher TS and Young's modulus (Alexandre & Dubois, 2000; Pavlidou & Papaspyrides, 2008; Sanchez-Garcia et al., 2010). Shojaee-Aliabadi, Mohammadifar, et al. (2014) reported that the incorporation of nanoclays up to a 10% (w/w) loading significantly increased the TS of carrageenan films and other investigations have also reported similar findings (Martins, Bourbon, et al., 2012; Zakuwan et al., 2013).

Due to its superior biodegradability, biocompatibility, as well as its economic advantages, carrageenan has been widely studied for use in a broad range of applications. Its hydrophilic characteristics along with high content of ester sulfate groups in its structure render carrageenan film suitable for edible food packaging, as well as for pharmacological, biomedical, and electrical applications. However, the hydrophilicity is also a major disadvantage, particularly in food containment applications where high moisture transfer and low water resistance of the film is of concern. Numerous physical and chemical techniques have been studied for the purpose of obtaining desirable film properties. Blending carrageenans with hydrophobic compounds, reinforcement with nanomaterials, or layering with other polymer films have resulted in significant enhancements to carrageenan film properties. These developments show that carrageenan possesses great potential for further development in the quest to produce eco-friendly, cost-effective food packaging materials.

Differences in the flexural properties of carrageenan films incorporated with nanoclay minerals have also been reported (Rhim & Wang, 2014) and instead of increasing the EAB some nanoclays have been shown to decrease the flexibility of the resultant film (Shojaee-Aliabadi, Mohammadifar, et al., 2014). This may be caused by different inherent characteristics of the various types of nanoclays with organically modified clays such as Cloisite®30B imparting higher hydrophobicity than the unmodified materials. The addition of such clays may result in stronger interfacial affinity and interactions between the clay and polymer matrix resulting in cross-linked networks that subsequently increase the flexural properties (Mirzataheri et al., 2010). In addition to the type of clay that is incorporated in the formulation, the degree of exfoliation/intercalation of the clay in the polymer matrix also substantially influences the mechanical properties of carrageenan films (Martins, Bourbon, et al., 2012).

Higher thermal stability of carrageenan films has also been obtained by the inclusion of nanoclays. The conformation of the carrageenan-nanoclay composite structure causes a more restricted thermal transmission through the silicate interlayers (Blumstein, 1965). The nanoclay structure may also act as an insulator and barrier to the transmission of volatile compounds that are produced during the decomposition of the composites (Kumar et al., 2010; Zulfiqar et al., 2008).

Nanocellulose

In comparison with the inorganic nano-materials that are used in carrageenan composites, cellulose nanoparticles facilitate some additional advantages including their sustainability and biodegradability in nature, lower density, and the ability to be incorporated into biopolymers using solvent casting methods (Angles & Dufresne, 2000; Kvien et al., 2005; Šturcová et al., 2005). Two major types of nanocellulose morphological structures have been applied in biopolymer matrices, namely cellulose-nanofibrils (CNFs) and cellulose-nanocrystals or nanowhiskers (CNCs). The CNCs have particle shapes like needle crystals that are 100-1000 nm in length and 4-25 nm in diameter and are obtained by removing most of the amorphous cellulose phase (Jonoobi et al., 2015; Xu et al., 2013a).

Similar to the incorporation of nanoclays, a relatively small amount of nanocellulose can effectively improve the mechanical properties of carrageenan films. Zakuwan et al. (2013) found that the optimum TS and Young’s modulus were obtained by reinforcing carrageenan with a 4% (w/w) of CNCs, with values of 36.8 and 1340 MPa respectively. This result was also supported by Zarina and Ahmad (2015), however, Savadekar et al. (2012) reported that the addition of only 0.4 % (w/w) CNFs produced the maximum TS value of the carrageenan film and any additional CNFs decreased the TS. These contrasting results may suggest that different cellulose structures may have different effects on the mechanical properties of carrageenan films. It appears that CNCs exhibit optimum effects on the tensile properties of carrageenan films at higher loadings in comparison with CNFs. An excessive amount of nanocellulose added in the formulation can lead to particle agglomeration within the carrageenan polymer matrix, which consequently reduces the mechanical properties of the final films (Sánchez-García et al., 2010). In addition, increasing the amount of these materials also detrimentally affects the opacity of the film (Abdollahi et al., 2013).

In general, nanocellulose reinforcement has been shown to produce typically similar effects on the physico-mechanical properties of carrageenan-based film as is produced by the nanoclays, with a demonstrated increase in water resistance, enhancement of the moisture and gas barrier properties, as well as an enhancement of the thermal stability of the film (Savadekar et al., 2012; Zarina & Ahmad, 2015). These properties are also affected by the degree of particle dispersion in the polymer matrix, as well as degree of crystallinity of the nanocellulose materials (Chen et al., 2009; Ljungberg et al., 2005; Luiz de Paula et al., 2011).

Chitin Nanofibers

Chitin is commonly extracted from the exoskeleton of marine crustaceans and insects, as well as the cell wall of fungi and yeast (Tharanathan & Kittur, 2003). Hence, its availability in nature is plentiful, and is the second most abundant biopolymer after cellulose (Azuma et al., 2014). The chitin structure in the crustacean exoskeletons forms microfibrillar arrangements wrapped by protein, and the chitin nanofibers (ChNFs) obtained from further chemical processing are usually a few micrometers in length and 10-100 nm in diameter (Li et al., 2016). Reinforcing carrageenan polymers with ChNFs results in smooth and flexible films with good homogenous dispersion of the fibres (Shankar et al., 2015). The addition up to 5% (w/w) ChNF into a carrageenan matrix can increase its tensile strength and Young’s modulus with a decrease in its WVP, however, this may cause a loss in flexural properties and transparency of the film. In addition to improving certain physico-mechanical properties, the inclusion of ChNF in carrageenan films can impart AM activity, particularly against the Gram-positive food-borne pathogens such as *Listeria monocytogenes* (Shankar et al., 2015).

2.7.5 Multilayer Carrageenan Films

Even though blending carrageenans with other biopolymers is considered to be the easiest and most effective method to improve the film properties, introducing synthetic hydrophobic polymers using this technique may not lead to desirable superior properties (Shojaee-Aliabadi et al., 2013). This is due to the high hydrophilicity of the carrageenan, which is immiscible with hydrophobic, synthetic polymers due to the low interfacial adhesion between the two phases in the matrix system (Avella et al., 2000). The hydrophilicity of carrageenan and other biopolymers is responsible for the inferior barrier properties and water sensitivity of these systems (Fabra et al., 2008) and this is of particular concern when these polymers are to be used for food packaging applications. The production of multilayer films may show potential for overcoming these limitations and as such films can be prepared without the problems of incompatibility that often exists when using blending methods (Rhim et al., 2006).

Research reported by Rhim (2013) demonstrated the effectiveness of multilayer carrageenan/agar film reinforced by nanoclays, which was laminated with PLA. The PLA layer appeared to firmly adhere to the carrageenan-based layer *via* hydrogen-bond interactions, and it increased the transparency of the film due to the high transparency of the PLA layer. Double-layer films (carrageenan/agar-PLA) and triple-layer (PLA-carrageenan/agar-PLA) of similar overall thickness exhibited an increase in the TS from 48.2 ± 4.5 MPa (carrageenan/agar alone) to 59.9 ± 0.5 and 50.7 ± 8.4 MPa respectively. However, the PLA significantly decreased the elongation properties from 26.3 ± 5.1% to 9.4 ± 2.6% and 17.9 ± 1.9% for the double-layer and triple-layer films respectively. Moreover, the PLA layer dramatically reduced the WVP of the carrageenan-based film more than 24-fold. Higher hydrophobicity was also shown by the increase in the contact angle values from 50° to 60° with the latter value comparable with those of synthetic plastic films such as low-density polyethylene (LDPE), Ecoflex™ and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (Rhim & Hong, 2007). Moreover, the PLA layer also significantly increased the water resistance and thermal stability of the carrageenan-based film (Rhim, 2013). Overall, these results demonstrate the effectiveness of using a multilayer approach to improve the barrier properties of carrageenan films, thereby rendering these systems suitable candidates for food packaging applications.

2.7.6 Crosslinking

Crosslinking through chemical or physical methods has been widely applied to enhance the properties of biodegradable films (Shahbazi et al., 2016). Crosslinking is a process of chain bonding within the chemical structure of the polymer to improve its properties. It can be performed using chemical reagents or a crosslinker with multifunctional groups that react with the functional groups of the modified polymer (Sonker et al., 2018). The formation of molecular chain linkages in the polymer matrix by crosslinking can efficiently limit the movement of polymer chains and increase the properties (Shuzhen et al. 2018).

The commonly performed physical crosslinking approaches include UV crosslinking (Gutiérrez & González, 2016; Liang et al., 2019), electron beam irradiation (Puhova et al., 2016; Shin & Han, 2013), and gamma ray crosslinking (Navarro et al., 2018). The chemical approach consists of using potassium or calcium salt crosslinking agents (Nickerson et al., 2004; Yuguchi et al., 2002), and genipin (Meena et al., 2007; Muhamad et al., 2011).

A report by (Paşcalău et al., 2012) shows that carrageenan/alginate films crosslinked using a calcium salt resulted in higher values of Young's modulus (E) and ultimate stress (σ_u) and the higher calcium incorporated films also demonstrated a greater flexibility as well as higher water resistance.

For physical crosslinking, UV irradiation is possibly the most versatile irradiation process used in the food industry as it is relatively inexpensive, favourable in large scale processing, and easy to operate (Zhou et al., 2008). Photo-crosslinking is generally performed by the photolysis of a sensitizer agent and in the absence of a sentisizer crosslinking will not occur (Miranda et al., 2001). One of the most efficient photosensitizers used for biodegradable composite modification is sodium benzoate, which is well known to be highly photolysed by UV radiation (Takakura et al., 1965). Moreover, the incorporation of sodium benzoate in a composite matrix will bring about benefit to its antimicrobial capacity which, of course, is favourable in food packaging applications. Furthermore, sodium benzoate is classified in the United States as Generally Recognized as Safe (GRAS) (Villarruel et al., 2015).

2.8 Applications of Carrageenan-Based Films

2.8.1 Edible Films and Coatings

Utilizing carrageenan for edible films and coatings covers a broad range of food industry applications such as fresh and frozen fish, meat, and poultry (Baldwin et al., 2011; Cha et al., 2002; Seol et al., 2009; Stuchell & krochta, 1995; Wu et al., 2000), various dried food products (Kampf & Nussinovitch, 2000), fruit coatings (Bico et al., 2009; Hamzah et al., 2013; Ribeiro et al., 2007), and microencapsulation (Bartkowiak & Hunkeler, 2001; Desai & Jin Park, 2005). The principal goal in these applications is the prevention of dehydration, undesired chemical reactions (Baldwin & Wood, 2006; Osorio et al., 2011; Valero et al., 2013), as well as the inhibition of spoilage and deterioration by microbial contamination (Arvanitoyannis, 2010; Soni et al., 2016b). Moreover, carrageenans can also be functionalized to improve the delivery and dispersion of food additives or to control the release of active agents, particularly in active packaging applications (Barreto et al., 2003; Campos et al., 2011; Soni et al., 2016b).

In the case of fresh-cut fruit, carrageenan coatings form an effective barrier between the cut surface of the fruit and gas from the surroundings thereby reducing respiration and minimizing discoloration (Baeza, 2007). When used as an edible coating, carrageenan films can maintain fruit texture and provide antibacterial protection during storage (Bico et al., 2009; Plotto et al., 2010). Although the use of hydrophobic substances such as waxes, resins, or water insoluble proteins imparts better moisture barrier properties to edible films or coatings, the application of water-soluble hydrocolloids usually results in better mechanical properties than those provided by the hydrophobic materials (Arvanitoyannis, 2010). In addition, water-soluble hydrocolloids also exhibit better gas barrier properties (Nussinovitch, 2009) due to their compact and tightly packed conformation that result from organized hydrogen bonded network structures (Atarés et al., 2011; Bonilla et al., 2012).

2.8.2 Pharmaceutical and Biomedical Applications

Carrageenan utilization in pharmaceutical and biomedical applications has been extensively investigated (Garcia & Ghaly, 2001; Hoffman, 2002; Park et al., 2001; Picker, 1999; Sankalia et al., 2006; Yamada et al., 2005), and it has been regulated recently in Europe, the United States and the United Kingdom (García-González et al., 2011; Li et al., 2014). The application of carrageenan-based films in these areas are primarily related to drug delivery systems and wound dressings.

In the area of drug delivery systems, a multilayered polymeric film is usually applied due to its ability to manipulate the release of active compounds. Incorporating pharmaceutical agents into a polymer film followed by organizing the layers of the film in a particular way may prevent the loss of the drug substances (Wang et al., 2007). Various multilayered films have been investigated to obtain the controlled release of active compounds which can be triggered by certain conditions such as temperature, pH, enzymes, or ionic strength (Pinheiro et al., 2012; Quinn & Caruso, 2004; Serizawa et al., 2002; Wood et al., 2005).

An early report by Nagai (1985) described that a multilayered drug-delivery films are typically comprised of two main components: carrier film(s) and barrier film(s). The carrier film contains the pharmaceutical or active compounds that are usually dissolved or dispersed homogenously in the polymer matrix. It was also reported that configuring the concentration of the active compounds to increase from the outer wall to the inner part of the film results in better control of the release of the compounds. The barrier film is joined to one surface of the carrier film to essentially "lock in" the active compounds and maintain stability until the release is triggered by certain conditions. Examples of this mode of delivery include film strips containing active ingredients such as mouth freshener, nutrients or herbs, caffeine, and pharmaceutical ingredients (Siah et al., 2015). Medicinal strips made from edible films containing drug substances can be administered without water, which is helpful for persons who have difficulty in swallowing tablets or capsules such as children and the elderly (Siah et al., 2015). In addition, the use of edible films for drug delivery may be convenient for administering prescribed drugs where different doses can be obtained by using different lengths of edible film (Nagai, 1985).

Another application of carrageenan films is wound healing aids (Boateng et al., 2013; Fouda et al., 2015; Lloyd et al., 1998; Pawar et al., 2013; Wu et al., 2001) with hydrocolloid films among the most widely applied due to their distinctive ability to adhere to both moist and dry surfaces (Heenan, 1998). These films have exhibited desirable properties in maintaining a moist environment, facilitating the drainage and evaporation of wound exudates, as well as allowing adequate gas exchange (Pawar et al., 2013; Sussman, 2010). Moreover, they have also been used for delivering pharmaceutical ingredients to wound surfaces and to oral cavities for medication purposes (Boateng et al., 2009; Cunha & Grenha, 2016; Zivanovic et al., 2007). In the preparation of these films, the polymer substances are usually cross-linked to enhance their water entrapping ability so that the films can highly absorb and retain the liquid upon contact with exuding wounds (Boateng et al., 2008).

2.8.3 Electrolytes/Electrically Conductive Materials

In addition to their environmental benefits, the high degree of compatibility with salts makes some biopolymers including carrageenan attractive in the development of electrically conductive materials (Barbucci et al., 2000; Rozali et al., 2012). The large number of hydroxyl groups in its molecular structure allows carrageenan to form cross-linking networks with other constituents, and substituents with other functional groups in polymer electrolytes (Yang et al., 2011). Finkenstadt (2005) reported that these polymers, with their extensive hydrogen bonding, usually exhibit a higher conductivity than those with a lesser extent of such bonding. Even though liquid electrolytes show better conductivity and the ability to transmit larger currents, flexible carrageenan-based films can be suitable for use in electrically conductive devices (Arof et al., 2010).

A study by Prasad and Kadokawa (2010) reported that the use of carrageenan-polymer film as the host polymer of two different ionic components, 1-(3-acryloyloxypropyl)-3-vinylimidazolium bromide and 1-vinyl-3-vinylbenzylimidazolium chloride, results in a material with good electrical conductivity and mechanical properties. It was further suggested that this composite has the potential to be applied for use in semiconductors or organic memory devices. Furthermore, the host polymer of the electrolyte composite which is prepared by blending carboxymethyl κ-carrageenan and carboxymethyl cellulose could also enhance the conductive properties of the material and showed favourable characteristics for fabrication of dye-sensitized solar cells (Rudhziah, Ahmad, et al., 2015). Another novel, electrical conductive device involving carrageenan-based composites is the microbial fuel cell which offers economic benefits compared with the commonly used polymer material, poly(pyrrole) (Esmaeili et al., 2014).

2.9 Conclusions

Due to its superior biodegradability, biocompatibility, as well as its economic advantages, carrageenan has been widely studied for use in a broad range of applications. Its hydrophilic characteristics along with high content of ester sulfate groups in its structure render carrageenan film suitable for edible food packaging, as well as for pharmacological, biomedical, and electrical applications. However, the hydrophilicity is also a major disadvantage, particularly in food containment applications where high moisture transfer and low water resistance of the film is of concern. Numerous physical and chemical techniques have been studied for the purpose of obtaining desirable film properties. Blending carrageenans with hydrophobic compounds, reinforcement with nanomaterials, or layering with other polymer films have resulted in significant enhancements to carrageenan film properties. These developments show that carrageenan possesses great potential for further development in the quest to produce eco-friendly, cost-effective food packaging materials.

CHAPTER 3

Influence of Glycerol on Semi-refined Carrageenan Film Properties

3.1 Overview

This chapter presents an investigation of SRC film preparation using a solution casting method, which involves the incorporation of glycerol as a plasticizer to reduce the inherent brittleness of the original SRC film, and subsequently to enhance its mechanical properties. In addition to this, the investigation is also conducted to obtain the optimum loading of glycerol in the SRC polymer film which is then used in all formulations in subsequent experimental works. The effects of glycerol incorporation on the optical and physical properties, as well as thermal stability of the resultant film are discussed.

A manuscript entitled: “Characterization of Semi-refined Carrageenan-Based Film for Primary Food Packaging Purposes”, Sedayu, B. B., Cran, M. J., Bigger, S. W., has been published in the *Journal of Polymers and the Environment*, **26**, 3754–3761, 2018. doi: https://doi.org/10.1007/s10924-018-1255-y.

3.2 Introduction

The majority of previous studies on the development of carrageenan-based film have utilized a refined carrageenan (RC) (Farhan & Hani, 2017; Paula et al., 2015; Rhim & Wang, 2013; Zarina & Ahmad, 2015), 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 (Farhan & Hani, 2017). By omitting these steps, SRC thus contains a cellulose residue that produces a less transparent gel product compared with the refined material (Ghosh et al., 2006). 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 (Zarina & Ahmad, 2015), which consequently limits their potential use for food packaging applications. Plasticizers are therefore typically added to the formulations to improve their flexibility (Vieira et al., 2011) and glycerol, a non-volatile polyol, is one of the more common plasticizers incorporated into carrageenan films to enhance their flexibility and processability (Farhan & Hani, 2017; Paula et al., 2015; Rhim & Wang, 2013). The amount of plasticizer added to carrageenan films must be carefully optimized in order to obtain the best overall film properties.

Among the reported studies on various plasticizers used in conjunction with natural biopolymers, glycerol is the most widely applied specifically in the preparation of carrageenan-based composites because of its compatibility, high retention as well as nontoxic characteristics (Farhan & Hani, 2017; Harumarani et al., 2016; Shankar et al., 2015). It is also known that glycerol is able to facilitate high flexibility to the formulated composite (Tian et al., 2018). Based on preliminary experiments published in the literature (Farhan & Hani, 2017; Shojaee-Aliabadi et al., 2014; Zarina & Ahmad, 2014), in the current investigation a variation of 0 – 50% (w/w) glycerol was chosen as the optimum amount incorporated in the carrageenan polymer film.

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.

3.3 Materials and Methods

3.3.1 Materials

The SRC used in this study was a commercial food-grade, semi-refined κ-carrageenan, E407a (W-Hydrocolloids Inc., The Philippines) derived from the red seaweed species, *Eucheuma cottonii*. The powder size of the SRC is < 89 μm with moisture content of 12% (w/w) and pH 8-11. Its average water gel strength is 300 g/cm^2 (1.5% (w/v) at 20°C) and the average potassium gel strength is 400 g/cm^2 (1% (w/v) at 20°C). Heavy metal impurity content is: Pb (≤ 5 ppm), As (≤ 3 ppm), Hg (≤ 1 ppm) and Cd (≤ 2 ppm). The total rough impurity content (mainly residual cellulose and other elements (Phillips, 1996) is *ca*. 21% (w/w) as determined after filtration through Whatman Grade 4 filter paper. The melting point range observed by DSC analysis using Mettler Toledo DSC-1 thermal analyser under a nitrogen atmosphere is *ca.* 163–185°C (see

App.1-S1 in the Appendix), and the percentage crystallinity measured using a Rigaku Miniflex 600 diffractometer over a 2θ range of 5−40° was found to be 47%.

Similar to other polysaccharide polymers, the weight average molecular weight, $<M_w>$, of carrageenan varies depending upon the seaweed species, geographical condition, and SRC production process (Phillips, 1996; Ramu Ganesan et al., 2018). The typical $<M_w>$ of SRC extracted from *Eucheuma cottonii* obtained from The Philippines waters is *ca.* 614 kDa. Typically, such carrageenans have an approximate chemical composition of galactose (50.1 mol%), 3.6–anhydrogalactose (43.6 mol%), glucose (4.3 mol%), 6–O–methylgalactose (0.9 mol%), xylose (0.9 mol%), and mannose (0.2 mol%) (Phillips, 1996).

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.

3.3.2 Film Preparation

Semi-refined carrageenan films were prepared using a casting technique (Rhim, 2012), 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 × 18 × 2.5 cm), and allowed to dry at room temperature for 36 h.

Film 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).

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 λ = 550 nm using a UV-visible spectrophotometer (Biochrom Libra S12). Three rectangular samples (14 mm × 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. (2009) 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 λ = 550 nm, and x is the thickness of the film sample (mm). The units of opacity are presented as absorbance units (AU) mm^{-1}.

Moisture Content

The moisture content of the film samples was measured according to the method described by Farhan and Hani (2017). 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.

Water Vapor Permeability

The WVP of the films was determined according to a method by Sobral et al. (2001). 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/tA was calculated from the slope of the weight gain *versus* time plot.

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 of 40-280°C at a heating rate of 10°C min^{-1}, with a nitrogen flow rate of 20 mL min^{-1}.

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 crosshead 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.

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 10^{TM} software.

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.

3.3.3 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. $p < 0.05$).

3.4 Results and Discussion

3.4.1 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 3-1**. 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 3-1**). Similar transparency results have been reported in previous studies (Farahnaky et al., 2013; Farhan & Hani, 2017; Sanyang et al., 2016) 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 (Jongjareonrak et al., 2005). In comparison, RC films have been reported to be more transparent, whiter films with minimal yellowness (Martins, Cerqueira, et al., 2012; Rhim, 2012). 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 (Masarin et al., 2016), which would otherwise obstruct the penetration of light through the film.

Table 3-1. 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 ± 0.42^{ab}	-0.79 ± 0.04^{a}	10.33 ± 0.79^{c}	16.57 ± 0.40^{a}	67.4 ± 1.8 ^{a}
10	86.30 ± 0.27^{ab}	-0.86 ± 0.04^{b}	10.94 ± 0.60^{c}	15.61 ± 0.78^{b}	68.3 ± 0.6 ^{a}
20	85.95 ± 0.56^{a}	-0.87 ± 0.04^{b}	10.98 ± 1.08^{c}	14.24 ± 0.69^{c}	72.2 ± 3.3 ^{b}
30	86.50 ± 0.23^{bc}	-0.93 ± 0.02^{c}	9.55 ± 0.42^{b}	11.74 ± 0.50^{d}	80.7 ± 1.2 ^{c}
40	86.71 ± 0.38^{c}	-1.01 ± 0.05^{d}	8.87 ± 0.59^{b}	10.56 ± 0.22^{e}	83.6 ± 0.4 ^{c}
50	87.14 ± 0.40^{d}	-1.07 ± 0.03^{e}	8.07 ± 0.77^{a}	10.22 ± 0.34^{e}	88.9 ± 1.1 ^{d}

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.

The thickness of the films also increased with the incorporation of glycerol as shown in **Table 3-1**. This is not unexpected since plasticizers may restructure the intermolecular polymer chains by increasing the free volume of the film matrix (Sanyang et al., 2016). The increasing film thickness may also be explained by the increase in the interstitial spacing between the polymer chains within the film matrix (Jongjareonrak et al., 2005).

3.4.2 Film Moisture Content and Water Vapor Permeability

As shown in **Fig. 3-1(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 (Farahnaky et al., 2013; Sothornvit & Krochta, 2000). Similar results have also been reported for previous carrageenan-based films as well as in the production of other water-soluble polymer films (Karbowiak, Hervet, et al., 2006; Martins, Cerqueira, et al., 2012). Moreover, Karbowiak, Hervet, et al. (2006) 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. 3-1(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.

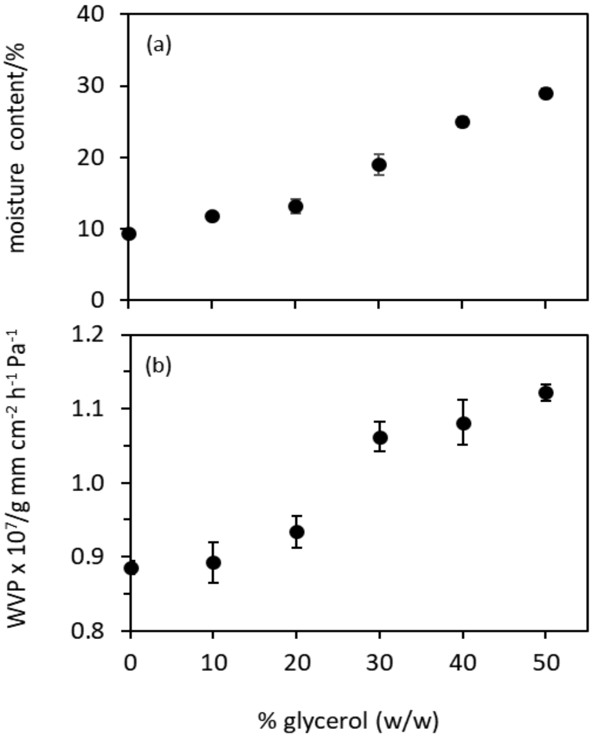


Figure 3-1. Effect of glycerol content on SRC film moisture content (a) and WVP (b)

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 (Rivero et al., 2016) and gelatin (Sobral et al., 2001). 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 (Rivero et al., 2016).

3.4.3 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. 3-2**, 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.

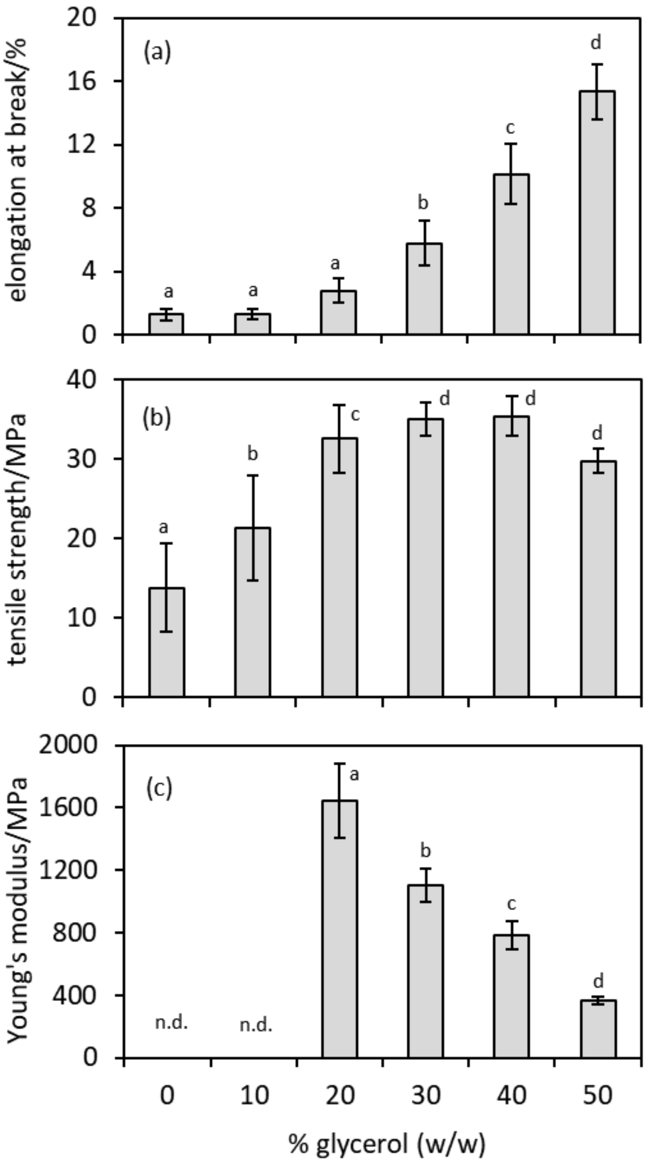


Figure 3-2. Effect of glycerol content on SRC film EAB (a), TS (b), and YM (c). Different letters between data bars represent significant differences, n.d. = not determined

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 (2000) 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 (Bertuzzi et al., 2012), 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 (Sanyang et al., 2016).

In addition to the TS and EAB, **Fig. 3-2** also shows the influence of glycerol addition on the YM of the film samples (**Fig. 3-2(c)**). For zero and 10% (w/w) glycerol content, the YM was not determined since the film was too brittle and easily cracked during the testing 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 (Farahnaky et al., 2013). Overall, the results of the present study indicate that the plasticized SRC films have good overall mechanical strength but with lower flexibility.

3.4.4 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. 3-3**. 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 (Paşcalău et al., 2012). 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 (Aranilla et al., 2012). 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–SO_3 bonds of the D-galactose-4-sulfate respectively which represent the typical bonds found in κ-carrageenan (Paşcalău et al., 2012; Paula et al., 2015). 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 (Kongjao et al., 2010). 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.

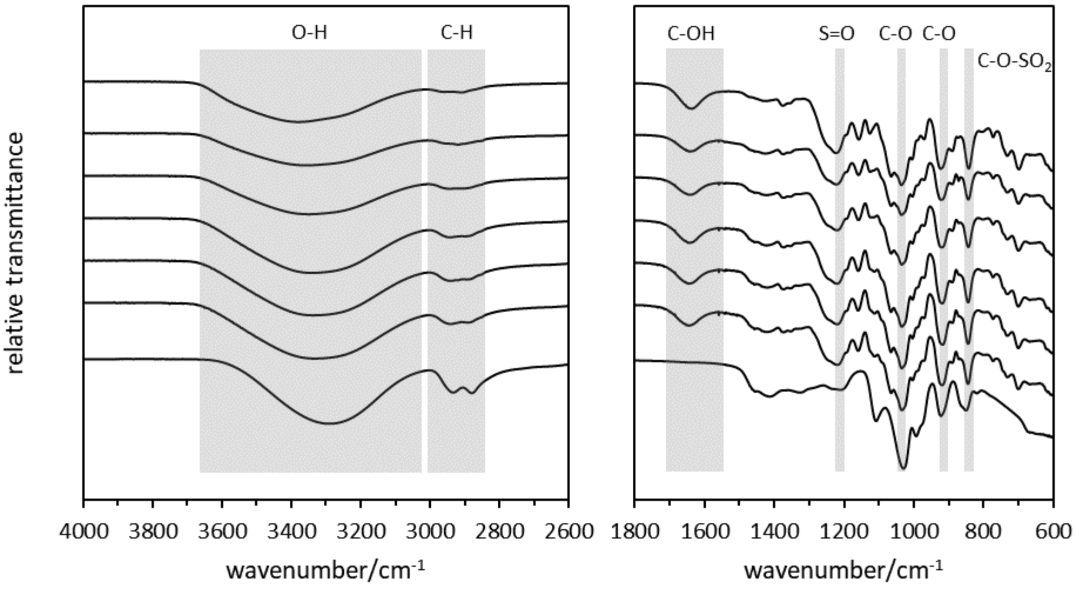


Figure 3-3. FT-IR spectra of SRC films with increasing glycerol content

3.4.5 Thermal Properties

The thermal transitions of SRC films incorporated with different glycerol concentrations were investigated using DSC as shown in **Fig. 3-4**. 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 (Rezaei & Motamedzadegan, 2015; van Soest et al., 1996).

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 (Xue et al., 2017).

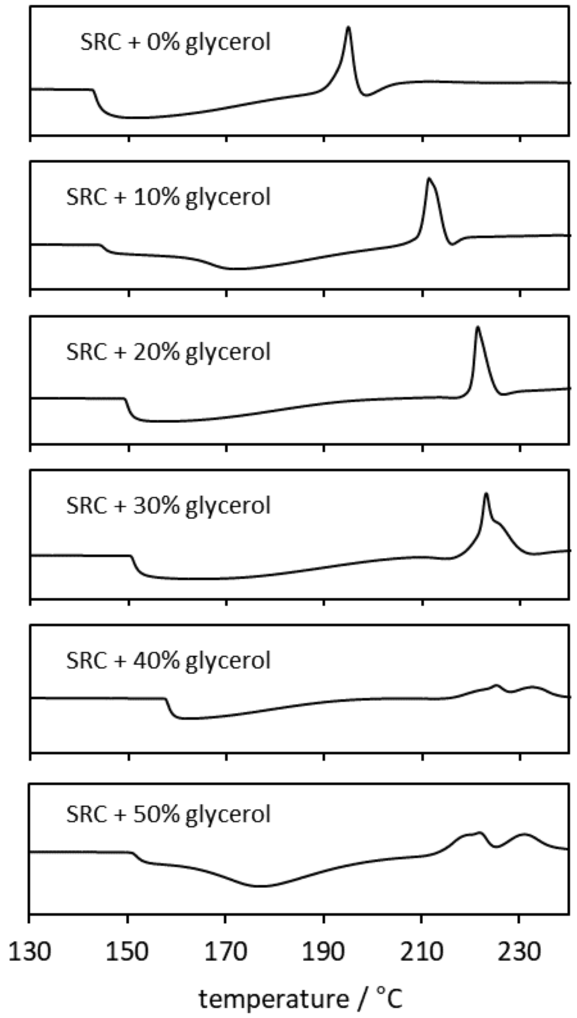


Figure 3-4. DSC thermograms of SRC films as a function of glycerol content

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 (Hejri et al., 2012; Pang et al., 2013).

3.4.6 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. 3-5**. 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 (Jiang et al., 2016; Zhang et al., 2015). Additionally, some platelets (*ca*. 10 µm dia.) can also be seen in the micrographs as indicated by arrows (see **Fig. 3-5**) 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 (Paşcalău et al., 2012).

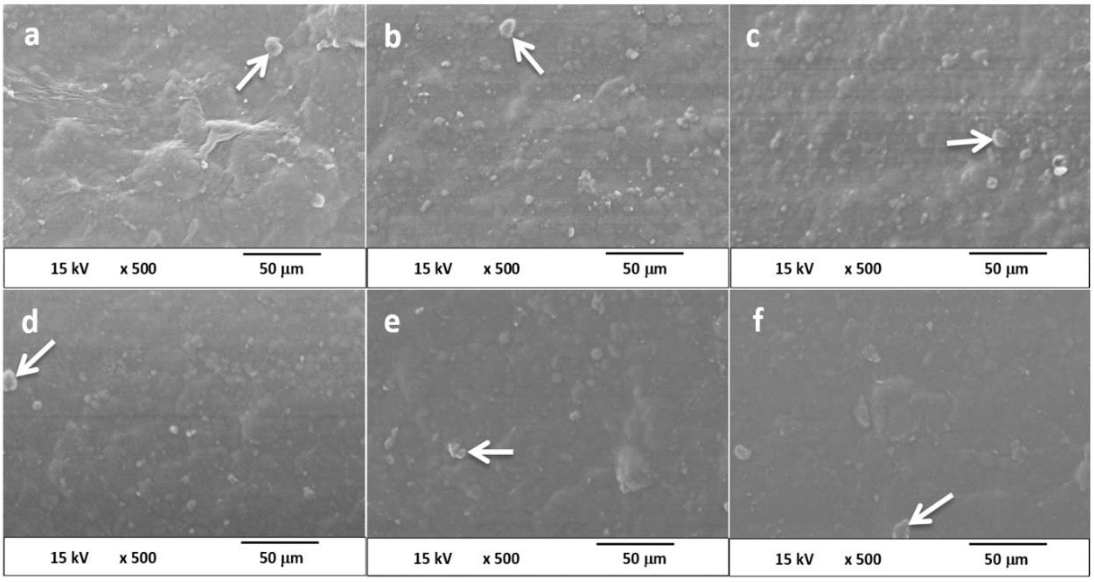


Figure 3-5. SEM micrographs (500× magnification) of SRC films incorporated with glycerol: (a) 0 %, (b) 10 %, (c) 20 %, (d) 30 %, (e) 40 %, (f) 50 %.

3.5 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 similar biopolymer 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.

CHAPTER 4

Nanocellulose Reinforcement to Improve Film Properties

4.1 Overview

This chapter presents an investigation of the nanocellulose fibril (NCF) reinforcement of semi-refined carrageenan (SRC) polymer films against a benchmark of similar films made from refined carrageenan (RC). The effects of the NCF reinforcement at various concentrations on the water sensitivity, physicomechanical and thermal properties of both SRC and RC polymer films is presented and discussed comprehensively.

The manuscript entitled: “Reinforcement of Refined and Semi-Refined Carrageenan Film with Nanocellulose”, Sedayu, B. B., Cran, M. J., Bigger, S. W., has been published in *Polymers, 12*(5). doi: https://dx.doi.org/10.3390%2Fpolym12051145.

4.2 Introduction

During the carrageenan production process, a large amount of the feedstock (*ca*. 60-70% w/w) ends up as solid waste. More than one third of the seaweed waste biomass from the carrageenan industry (SWBC) is comprised of cellulose with a small amount of lignin (4.5% w/w) and hemicellulose (4.5% w/w) (Uju et al., 2015). The cellulose component can be recovered from the SWBC and processed further into nanocellulose (NC) (Chen et al., 2016; Feng et al., 2015; Liu et al., 2017; Uju et al., 2015). The NC can potentially be reutilized in the production of carrageenan or other bio-based films and it may therefore be possible to obtain enhanced film properties in addition to gaining further environmental benefits to the carrageenan industry. Indeed, the successful recovery of cellulose from SWBC and the production of NC from seaweed have also been recently reported (Chen et al., 2016; Feng et al., 2015; Gao et al., 2018; Liu et al., 2017; Uju et al., 2015).

Carrageenan-based films show some desirable properties, particularly for the development of packaging films. However, the inherent hydrophilic nature of these films is a major issue that is responsible for the poor moisture barrier properties and low water resistance of the film (Sedayu et al., 2019) and this is also observed in the case of other bio-based polymer films such as those based on starch and chitosan (Khan et al., 2012). Since good moisture and barrier properties are required to protect and maintain many packaged foods, one technique to improve these properties involves reinforcement using nanocellulose fibrils (NCF) (Lopes et al., 2018; Savadekar et al., 2012; Xu et al., 2013b). In comparison with the other type of nanocellulose *i.e.* cellulose nanocrystal/nanowhisker (CNC/CNW) that are used for composite reinforcement, NCF's are longer, have a higher aspect ratio (length to diameter) and contain more amorphous regions in their molecular structure (Abitbol et al., 2016) facilitating greater mechanical strength and flexibility (Xu et al., 2013b), and also exhibit high optical clarity (Carla et al., 2017). More importantly, the processing of NCF's is considerably cheaper than that of the CNC/CNW (Dufresne, 2012).

In synthetic polymer composites, NC inclusion in the film matrix is reported to render more ecofriendly, light-weight composites (Hubbe et al., 2017), whereas in bio-based polymers, NC inclusion has been reported to enhance the barrier, mechanical, and thermal properties of the resultant films (El Achaby et al., 2018; Teixeira et al., 2011). Although previous studies have reported some positive outcomes of NC inclusion into RC films (Savadekar et al., 2012; Yadav & Chiu, 2019; Zakuwan & Ahmad, 2018), no studies have been conducted using SRC to the best of our knowledge. Even though the main component in SRC, namely κ-carrageenan, is the same as that in conventional RC, the overall composition of these two products have shown quite distinct molecular structures particularly in regard to the presence of insoluble components in their polymer matrices (Jurasek & Phillips, 1998; Takigami et al., 2000).

In view of the potential to utilize the less expensive SRC in composite film production, the present investigation aims to observe the effects of NC reinforcement on the properties of SRC film. A comparison with RC film reinforced with NC is presented with an emphasis on the evaluation of the physicomechanical and barrier properties. The range of NCF concentrations used to reinforce the SRC and RC polymer films i.e. 1, 3, 5, 7% (w/w) was selected in accordance with typical concentrations used in previous research studies (Abdul Khalil et al., 2017; Yadav & Chiu, 2019; Zakuwan & Ahmad, 2018; Zarina & Ahmad, 2014).

4.3 Materials and Methods

The semi-refined carrageenan used in this investigation was similar to the material described in **Sec. 3.3.1**.

Refined carrageenan (RC), type 22048 κ-carrageenan, was purchased from Sigma Aldrich (Australia). This κ-carrageenan has moisture content of 5.7% (w/w) with viscosity of 5.2 mPas in 0.3% (w/v) water at 25°C. Reported by Dul et al. (2015), the $\langle M_w \rangle$ of this material is 672 kDa with sulfate content of 20.3% and pH 6.7–7.2. The melting point range determined using the same method to that used above for the SRC is *ca.* 176–190°C.

Nanocellulose fibrils (NCF) that were used to reinforce the films were obtained from Nanocellulose Pty Ltd (Australia). Glycerol (99%) and KNO_3 ($\geq$ 99.0%) were purchased from Sigma-Aldrich (Australia), and $Mg(NO_3)_2$ was purchased from Ajax Finechem (Australia). Milli-Q water was used in the preparation of the films and subsequent experiments.

4.3.1 Film Preparation

The SRC film preparation was performed following the method by Sedayu et al. (2018) with slight modification in which 5 g of SRC was initially dissolved in 250 mL water and was stirred vigorously for 15 min to dissolve the powder. Heating was applied until a temperature of 90°C was attained, and the solution was stirred at this temperature for 30 min. During heating, glycerol was added to the solution to obtain a 40% (w/w) mixture of glycerol in SRC. The mixture was then cooled for 5 min at room temperature to remove any air bubbles. A 40 mL aliquot of the resultant SRC solution was then poured onto an acrylic casting tray (200 × 150 × 3 mm) and allowed to dry at ambient temperature (*ca*. 22°C) for 36 h before the film was peeled away from the tray.

To prepare the NCF incorporated SRC films, various loadings of NCFs (i.e. 1%, 3%, 5% and 7% (w/w)) relative to SRC were used. In each case, the corresponding mass of NCF was firstly dispersed in 150 mL of distilled water by vigorous stirring for 15 min followed by shearing for 10 min at 20,500 rpm using a Unidrive X1000 homogenizer (CAT, Germany). The homogenized solution was then sonicated at 50 Hz and 80% amplitude using an Ultrasonic processor UP400S (Hielcher, Germany).

Separately, for each of the formulated samples, 5 g of SRC was dispersed in 100 mL of water by vigorous stirring for 15 min. The two prepared solutions were then mixed and stirred for a further 15 min along with the temperature being raised to 90°C and maintained for 30 min. The addition of glycerol and the casting procedure was the same as that used above in the preparation of the SRC film.

All the RC films and RC/NCF films were prepared in accordance with the same procedures as above. Before all film samples were subjected to further testing, they were conditioned for 48 h at 22°C in a 53% RH environment by placing them in a desiccator containing saturated $Mg(NO_3)_2$.

4.3.2 Physical, Optical and Structural Properties

A digital gauge micrometer (DML, UK) was used to measure the thickness of the film samples with a precision of 0.001 mm. A minimum of five measurements were taken and averaged with the results later used in subsequent property calculations.

The surface color and opacity of the film samples, structural properties (FTIR), and statistical data analysis were performed according to the methods described in **Sec. 3.3.2**.

4.3.3 Surface Morphology

A Hitachi Tabletop TM-3030Plus scanning electron microscope (SEM) was used to observe microstructural images of the surface of the films in backscattering mode using an accelerating voltage of 5 kV under a low vacuum. Prior to imaging, the samples were coated with iridium using a Cressington (Great Britain) sputter coater.

4.3.4 Moisture Uptake, Water Resistance and Moisture Barrier

The moisture uptake was determined gravimetrically in accordance with the method devised by Rhim and Wang (2013) using a rectangular 50 mm × 25 mm film sample. Prior to measurement, the film samples were dried in an oven at 60°C for 48 h. The moisture uptake was then determined by placing the films in a desiccator containing a saturated solution of KNO_3 to obtain a 98% RH environment. The desiccator was then placed in controlled temperature conditions at 25°C for 24 h and the moisture uptake was calculated based on the mass change before and after exposure to the high humidity.

The moisture content and WVP of the films were determined following the method described in **Sec. 3.3.2**.

4.3.5 Water Solubility

To measure the percentage water solubility (WS), a gravimetric analysis was performed in accordance with a method by Rhim and Wang (2013) with slight modification. A square piece of film (12.5 mm^2) was firstly dried in an oven at 60°C for 48 h to obtain the initial dry mass (W_0), and immediately placed in a 50 mL tube containing 30 mL of water at 25°C. The tube was then placed in a shaker water bath (Ratek SWB20D, Australia) to maintain the temperature at 25°C with a constant gentle shaking for 30 min. The undissolved film was carefully removed and its surface gently blotted dry with a tissue before it was dried in an oven at 105°C for 24 h to obtain the final mass (W_F). The value of WS of the film was calculated using the following equation:

$$WS\ (\%) = ((W_0 - W_F)/W_0) \times 100$$

4.3.6 Water Contact Angle

Water contact angle (WCA) measurements were taken to evaluate the wettability or hydrophobicity of the surfaces of the films. The measurements of WCA were performed using a drop shape analyser DSA30S (Kruss, Germany). Samples of film were adhered to a glass slide with double-sided tape to obtain a flat film surface for the measurement. A 4 μL water droplet was placed onto the film surface using a micro-syringe and the WCA was determined by measuring the angle between the horizontal baseline of the droplet and the tangent at the droplet boundary. The angle was obtained using the Advance 1.6.1.0 (Kruss, Germany) software.

4.3.7 Mechanical Properties

The tensile strength (TS), elastic modulus (EM) and elongation at break (EB) of the sample films were tested using an Instron Universal Testing Machine (Model 4301) with a 5 kN load cell and in accordance with the ASTM D882 (2012) method. Testing was performed on a minimum of four film strips (100 mm × 15 mm) taken from each sample. A crosshead speed of 10 mm min^{-1} was used with a gauge length of 50 mm. The mechanical properties evaluations and calculations were obtained by using BlueHill Series IX software.

4.3.8 Thermal Analysis

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) experiments were performed using a Mettler Toledo TGA/DSC1 and a Mettler Toledo DSC-1 thermal analyzer (Schwarzenbach, Switzerland) respectively to evaluate the thermal properties of the sample films. For the TGA analyses, samples of 8-12 mg were weighed, placed in alumina crucibles and heated from 30 to 400°C at a heating rate of 10°C min^{-1} under a nitrogen atmosphere (20 mL min^{-1} flow rate). The DSC measurements were performed by loading a 5-10 mg of sample into a 40 µL aluminium crucible that was hermetically sealed prior to heating from 50 to 320°C at a heating rate of 10°C min^{-1} under a nitrogen atmosphere (20 mL min^{-1} flow rate). An empty crucible was used as the reference.

4.3.9 X-ray Diffraction

The X-ray diffraction (XRD) patterns of the SRC and RC film samples were measured using a benchtop X-ray diffractometer (Rigaku Miniflex 600). For each measurement, a rectangular piece of film was mounted on a sample glass slide and the diffraction spectrum was recorded using Cu Kα radiation ($\lambda = 0.154$ nm) at 40 kV and 20 mA over the 3–40° range.

4.4 Results and Discussion

4.4.1 Physical and Optical Properties

During the process of film casting, the same volumes of SRC and RC solutions were poured into the casting trays. This resulted in the formation of SRC films that were *ca.* 40% thicker than the those of the RC films (see **Table 4-1**), however, this was not unexpected since the SRC contains residual insoluble solid particles that are not present in the RC. The addition of 1% to 7% (w/w) of NCF to the SRC and RC films did not significantly affect the resulting film thicknesses with the exception, perhaps of the SRC film containing 7% (w/w) of NCF which appears to be slightly thinner than the others in its series. It is expected that the range of loadings of NCF used and/or its dimensions and compatibility in relation to the polymer matrix are such that the additive will not affect the thickness of the resultant films prepared under the conditions used in this study.

As expected, the moisture content increased with film thickness (see **Fig. 4-1,** see also **App.1-S2**), and a similar observation has also been found in the case of SRC films incorporated with glycerol (Sedayu et al., 2018). From the obtained linear regression of those two parameters (moisture content *vs* film thickness) we can assume that the inherent moisture content of the SRC/NCF film found to be around 3.36%, which is much lower than that of the RC/NCF of *ca.* 21.14%. Moreover, we can also find that the SRC film seems greatly more sensitive to the NCF addition in compared with the RC film suggesting a more hygroscopic characteristic of the SRC film.

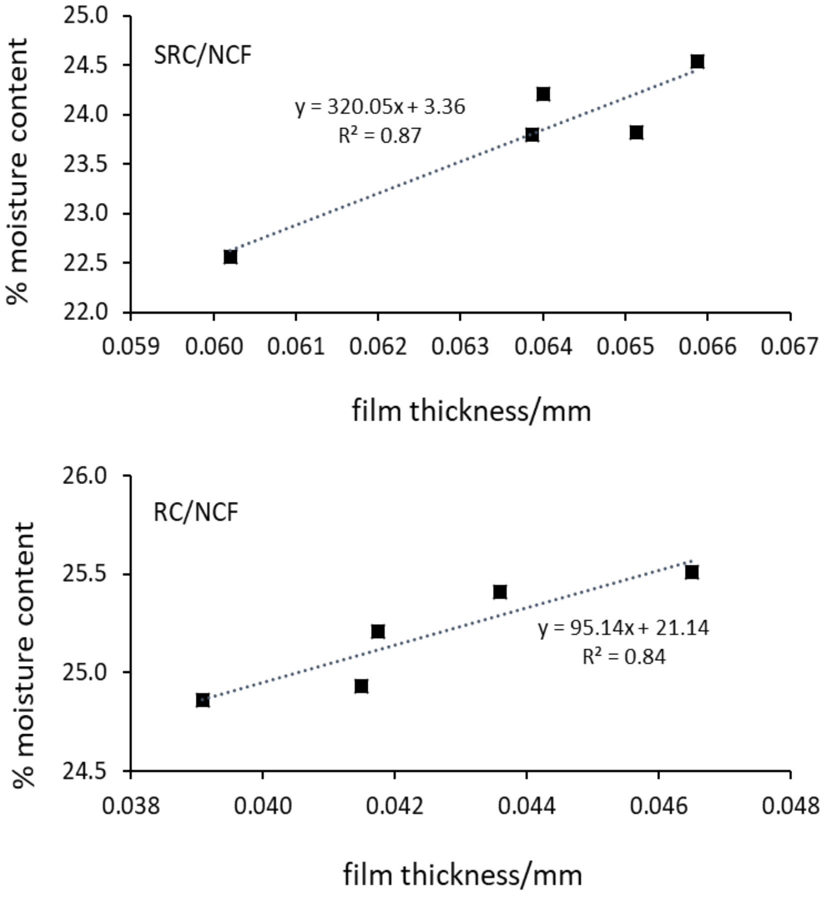


Figure 4-1. Plots of moisture content against film thickness for the SRC/NCF and RC/NCF films.

Figure 4-2 shows plots of the moisture content against the NCF loading for the SRC/NCF and RC/NCF formulations. Interestingly, the two materials show opposite trends with the moisture content of the SRC/NCF formulation decreasing and that of the RC/NCF slightly increasing with increasing NCF loading. The downward trend in the moisture content of the SRC/NCF formulations continued over the range of NCF loadings that were tested with no threshold value being observed. The RC/NCF formulations seemed to reach a plateau at ca. 5% (w/w) NCF loading. The observed decrease in the moisture content of the SRC/NCF formulation may be due to the NCF displacing water molecules within the voids in the SRC structure. The RC material, on the other hand appears to have a considerably higher inherent water content and it is possible that the addition of NCF to it may attract further water molecules through hydrogen bonding with the NCF particles. Clearly, in the case of the SRC material it appears the latter effect is overshadowed by the propensity of the material to replace water molecules in its voids with NCF.

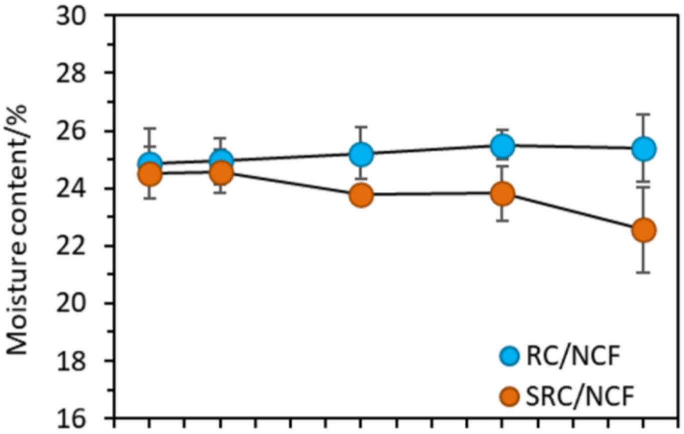


Figure 4-2. Plot of moisture content against NCF loading for the SRC/NCF and RC/NCF films.

Table 4-1 lists the color properties of the SRC/NCF and RC/NCF films in terms of the lightness (L^*), a^* and b^* values. The presence of solid residues in the SRC film formulations resulted in lower lightness values than those observed for the RC films. Unlike the RC/NCF films that were comparatively colourless and transparent, the SRC/NCF films were more red/yellowish in appearance with higher a^* and b^* values that are attributable to some of the residual components in SRC such as cellulose, minerals, and insoluble aromatic compounds (Masarin et al., 2016). The incorporation of NCF into the formulation contributed to an increase in the redness of the SRC and, in general, slightly reduced the lightness of the SRC/NCF and RC/NCF films.

Table 4-1. Color properties of the SRC and RC films

Film samples	*L**		*a**		*b**	
	SRC	**RC**	**SRC**	**RC**	**SRC**	**RC**
NCF 0%	88.19 ± 0.17^{a}	94.68 ± 0.22^{A}	0.12 ± 0.03^{a}	-0.51 ± 0.09^{A}	7.60 ± 0.21^{a}	4.53 ± 0.13^{A}
NCF 1%	88.06 ± 0.22^{b}	94.49 ± 0.17^{B}	0.28 ± 0.04^{b}	-0.70 ± 0.02^{B}	5.94 ± 0.29^{bc}	4.93 ± 0.10^{B}
NCF 3%	88.29 ± 0.22^{b}	94.39 ± 0.16^{BC}	0.23 ± 0.05^{c}	-0.61 ± 0.25^{AB}	5.71 ± 0.29^{b}	4.87 ± 0.11^{B}
NCF 5%	88.07 ± 0.35^{b}	94.41 ± 0.10^{B}	0.25 ± 0.04^{c}	-0.70 ± 0.02^{B}	6.06 ± 0.50^{c}	4.90 ± 0.07^{B}
NCF 7%	88.12 ± 0.38^{b}	94.27 ± 0.13^{C}	0.23 ± 0.03^{c}	-0.67 ± 0.02^{B}	6.17 ± 0.48^{c}	4.88 ± 0.06^{B}

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

The presence of residual components in the SRC/NCF films also contributed to the greater opacity than that of the RC/NCF formulation, and the opacity in the case of both the SRC/NCF and RC/CNF formulations was further increased with the addition of higher levels of NCF due to the dispersed NCF particles obstructing the passage of light through the films (see **Fig.4-3**). Previous investigations on other biopolymers have reported similar results in that nanocellulose distribution in the matrix has lowered film clarity (Dehnad et al., 2014; Oun & Rhim, 2015; Pereda et al., 2011).

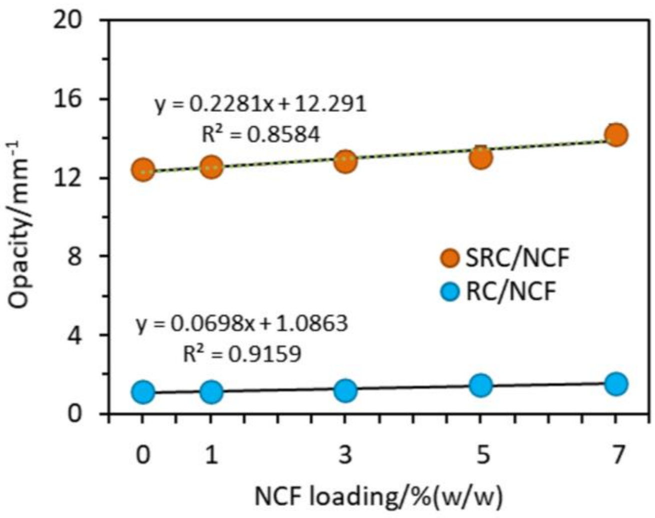


Figure 4-3. Plot of opacity against NCF loading for the SRC/NCF and RC/NCF films.

In **Fig. 4-3**, opacity is plotted versus NCF loading to show the trend of apparent absorbance of the formulated films. Adding a given amount of NCF to SRC produces a slightly greater increase in the opacity of the resulting film than in the case of the RC/NCF (see also **App. 1-S3**). This is an interesting result possibly due to the NCF particles may for some reason induce a greater extent of crystallization in the matrix of the SRC in the presence of the impurities than they do in the RC/NCF film formulation, which in turn reduces the light scattering (De Santis & Pantani, 2013; Raymond & Ronca, 2017).

4.4.2 Water Sensitivity and Moisture Barrier Properties

In food packaging applications, a certain level of water resistance is crucial to prevent the packaging material from disintegration when in contact with high moisture-containing foods such as fruits and meat (Yadav & Chiu, 2019). The moisture sensitivity and barrier properties shown in **Fig. 4-4** suggest that in general, the SRC/NCF film formulations are more sensitive to water than the RC/NCF formulations. This may be due to the impurities within the matrix of the SRC that disrupt and/or weaken the intermolecular carrageenan chain structure in the matrix thus enabling greater water ingress (Jamróz et al., 2019). As a consequence, the SRC/NCF films are more susceptible to high moisture environments compared with the RC/NCF films.

Figure 4-4(a) shows the WS values against the NCF loading for the SRC/NCF and RC/NCF film formulations. The WS values exhibit a general downward trend with increasing NCF loading in the case of the SRC. However, in the case of the RC material the downward trend appears to be halted at a NCF threshold (*i.e.* optimum loading in this case) of *ca*. 4-5% (w/w) with a possible upward trend thereafter suggesting that agglomeration of the NCF may occur in this material at sufficiently high levels (Santos et al., 2016). Such agglomeration may disrupt the coherence of the polymer matrix thereby facilitating the ingress of water (Jamróz et al., 2019). The continued downward trend in WS values at high NCF loadings observed in the case of the SRC material suggests that the NCF may be occupying voids created in the material by the impurities that are present and that a threshold loading of NCF with respect to the WS parameter was not reached. The reduction in WS by NCF incorporation may be due to the presence of relatively stable cellulose polymers that do not readily dissolve in water (Lan et al., 2011), it consequently reduced "swellability" and insolubility of the reinforced film in water, also attributed to lower absorption in the surrounding water (Kumar & Singh, 2008). Similar findings have been reported for the NCF reinforcement of starch and carrageenan (Lopes et al., 2018).

The moisture uptake data plotted in **Fig. 4-4(b)** shows a similar downward trend with increased NCF loading for both the SRC/NCF and RC/NCF formulations as was observed in the case of the WS measurements. However, the SRC/NCF formulations are inherently more hygroscopic than the RC/NCF formulations and a levelling out in the trend of the moisture uptake values at higher NCF loadings above *ca*. 5% (w/w) is once again observed in the case of the RC/NCF formulations. There appears to be a levelling out of the moisture uptake values for the SRC/NCF commencing at *ca*. 5% (w/w) NCF loading, however the data suggest that further improvement may have been reached at a level of 7% (w/w) loading.

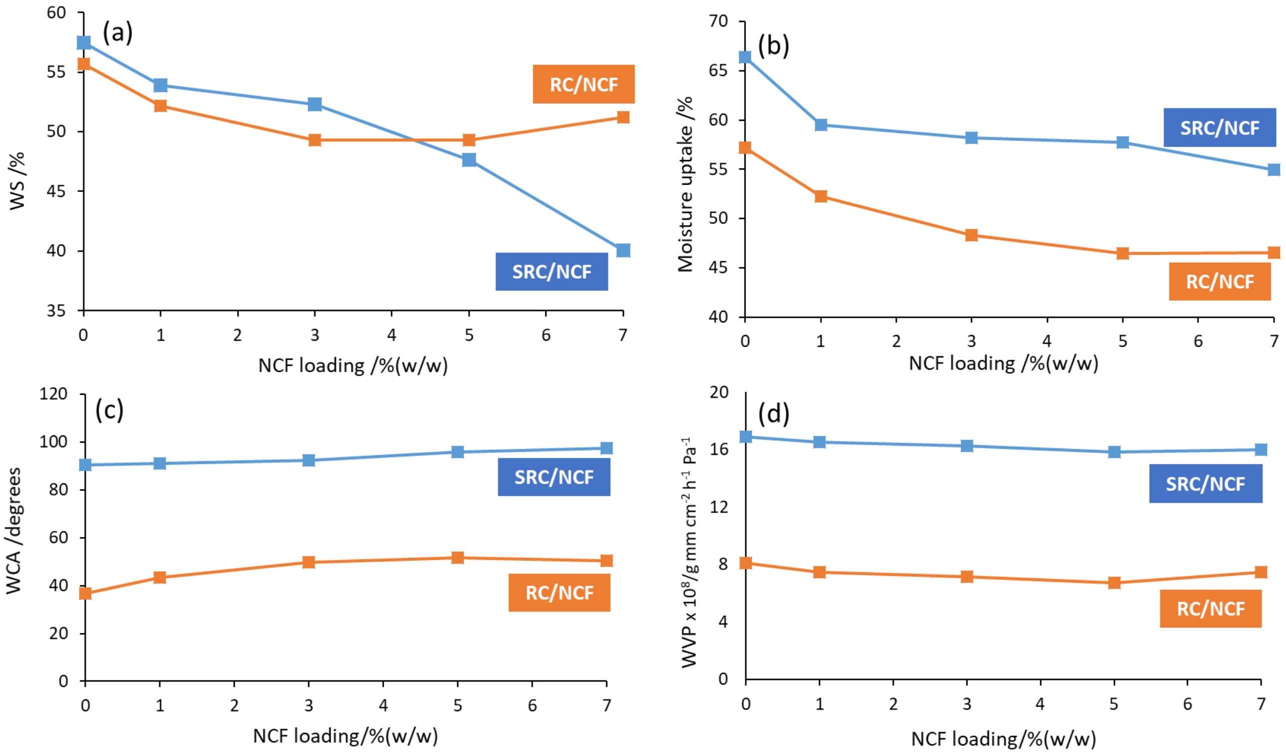


Figure 4-4. Plots of: (a) WS, (b) moisture uptake, (c) WCA and (d) WVP against NCF loading for the SRC/NCF and RC/NCF film formulations.

Figure 4-4(c) shows plots of the WCA values against the NCF loading for the SRC/NCF and RC/NCF formulations. The SRC/NCF films exhibit consistently higher WCA values than those of the RC/NCF formulations suggesting the surface of the SRC/NCF films are more hydrophobic. The randomly distributed impurities in the SRC that are mainly comprised of undissolved particulate cellulose and some minerals may disrupt the interaction between water and the substrate at the interface. The plot also shows the levelling off in the WCA commencing at the higher NCF loadings (*ca*. 5% (w/w)) and is therefore consistent with the data plotted for this formulation in **Figs. 4-1(a)** and **4-1(b)**.

Shown in **Fig. 4-4(d)** are plots of the WVP values against the NCF loading for the film formulations. The WVP values of the SRC/NCF films are about double those of the RC/NCF films. This may be due to the impurities in SRC that create pores or voids which can facilitate the penetration of water molecules through the film (Sampath et al., 2016). The data are also consistent with the higher water solubility, moisture uptake and hygroscopic nature exhibited by the SRC/NCF formulations compared with the RC/NCF formulations.

Upon considering overall the water sensitivity and moisture barrier results shown in **Fig. 4-4**, the incorporation of NCF in the formulation was found to improve the water sensitivity of the films at loadings up to *ca*. 7% (w/w) in the SRC polymer whereas the maximum effect was observed at *ca*. 5% (w/w) loading in the case of the RC polymer. With the addition and increased loading of NCF, the moisture uptake and WS decreased and the WCA increased the case of both the SRC/NCF and RC/NCF films. These results were found to be significant ($p < 0.05$) in all cases at NCF levels above 1% (w/w).

The threshold NCF loadings in the SRC/NCF and RC/NCF formulations resulted in respective decreases in the WS of 30% and 11%, decreases in the moisture uptake of 17% and 19%, increases in the WCA of 8% and 40%. For food packaging purposes, the WVP should be as low as possible to prevent the transfer of water molecules from the food to the environment and vice versa in order to maintain the quality of the packaged food. In addition to the improvement in the water sensitivity of SRC and RC films imparted by NCF inclusion, a decrease in the WVP was observed for both film formulations with a maximum reduction obtained at a level of 5% (w/w) NCF. This loading decreased the WVP of the SRC/NCF and RC/NCF films by *ca*. 6% and 17% respectively. The inclusion of NCF may hinder the diffusion of water molecules through the polymer matrix as a result of the tortuous blocking caused by the impermeable part of the cellulose particles in the matrix (Lopes et al., 2018). The reduction in WVP upon NCF inclusion observed in the current study is consistent with other investigations on various biocomposites utilizing NC reinforcement (Dehnad et al., 2014; El Miri et al., 2015; Khan et al., 2012).

The enhanced properties of SRC/NCF and RC/NCF films may be further explained by the interaction between the hydroxyl groups of NCF with the hydroxyl and/or carboxyl groups in the carrageenan structure through strong hydrogen bonding that results in an improvement in polymer cohesiveness within the matrix (Abdollahi et al., 2013). Similar observations have also been made in other NC-reinforced biopolymers including starch, alginate, and chitosan films (Abdollahi et al., 2013; Cao et al., 2008; Li et al., 2009).

An interesting finding in the present study is that the loading of NCF into neat RC was found to be optimized at 5% (w/w). Increasing the NCF above this level was ineffective at further improving the WS, moisture uptake, hygroscopic nature or WVP of the RC/NCF formulation. This may be due to the possible agglomeration of the cellulose fibres in the matrix (Santos et al., 2016). Such agglomeration has also been reported by Sánchez-García et al. (2010) upon the incorporation of fibrillated cellulose into carrageenan-glycerol polymer film at loadings above 3% (w/w). In the case of the SRC/NCF film, however, incorporation of NCF up to 7% (w/w) still showed enhancements in the water sensitivity parameters in most cases. It seems that the particulate impurities in this material interact with NCF resulting in a delayed self-agglomeration of the NCF particles in the SRC film matrix.

4.4.3 Mechanical Properties

Food packaging films require adequate mechanical properties to prevent failure or cracking during manufacture, handling, storage, and application to food products (Sothornvit & Rodsamran, 2008). As shown in **Fig. 4-5**, the TS and EM values observed for the neat SRC films are, in general, similar to those observed for the neat RC films. The significant difference in the EB values of these materials, however, suggest that the SRC material is of considerably inferior elasticity to the RC material. The lower EB value may be due to the presence of the particulate impurities in the SRC that interrupt the intermolecular structure of the κ-carrageenan base polymer thereby weakening its cohesiveness and resulting in a less compact material compared with the RC polymer. Conversely, the particulates presence in matrix of the SRC may also lead to greater stiffness and less flexibility in the produced film (Pereda et al., 2011).

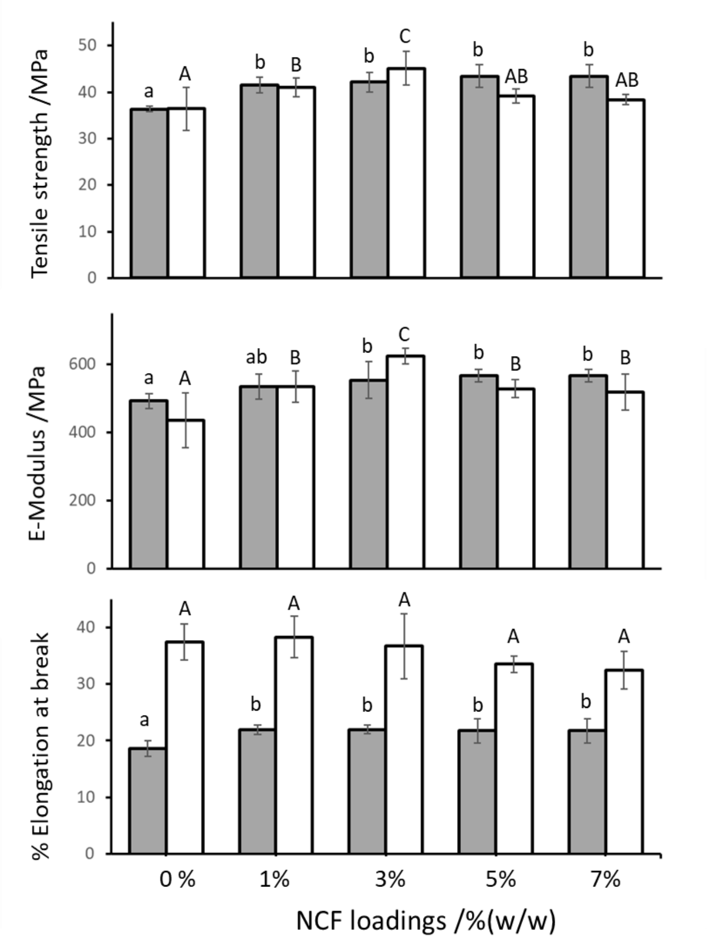


Figure 4-5. The tensile strength, elastic modulus and elongation at break of: (a) ■ SRC/NCF and (b) □ RC/NCF films. Values are plotted as the mean with one standard deviation error bars. Any two labelled with the same letter are not significantly different ($p > 0.05$) as determined by a Duncan test.

The incorporation of NCF into the SRC at a level of 1% (w/w) resulted in a *ca*. 20% increase in the TS of the material which was found to be significant at the $p < 0.05$ Increasing the NCF loading beyond this level had no further significant effect on the TS in this case. For the RC material the TS increased with increased loading of NCF up to a level of 3% (w/w) NCF after which no further improvement was observed. At a level of 3% (w/w) NCF the TS of the RC material was increased by *ca*. 23% and at higher loading the TS of this material was observed to decrease (see **Fig. 4-5**).

Similar trends to those observed in the case of the TS results were also observed in the EM results for both the SRC/NCF and RC/NCF formulations, namely an initial significant increase in the EM upon the addition of the NCF followed by a plateau in the value being reached at a certain threshold level of additive. In the case of the SRC/NCF formulation between 1-3% (w/w) loading of NCF appeared to be optimal resulting in a *ca*. 15% increase in the EM at a loading of 3% (w/w) NCF. For the RC/NCF formulation the threshold NCF level appears to be 3% (w/w) which delivers a *ca*. 43% increase in the EM value (see **Fig. 4-5**).

The trends in the TS and EM values with increased loading of NCF reflect those observed for the water sensitivity and moisture barrier properties above in that the given parameter reaches a threshold value upon the addition of NCF after which it either reaches a plateau or, as in the case of the WS value and possibly the WVP value of the RC/NCF material, trends towards a less than desirable value.

The addition of 1% (w/w) NCF to the SRC material resulted in a *ca*. 17% increase in the EB value. At loadings greater than this there appears to be no further improvement in this property. The addition of NCF to the RC material had no measurable effect on its EB (**see Fig. 4-5**). A similar reduction in the EB value upon NCF incorporation has also been reported by Kumar and Singh (2008) in the preparation of starch films.

The improvements in mechanical properties brought about by the addition of NCF may be attributed to the highly interfacial interaction between the high-strength cellulose and carrageenan polymer (Siqueira et al., 2010), in conjunction with an even dispersion of the cellulose particles within the polymer matrix that subsequently strengthens the film structure (Oun & Rhim, 2015). Such improvement is also attributed to the high degree of compatibility between cellulose and carrageenan (Abdul Khalil, Tye, et al., 2017). Beyond the threshold loadings of NCF into the SRC and RC materials, no further improvement in the mechanical properties are observed suggesting these may be optimum levels and that higher NCF loadings may result in aggregation (Oun & Rhim, 2015; Savadekar et al., 2012) or other detrimental effects. Several investigations have reported NC aggregation in κ-carrageenan polymer film (Savadekar et al., 2012), as well as in several other biocomposite film preparations such as gelatine (Hivechi et al., 2019), polyvinyl alcohol (Yuwawech et al., 2015), and alginate (Abdollahi et al., 2013) with typical optimum NC loadings of *ca.* 5% (w/w). Furthermore, the higher NCF loading needed in the SRC formulations to reach this critical point may be due to the solid particulates in the matrix impeding the formation of aggregates.

4.4.4 Thermal Behaviour

The thermal stabilities of the SRC/NCF and RC/NCF films were evaluated using TGA and the results are shown in **Fig. 4-6**. There are three stages of thermal decomposition of the SRC material and the addition of NCF did not change to any considerable extent the decomposition temperatures associated with each stage with the possible exception of a slight shift to lower temperatures by *ca.* 2°C at the peak of the second stage of decomposition (see dTGA in **Fig. 4-6**).

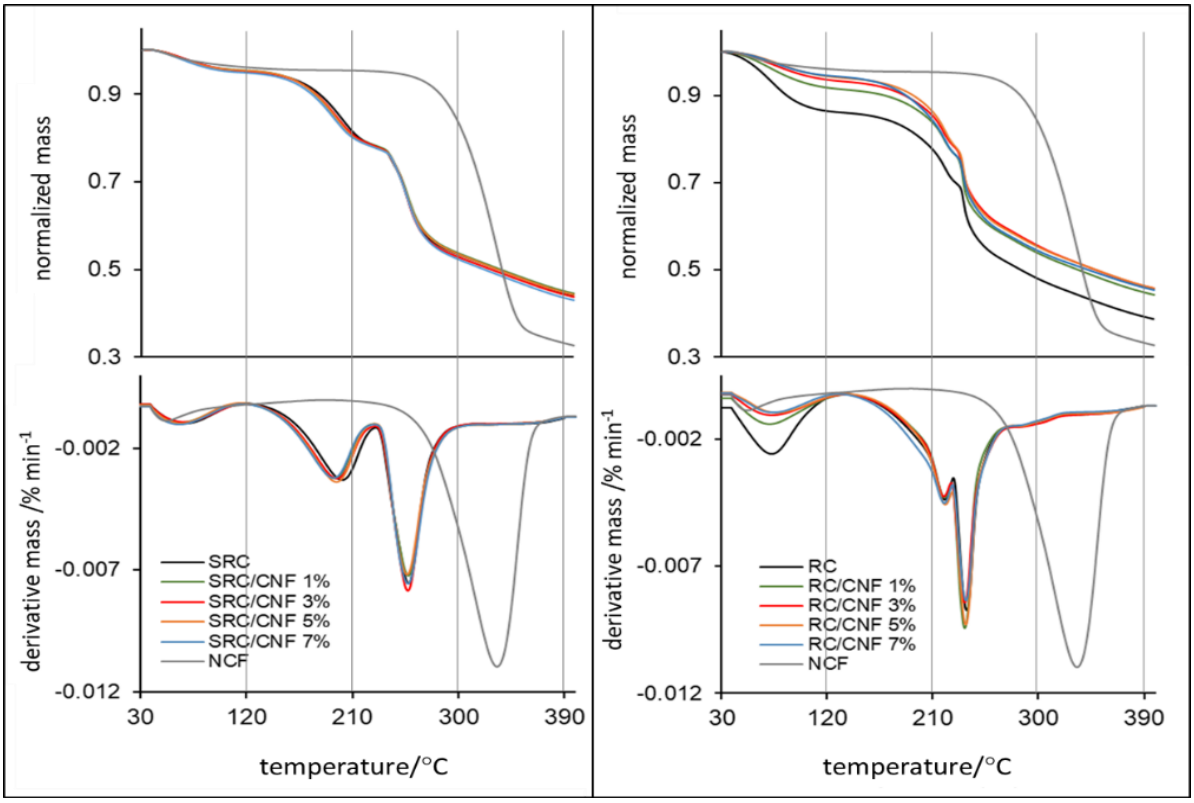


Figure 4-6. Thermograms of SRC/NCF and RC/NCF film formulations: (a) TGA thermogram and (b) derivative dTGA thermogram. Samples were heated from 30 to 400°C at a heating rate of 10°C min^{-1} under a nitrogen atmosphere (20 mL min^{-1} flow rate).

The first stage of decomposition occurs between 50 and 120°C and corresponds to the evaporation of water molecules from the film matrix. These water molecules are bound by the hydroxyl groups of the glucosyl units along the carrageenan structure and/or by the hydroxyl groups of the plasticizer (glycerol) (Zarina & Ahmad, 2014). The second stage occurs between 170 and 225°C and corresponds to the volatilization of the plasticizer from the polymer (Rhim, 2013). It is followed by the third stage between 240 and 275°C that is attributed to the decomposition of the carrageenan polymer chains.

In the case of the RC/NCF films, the thermograms were similar across all stages of decomposition. However, the inclusion of NCF considerably delayed the mass loss during thermal decomposition suggesting that NCF improved the thermal stability of the RC material (Gan et al., 2020). The mass losses of the RC/NCF film formulation during the first stage of the heating profile decreased with higher NCF loadings in the matrix of the film, and the optimum reduction of the mass loss was found at a level of 3% (w/w) NCF in RC. At the second stage of the profile (*i.e*. 225°C), the 3% (w/w) NCF loading delayed the mass loss of neat RC film by 7.9%, whereas after the third stage of the profile (*i.e*. 400°C), the mass loss was delayed by 7.1% (See **App.1-S4** in the Appendix) These data suggest that in the case of the RC/NCF formulations the presence of NCF retards the egress of water and glycerol from the polymer matrix and further supports the notion that the NCF thermally stabilizes to some extent the base polymer.

It can be observed from the dTGA thermograms in **Fig. 4-6** that the second decomposition stage peaked at a considerably lower temperature in the case of the SRC/NCF films (*ca*. 195°C) compared with the RC/NCF films (*ca*. 220°C). This may suggest a stronger bonding between the glycerol plasticizer molecules and the carrageenan polymer chains in the RC matrix compared to those in the SRC matrix (Sedayu et al., 2018). The particulate impurities distributed in the SRC matrix may disrupt or impede the hydrogen bonding between the carrageenan and the plasticizer rendering more readily volatilized plasticizer molecules (Jamróz et al., 2019). However, compared to the RC/NCF films, the SRC/NCF films demonstrated a higher peak degradation temperature at the next stage of decomposition that corresponds to the degradation of the main carrageenan polymer structure. In this case, the peak degradation temperature of the SRC/NCF films was *ca.* 257°C whereas that of the RC/NCF films was *ca*. 236°C. This may indicate that some impurities contained in the SRC matrix such as cellulose, minerals, or other inorganic materials have played a role in retarding thermal degradation of the carrageenan polymer (Roman & Winter, 2004).

The DSC thermograms for all film formulations are shown in **Fig. 4-7**. In general, the inclusion of NCF in the SRC polymer formulations had little effect on the thermal transitions of the material. The glass transition temperature (T_g) of neat SRC sample, which is also associated with its melting temperature, was observed to shift slightly from 172°C to 178°C at 3% (w/w) NCF loading. In the case of the RC material, a significant increase in the peak melting temperature occurred with the incorporation of 1% (w/w) NCF where a shift from 162°C to 178°C was observed. This may indicate that the nucleation of nanoscale NCF in the RC matrix was greater than that which occurred in the SRC and which further improved the integrity of the RC film crystallization compared to that of the SRC film (Zhang et al., 2019). A similar observation of enhanced thermal properties was also reported by Zhang et al. (2019) in polyhydroxybutyrate (PHB) composite as a result of reinforcement with NCF.

There was no further significant increase in the peak melting temperature observed with higher NCF loadings (See **App.1-S5** in the Appendix).

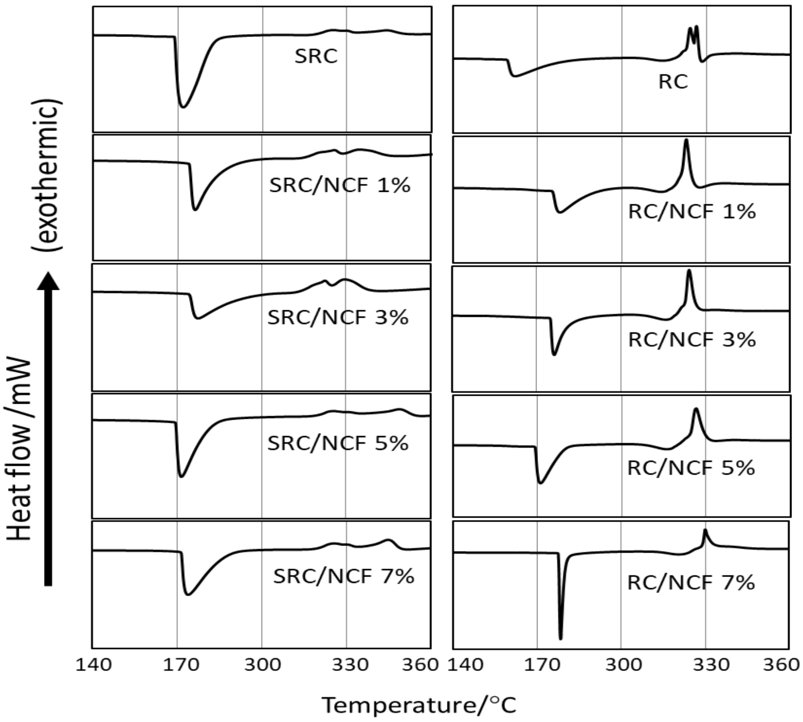


Figure 4-7. The DSC thermograms of the SRC/NCF and RC/NCF film formulations. Samples were heated under nitrogen (flow rate 20 mL min^{-1}) from 50 to 320°C at a heating rate of 10°C min^{-1}.

Furthermore, the thermal decomposition of the samples that is depicted by the exothermal peaks in the thermograms occurred over a noticeably broader range of temperatures in the SRC/NCF samples than in the RC/NCF samples, suggesting a more complex structure and subsequently a more complex degradation sequence, of the SRC/NCF samples presumably caused by the presence of impurities. In the case of the RC/NCF samples, a slight shift of the decomposition peak to a higher temperature was observed as the NCF loading in the matrix was increased. These results confirm the ability of the NCF to act as a stabilizer for this material as suggested by the thermogravimetric results shown in **Fig. 4-6**.

4.4.5 Surface Imaging

Figure 4-8 shows SEM images of the surfaces of the SRC/NCF and RC/NCF films containing various loadings of NCF. As expected, the SRC/NCF samples showed a rougher surface contour in comparison with the RC/NCF samples. The incorporation of NCF into SRC/NCF formulations up to a 3% (w/w) loading did not result in any significant changes to the film surface but at a higher loading of 7% (w/w) NCF, a suspected cellulose agglomeration was identified in some parts of the film (**see Fig. 4-8(c)**). The agglomeration of NCF particles at above a threshold level may explain the turning point in the improvement of the overall properties of the SRC film formulation as observed above (see **Sec. 3.2** and **Sec**. **3.3**).

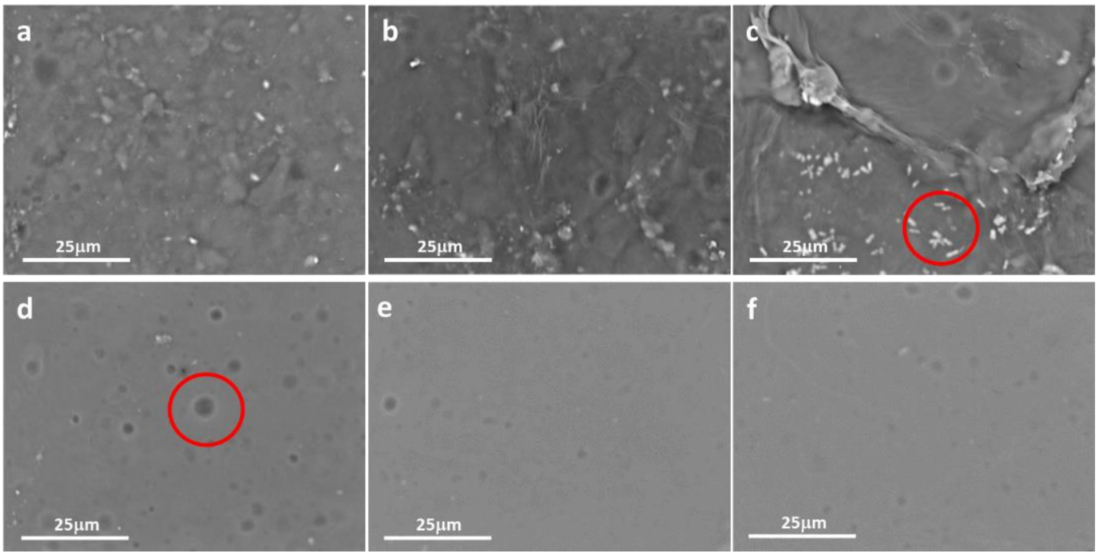


Figure 4-8. Scanning electron micrographs (2000×) of SRC reinforced with: (a) 0%, (b) 3% and (c) 7% (w/w) NCF and RC films reinforced with: (d) 0%, (e) 3% and (f) 7% (w/w) NCF. The figure shows evidence of cellulose agglomeration (image (c)) and glycerol domains (image (d)). The images were obtained in backscattering mode using an accelerating voltage of 5 kV under a low vacuum.

In the case of the RC/NCF film formulations, no noticeable changes in the film surface was caused by the inclusion of the NCF in the film matrix. The absence of impurities in the RC polymer presumably reduces the number of nucleating sites that facilitate

NCF agglomeration that can contribute to the surface roughness observed in the case of the SRC material.

An interesting observation can be made in relation to the effect of NCF on the dispersion of the plasticizer (glycerol) within the RC film matrix. It appears that the addition of NCF reduces the size of the glycerol domains that are seen clearly in **Fig. 4-8(d)** which suggests that the presence of NCF in the matrix may increase the miscibility of the plasticizer and may prevent the aggregation of glycerol, thus facilitating a more homogenous film structure (Sánchez-García et al., 2010). A similar phenomenon was also reported by Sánchez-García et al. (2010) when incorporating cellulose nanowhiskers and glycerol in hybrid-carrageenan film. Furthermore, this behaviour is also reflected in the DSC thermograms (**see Fig. 4-7**) whereby the neat RC film exhibited two exothermal peaks, corresponding to two separate decomposition processes of the carrageenan polymer and glycerol, respectively. The NCF incorporated RC/NCF samples exhibit only a single exothermal decomposition peak that is indicative of a more homogeneous polymer matrix.

4.4.6 Film Crystallinity by X-ray Diffraction

The XRD spectra of the sample films are shown in **Fig. 4-9**. The spectra observed for the SRC/NCF and RC/NCF formulations along with the SRC and RC materials and NCF additive all exhibited similar profiles at the 2θ diffraction angle. This is to be expected as all the materials are based on a polysaccharide structure that typical major peaks exhibited at 2θ = 14.5°, 17.3°, 18.9°, 26.9° and 29.0°.

These peaks correspond to the predominant diffraction patterns of Type I cellulose (El Achaby et al., 2018). The XRD spectrum of the SRC/NCF formulations also showed a distinct broad shoulder observed in the range between 2θ = 20° to 25° indicating the presence of amorphous regions in the SRC base polymer (Kanmani & Rhim, 2014a), as well as some other minor peaks at around 2θ = 30° and 37° that may be due to the presence of inorganic salt residues (e.g. NaCl), other minerals and unknown impurities found in seaweed (Corvaglia et al., 2016; El Achaby et al., 2018).

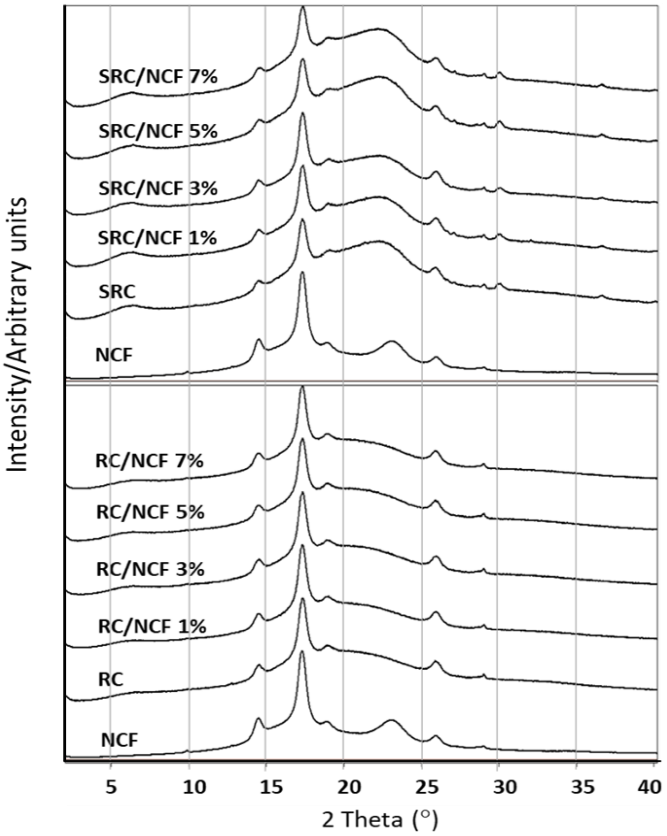


Figure 4-9. X-ray diffraction spectra of the SRC/NCF and RC/NCF film formulations. The diffraction spectra were recorded using Cu Kα radiation (λ = 0.154 nm) at 40 kV and 20 mA.

The impurities in the SRC material can be present in both the amorphous and crystalline regions of the carrageenan polymer and can further influence the formation of the crystalline regions. Since there are more solid particulates in SRC compared to RC, the degree of crystallinity of the neat SRC film (56%) was found to be slightly lower than that of the neat RC film (61%). Furthermore, the degree of crystallinity is influenced by inter- and extra-molecular hydrogen bonding of the carrageenan chains in the matrix, and it can be decreased by the presence of impurities that may disrupt these hydrogen bonds (Jamróz et al., 2019). The lower crystallinity of the SRC material may therefore be caused by a reduced cohesiveness within its polymer structure. The crystalline regions of SRC/NCF and RC/NCF samples were observed to increase with an increasing level of NCF in the formulation with maximum increases in crystallinity observed for the SRC/NCF and RC/NCF formulations at 3% and 1% (w/w) NCF respectively. These levels resulted in a degree of crystallinity of 71% and 66% for the SRC/NCF and RC/NCF samples respectively (see **App.1-S6**) whereas above these levels, the crystallinity reached a plateau possibly due to agglomeration. It is likely that the increased crystalline regions in the SRC/NCF and RC/NCF polymer structures has contributed to the enhancement of the overall physico-mechanical properties and thermal stability in these systems (Kanmani & Rhim, 2014a; Savadekar et al., 2012) as observed elsewhere in this study.

4.4.7 Structural Analysis by Fourier Transform Infrared Spectroscopy

The FTIR spectra shown in **Fig. 4-10** were used to observe any differences in the structures of the materials brought about by the addition of NCF to the formulation. Due to the high miscibility of NCF molecules in the SRC and RC polymer matrices, as well as the relatively low levels of NCF added in the formulations (up to 7% (w/w)), there are, as expected, few differences between the neat films and those containing NCF.

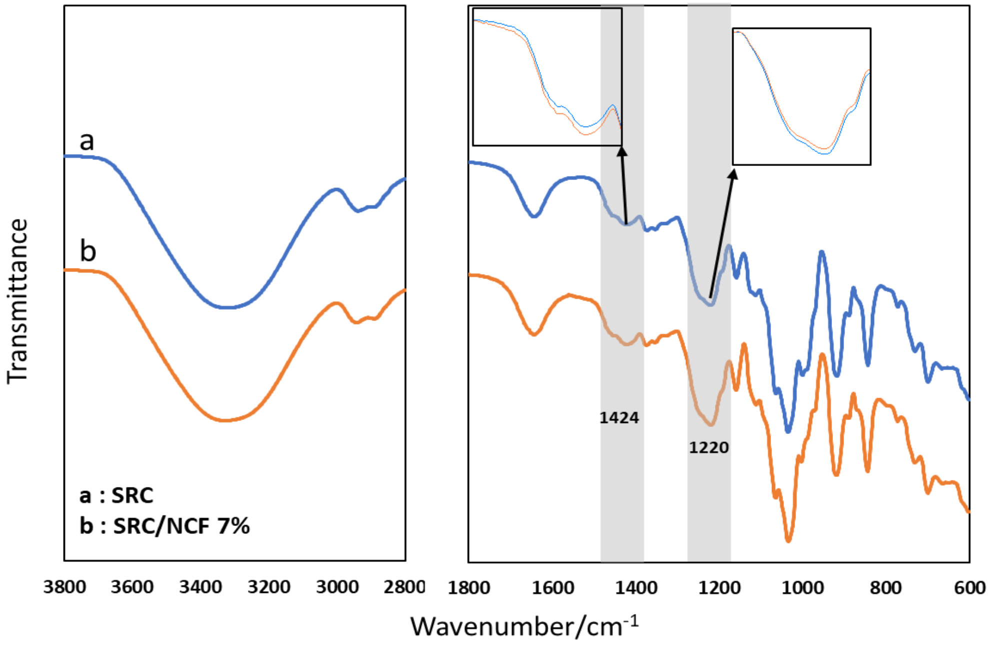


Figure 4-10. FTIR spectra of the SRC/NCF and RC/NCF film formulations. Spectra were recorded using an average of 64 scans at 4 cm^{-1} resolution.

In the NCF incorporated SRC film, a small increase in the intensity of the peak is observed at 1424 cm^{-1} that correspond to –CH_2– alkyl bending vibrations (Schwarzova et al., 2015; Zarina & Ahmad, 2014) also a small decrease in the peak intensity at 1220 cm^{-1} assigned to the ester sulfate O=S=O symmetric vibration (Ghani et al., 2019) from the SRC. The slight increased symmetric CH_2 bending mode reflects the increasing degree of crystallinity of the sample film by the NCF incorporation as this vibration mode is also known as the “crystallinity band” (Abdulkhani et al., 2013), thereby increasing the intensity of that CH_2 peak may facilitate the reduction of the O=S=O bending in some way. This ester sulfate group is also associated with the amorphous structure of carrageenan polymer (Ghani et al., 2019). These results confirm the crystalline structure formation described in previous XRD spectra (see **Sec. 3.6**). Whilst in the case of the RC films, there is no considerable change identified on the formulated films (see **App.1-S7** in the Appendix).

4.4.8 Towards Optimization of NCF Loading

Table 4-2 summarizes the results obtained in each of the experimental sections in terms of the threshold values that were observed for the NCF loading in each case for the SRC/NCF and RC/NCF formulations. The table was constructed with a view to identifying a possible optimum loading of NCF in each of the SRC and RC materials that would produce the best overall performance as packaging film candidates.

Table 4-2. Threshold NCF loadings for the tested properties of the SRC and RC materials.

Properties	**Threshold NCF Loading/%(w/w)**	
	SRC/NCF	**RC/NCF**
Physical and Optical		
Film Thickness	~ 7	Note 1
Opacity	Note 2	Note 2
Crystallinity	3	1
Surface Texture	3 (7 max)	Note 3
Water Sensitivity and Moisture Barrier		
Moisture Content	Note 4	5
Water Solubility	Note 4	4–5
Moisture Uptake	~ 7	5
Water Contact Angle	5	5
Water Vapour Permeability	5	5
Mechanical		
Tensile Strength	1	3
Elastic Modulus	1–3	3
Elongation at Break	1	3 max
Thermal		
Thermal Mass Loss	Note 5	3
Melting Temperature	Note 5	1

Notes

1. NCF loading found not to affect resultant film thickness.
2. Opacity of SRC and RC continues to increase with increased NCF loading.
3. No noticeable change in surface texture observed over range of NCF loadings tested.
4. No threshold value was observed in the case of the SRC/NCF formulation.
5. No observable effect of NCF found in the case of the SRC/NCF formulation.

The threshold values given in **Table 4-2** represent either levels where no further improvement in the property was observed or a turning point in the property towards a less desirable value of its measured parameter. Since for those properties where no threshold was observed and the associated value of the measured parameter continued with the observed trend, only those properties where a threshold was observed need to be considered. Whence upon consideration of the data in **Table 4-2** along with the trends in the individual properties with NCF loading, it is suggested that a loading of 5% (w/w) of NCF in the SRC and 3% (w/w) in the RC material will produce the optimum overall performance of these as candidates for packaging materials.

4.5 Conclusions

An investigation of the addition of NCF to SRC and RC film formulations as a reinforcement showed general improvements in the water sensitivity, mechanical properties, and thermal properties of these materials. The presence of natural impurities in the SRC resulted in slightly poorer optical properties of the resultant films compared with those produced with the RC material, however, the distribution of solid impurities (*i.e.* cellulose and minerals) within the structural matrix resulted in the SRC/NCF films having a more hydrophobic surface than the RC/NCF films. The impurities in SRC material also render the water resistance and barrier properties of SRC/NCF films lower than those of RC/NCF films due to the less compact matrix structure within the SRC formulation. On the other hand, these impurity particulates result in the SRC/NCF films being considerably more rigid than their RC/NCF counterparts. The impurities in the SRC matrix were found to interact with NCF particles contributing a delayed self-agglomeration of the NCF particles in the SRC polymer film. The optimum levels of the NCF additive required to effectively enhance the overall properties of the SRC/NCF and RC/NCF formulations were found to be 5% (w/w) and 3% (w/w) respectively. With the exception of the reduced optical properties and the WVP values being greater than those of the RC/NCF films, the SRC/NCF films have generally shown a reasonable performance compared to that found in the case of the RC/NCF film formulations, and as such can be further recommended as a rigid-opaque film material for food packaging applications at significantly lower cost.

CHAPTER 5

Improving the Moisture Barrier and Mechanical Properties with Nanoclay and Poly(caprolactone)

5.1 Overview

Chapter 5 presents an investigation of the reinforcement of SRC film with nanoclay (hydrophilic bentonite), and in combination with a thin lamination onto the surface of the film using hydrophobic poly(caprolactone). The resulting effects on the water sensitivity, moisture barrier, and mechanical properties are presented and discussed.

The manuscript entitled: “Improving the Moisture Barrier and Mechanical Properties of Semi-Refined Carrageenan Films”, Sedayu, B. B., Cran, M. J., Bigger, S. W. has been published in the *Journal of Applied Polymer Science* (available at: https://doi.org/10.1002/app.49238).

5.2 Introduction

The reinforcement of SRC film by nanocellulose in the previous investigation resulted in an overall enhancement of the film properties. However, the use of nanocellulose, which is considered as a hydrophilic substance, did not improve the water sensitivity of the formulation. Thus, further investigations are needed to explore the use of a more hydrophobic reinforcing material to improve the properties of the film in particular regard to overcoming the inherent hydrophilic characteristics of the SRC.

Hydrophilicity can pose a critical problem in food packaging applications since the major quality losses of packaged foods are associated with water vapor and gas transfer through packaging films (Karel & Lund, 2003). In addition, the inability of these materials to accommodate high water content produce such as meats, fruits, and liquid products severely constrains their implementation in the packaging of these food items. Plasticizers are usually incorporated into carrageenan polymers to obtain desirable mechanical properties because films comprised entirely of carrageenan are inherently brittle (Cian et al., 2014; Zarina & Ahmad, 2015). Nonetheless, the addition of plasticizers is also well known to exacerbate the moisture sensitivity of polysaccharide films (Sedayu et al., 2018; Wittaya, 2012a). To address this and other drawbacks, reinforcing nanoclays (NCs) and blending of hydrophobic materials into carrageenan-based film have been studied and have demonstrated positive outcomes (Rane et al., 2014; Rhim & Wang, 2013; Shojaee-Aliabadi, Mohammadifar, et al., 2014).

The inclusion of natural NCs into bio-based polymers has been reported to successfully enhance the barrier and mechanical properties of the produced films (Gabr et al., 2013; Rhim, 2011; Shojaee-Aliabadi, Mohammadifar, et al., 2014). Although hydrophilic NCs may increase the water sensitivity of bio-based films, they are more compatible with such polymers than hydrophobic NCs and can therefore form a highly even dispersion (intercalation) within the matrix (Martins, Bourbon, et al., 2012). The enhanced barrier properties can be explained by the tortuous conformation of the clay particles that obstruct the penetration of moisture or oxygen passage through the films (Rhim, 2011; Sorrentino et al., 2007b). In addition to this, Müller et al. (2012) have also observed that bio-based films containing a hydrophobic type of NC have inferior water vapor and mechanical properties compared with those that contain a hydrophilic clay.

The incompatibility of hydrophilic polymers in blends with hydrophobic substances has been a major constraint in obtaining desirable products (Avella et al., 2000). This often results in poor dispersion within the mixture, and consequently brings undesirable impacts to the films that are produced such as agglomeration, poor optical properties and/or poor mechanical properties (Rekemin & Abedin, 2017; Sánchez-González et al., 2009; Shojaee-Aliabadi, Hosseini, et al., 2014). With regard to the latter, the mechanical property requirements of a given packaging material depend on its specific application. For example, semi-rigid food containers, in general, do not require high elongation properties but need higher TS to ensure the structural integrity. In the case of films, stretchability or high EB is often required, particularly in the case of stretch-wrap films (Ferreira et al., 2016).

An alternative to blending that can potentially achieve better outcomes is layering or lamination of hydrophilic films with a hydrophobic substance (Rhim et al., 2006). Examples of common bilayer products that are readily found in the global market include takeaway paper coffee cups and food containers where a hydrophobic layer (polymer film) is applied to be in contact with the food product as this layer is able to withstand water or high moisture levels. The current barrier materials used in layering biodegradable packaging films commonly originate from synthetic polymers such as poly(vinyl alcohol), polyethylene, fluorocarbon polymers, or ethylene vinyl alcohol copolymers (Hong & Krochta, 2003; Khwaldia et al., 2010). Using such materials may bring disadvantages in terms of their ecological aspects because they are not readily biodegradable (Myllymaki et al., 1998) and may not be recycled due to difficulty in separating the layers (Tihminlioglu et al., 2010). Poly(caprolactone) (PCL), an aliphatic polyester, is a commercially available synthetic biodegradable polymer with a crystallinity of around 50% and a low melting point of 60°C (Ortega-Toro, Collazo-Bigliardi, et al., 2016; Yasin & Tighe, 1992). Duarte et al. (2006) used PCL as a barrier layer for polysaccharide-based film and found the laminates to be environmentally biodegradable. Poly(caprolactone) also has high flexibility and films prepared by heat press-molding have shown a tensile strength (TS) of 33 MPa and an elongation at break (EB) of more than 1100% (Koenig & Huang, 1995). Increases in the mechanical strength of pea starch films has also been reported upon the incorporation of PCL (Fabunmi et al., 2011; Ortega-Toro, Muñoz, et al., 2016).

In view of the desirable environmental and economic benefits of using SRC as a substrate for food packaging polymer systems, the current paper investigated the possible enhancement of its hydrophobicity as well as its barrier and physicomechanical properties by the incorporation of nanoclay (NC) in combination with surface lamination using a thin layer of PCL. It is envisaged that an improvement in these properties of SRC would enable its use as a packaging material for high moisture-containing foods in particular.

5.3 Materials and Methods

5.3.1 Materials

Semi-refined and refined carrageenan used in this study were similar to those used in the previous investigations described respectively in **Sec. 3.3.1** and **Sec. 4.3.**

Hydrophilic bentonite nanoclay (MW = 180.1 g mol^{-1}), PCL (MW = *ca*.14 kDa), glycerol and KNO_3 (≥ 99.0%) were purchased from Sigma-Aldrich, Australia. The choice of a hydrophilic NC was made in order to achieve a high dispersion of the clay particles within the matrix of the polymer. Milli-Q water was used as a solvent for the SRC and NC, whereas tetrahydrofuran (THF, ≥99.0%, Sigma Aldrich, Australia) and

$Mg(NO_3)_2$ (Ajax Finechem, Australia) were used as a solvent in the preparation of PCL and conditioning of the samples.

5.3.2 Film Preparation

The SRC films were prepared according to the method described in **Sec. 4.3.1**. A second preparation of NC-reinforced SRC film (SRC/NC) was performed using the method proposed by Rhim (2013) with some modifications. Relative to SRC, a solution of 6% (w/w) hydrophilic bentonite NC was dispersed/swollen in 150 mL of water with constant stirring for 24 h at 45-50°C. The NC solution was then sheared at 20,000 rpm using a homogenizer (CAT Unidrive ×1000, Germany) for 10 min, followed by a further 10 min of sonication at 80% amplitude and 50 Hz. Separately, 5 g of SRC in 100 mL was prepared as described above and both solutions were then mixed and stirred for 15 min followed by heating at 90°C for 30 min. The glycerol addition and casting procedure was similar to that used in the preparation of the SRC film (see above). The selection of 6% (w/w) NC was based on a screening process whereby different levels of NC were added to the formulation and the tensile strength tested (**see App.2-S1** in the Appendix). A level of 6% (w/w) NC showed the greatest improvement with higher levels resulting in lower tensile strength values.

A third preparation of SRC and NC reinforced films laminated with PCL (SRC/NC/PCL) was obtained by pouring a 6% (w/w) PCL solution dissolved in THF onto the upper surface of the SRC/NC films. The PCL solution was poured evenly onto the surface of the dried films, and the excess solution was immediately removed from the film by holding the tray upright before the films were left to dry at 22 °C.

5.3.3 Film Conditioning and Thickness

Prior to characterization testing and measurement, all film samples were conditioned for 48 h at 22 °C in a desiccator containing saturated $Mg(NO_3)_2$ solution to obtain a 53% RH environment. The thickness of each sample was measured using a digital micrometer (Schut IP54, The Netherlands) with a 0.001 mm precision. The thickness measurements were used in the calculations of the water vapor permeability and mechanical property parameters.

5.3.4 Barrier Properties and Water Resistance

The moisture content and WVP were performed according to the method described in **Sec. 3.3.2**. The moisture uptake, water solubility, and WCA of the films were conducted following the previous methods respectively described in **Sec. 4.3.4**, **Sec. 4.3.5** and **Sec. 4.3.6**.

5.3.5 Optical and Mechanical Properties

The optical and mechanical properties of the SRC film samples were measured respectively in accordance with the methods described in **Sec. 3.3.2** and **Sec. 4.3.7**.

5.3.6 Thermal and Structural Analysis

The thermal stability of each film sample was investigated by thermogravimetric (TG) analysis using a Mettler-Toledo TGA/DSC1 thermal analyzer (Mettler-Toledo, Schwarzenbach, Switzerland) according to the method described in **Sec. 4.3.8** and the structural analysis was performed following the method described in **Sec**. **3.3.2**.

5.3.7 Scanning Electron Microscopy

The imaging of the surfaces of the films and cross section analyses were conducted using a Hitachi Tabletop (TM 3030 Plus) scanning electron microscope (SEM) equipped with an energy dispersive X-ray (EDX) spectrometer. The instrument was operated as described in **Sec. 4.3.3**. To observe the NC dispersion within the matrix of the film the silica spectra were produced by EDX mapping using AztechOne software (Oxford Instruments, UK).

Statistical analysis of the data collected from the samples was performed in accordance with the method described in **Sec. 3.3.3**.

5.4 Results and Discussion

5.4.1 Morphological and Physical Properties

The film samples were relatively uniform in thickness and were flexible with a yellowish translucent surface. As expected, the addition of NC slightly increased the film thickness from 0.066 to 0.069 mm (see **Table 5-1**) with similar results reported by Shojaee-Aliabadi, Mohammadifar, et al. (2014) and Rhim and Wang (2013) for κ-carrageenan films. Interestingly, the PCL-layered SRC films showed a negligible change in thickness in comparation with the unlayered films, thus indicating the formed PCL layer was very thin.

Figure 5-1(a) shows the cross-sectional image of a laminated SRC/PCL film where the PCL layer is 2-3 μm thick. There is also no visible delamination (**see Fig. 5-1(a)**) indicating strong interfacial adhesion between the SRC and PCL film layers. This adhesion may be initiated by hydrogen bonding interactions between the carbonyl groups of the PCL and the hydroxyl groups of the SRC and/or the plasticizer (glycerol). Similar hydrogen bonding between PCL and polysaccharides has been reported previously by Honma et al. (2003).

Table 5-1. Physical properties of the SRC/NC/PCL film formulations

Film samples	Thickness/mm	Moisture content /%	L^*	a^*	b^*	Opacity
SRC	0.066 ± 0.001^{c}	25.90 ± 0.76^{b}	88.22 ± 0.15^{c}	0.12 ± 0.04^{c}	7.57 ± 0.21^{d}	12.45 ± 0.37^{d}
SRC/NC	0.069 ± 0.001^{a}	23.87 ± 0.61^{a}	86.90 ± 0.18^{b}	0.21 ± 0.03^{b}	9.68 ± 0.18^{c}	13.67 ± 0.29^{c}
SRC/PCL	0.066 ± 0.002^{bc}	23.62 ± 0.72^{a}	88.32 ± 0.32^{c}	0.16 ± 0.03^{a}	6.96 ± 0.32^{b}	10.86 ± 0.37^{b}
SRC/NC/PCL	0.068 ± 0.002^{ab}	22.94 ± 0.94^{a}	87.31 ± 0.20^{a}	0.16 ± 0.03^{a}	8.86 ± 0.39^{a}	13.13 ± 0.59^{a}

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 ($p > 0.05$) as determined by a Duncan's test.

The surface image of the SRC film (**Fig. 5-1b**) shows some segregated domains as well as the presence of aggregated particles. These particles are mainly impurities commonly found in the raw SRC such as cellulosic residues. The inclusion of NC in SRC film formulation resulted in a rougher surface but with smaller domains than those of the SRC film and irregular surface aggregates (see **Fig. 5-1c**). Impurities present in raw SRC may interact with the NC particles during film preparation and consequently cause agglomeration. These aggregates may further impact upon the final physical-mechanical, and barrier properties of the film.

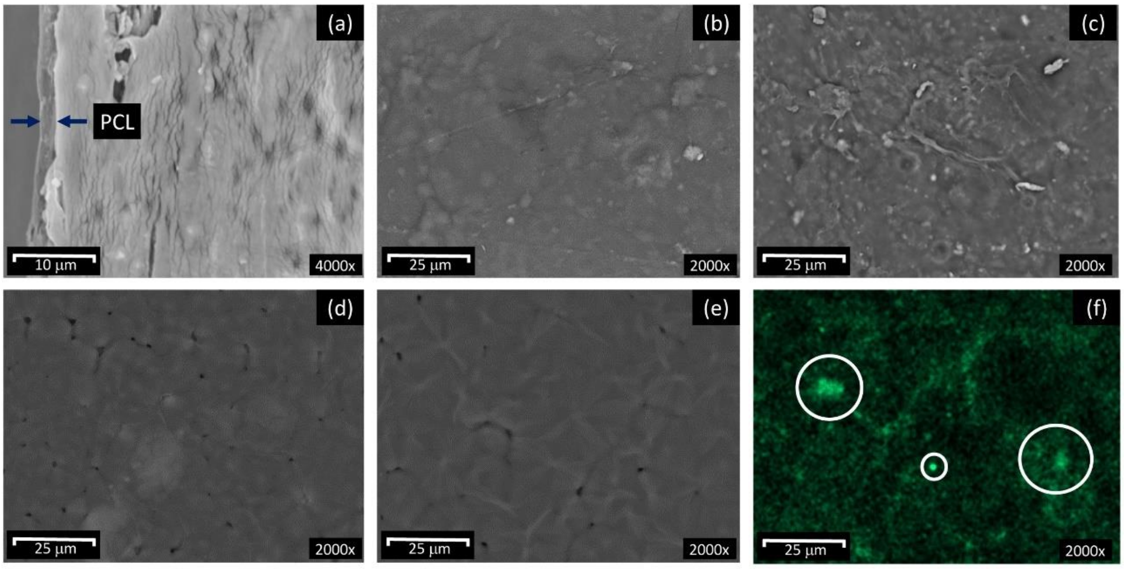


Figure 5-1. Scanning electron micrographs and EDX images of SRC/NC/PCL film formulations: (a) cross-sectional image of SRC/PCL, (b) SRC, (c) SRC/NC, (d) SRC/PCL, and (e) SRC/NC/PCL film; (f) EDX image of Si dispersion in SRC/NC film based on image The aggregation of some of the NC particles is indicated by the circles in image (f).

In the case of the PCL laminated films, both the SRC/PCL and SRC/NC/PCL films display similar surface images as shown in **Figs. 5-1(d)** and **5-1(e)** respectively. In the case of the SRC films containing NC, larger, more regular domains are observed compared with the neat SRC film, thus making the surface appear smoother. The smoothness of the surface is affected by the presence of NC in the matrix that facilitates a rougher surface contour thereby producing a stronger bond between the SRC and PCL film surfaces. Additionally, the surface smoothness might also be affected by the interaction between the hydrophilic clay particles on the film surface with the PCL polymer which creates a more compatible interfacial adhesion between the layers. Such a phenomenon has also been reported by Eng et al. (2013) in an investigation of hydrophilic clay/PCL blends.

The incorporation of the hydrophilic NC in the SRC film formulation resulted in greater homogenous dispersion in comparison with a less hydrophilic type of NC (see **App.2-S2** in the Appendix). The dispersion of NC particles represented by the silica elemental distribution showed a relatively even dispersion over the surface of the SRC film shown in **Fig. 5-1(f)**. However, some clay particle aggregates are also identified within the matrix and this aggregation might be caused by an excessive concentration of NC in the matrix of the SRC. Even though the addition of 6% (w/w) of NC into

SRC produces a film with optimum mechanical strength, this concentration may have initiated agglomeration.

Table 5-1 lists the film thickness, moisture content, color and opacity of the SRC film samples. Many of these properties are important considerations for packaging films and are particularly related to consumer acceptance. The data suggest that the moisture content of SRC films is significantly decreased ($p < 0.05$) by the inclusion of NC as well as by PCL lamination. This behavior may be explained by a reduction in the amount of available hydroxyl groups in the structure of the SRC film that can interact with water since these groups also interact with the clay particles (Shojaee-Aliabadi, Mohammadifar, et al., 2014) and PCL molecules.

The incorporation of the NC into the matrix of the SRC polymer increased the opacity of the film due to the dispersion of particles through the matrix obstructing the transmission of light (Rhim & Wang, 2014). The thickness of the SRC and SRC/PCL laminated films are the same (see **Table 5-1**) and the optical density of the SRC is much greater than that of the PCL. Whence the observed opacity difference between these two films may be explained by the effective decrease in the optical pathlength of light through the more optically dense SRC medium that is achieved upon lamination.

The surface color of the SRC film was affected by the NC incorporation and the lamination with PCL. The incorporation of NC into the SRC resulted in a significant decrease in the lightness (*L**), redness/greenness (*a**), and yellowness/blueness (*b**) values whereas lamination of SRC with PCL resulted in no significant change in the lightness but an increase in the *a** value and decrease in the *b** value (see **Table 5-1**). The decrease in lightness may be a result of the NC incorporation into the SRC polymer matrix rendering a matt finish to the film surface and a subsequent enhancement of the natural yellowish tint of SRC film. Similar observations of increased yellowness were also observed in alginate and whey protein isolate nanocomposite films reinforced with hydrophilic montmorillonite clay (Alboofetileh et al., 2013; Sothornvit et al., 2010). In comparison with clear transparent films obtained using refined carrageenan, the yellowish appearance of SRC films may originate from residual substances including glucan, insoluble aromatic compounds, and minerals (Masarin et al., 2016).

5.4.2 Moisture Barrier Properties and Hydrophobicity

Moisture barrier properties are among the more important properties that must be considered when developing films for food packaging applications. The WVP of any polymeric film, for example, depends on the chemical conformation and morphology of the polymer matrix, the nature of the permeant film, and the temperature of the surrounding environment (Siripatrawan & Harte, 2010).

The WVP, moisture uptake, WCA, and WS values of the different SRC film samples are shown in **Table 5-2**. The results suggest that the WVP of the SRC substrate is increased slightly upon the addition of NC to the formulation but is considerably decreased, as expected, by the addition of the PCL layer. In the case of the SRC/NC film, the lack of improvement in WVP may be due to the heterogeneous dispersion of the NC particles within the matrix since some agglomeration occurs as described above. This can lead to a less effective barrier to water vapor transmission through the matrix. The results also suggest that the hydrophobicity of the films increases upon addition of NC and lamination of the SRC as indicated by the lower moisture uptake values.

The WCA values indicate a slight decrease in hydrophobicity occur upon the addition of NC to the SRC possibly due to surface effects (see **Fig. 5-1c**) caused by the impurities in the formulation and which facilitate a higher interfacial adhesion between the film surface and the water drop. However, this effect is very much compensated for by the lamination of the SRC where a considerable increase in the WCA values is observed.

Table 5-2. Barrier properties and hydrophobicity of the SRC/NC/PCL film formulations

Film samples	**WVP/g mm cm^{-2} h^{-1} Pa^{-1} (× 10^{-7})**	**Moisture uptake/%**	**WCA/°**	**WS/%**
SRC	10.47 ± 0.09^{b}	49.07 ± 2.12^{c}	72.17 ± 3.98^{c}	49.12 ± 2.26^{c}
SRC/NC	10.67 ± 0.03^{b}	44.90 ± 1.55^{b}	69.77 ± 3.79^{b}	49.65 ± 1.80^{b}
SRC/PCL	8.43 ± 0.45^{a}	44.21 ± 1.15^{b}	95.78 ± 2.82^{a}	46.86 ± 0.97^{ac}
SRC/NC/PCL	8.36 ± 0.48^{b}	37.21 ± 1.84^{a}	95.20 ± 2.45^{a}	43.73 ± 3.76^{a}

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 ($p > 0.05$) as determined by a Duncan's test.

The WS values also reflect the resistance of the film formulation to water that may be present on the surface of foods when the film is applied as a food packaging medium, and may also reflect its inherent biodegradability (Cerqueira et al., 2012).

Also shown in **Table 5-2**, the WS value was slightly increased respectively upon the incorporation of the NC into the SRC film. This may be a result of the inclusion of the hydrophilic NC rendering the film to be more readily degraded in water. Similar behavior has also been reported by Alboofetileh et al. (2013) in alginate films prepared using hydrophilic montmorillonite clay.

The seemingly unexpected decrease in the moisture uptake from 49 to 45% that was observed when a hydrophilic additive such as bentonite was added to the matrix of the SRC can possibly be explained by an interaction between the clay particles with SRC and glycerol in the formulation. This occurs through ion-dipole interactions between sodium ions in the NC and the hydroxyl groups from the SRC and glycerol that reduce the availability of hydroxyl groups that are capable of interacting with surrounding water molecules. The overall effect thereby results in a less hygroscopic film (Müller et al., 2011).

The addition of NC alone did not alter the WVP considerably, however, layering the films with PCL generally improved the moisture barrier and surface hydrophobicity. The PCL layer reduced the WVP and WS values by *ca.* 19% and 10% respectively for the SRC/PCL samples compared with the SRC samples and by *ca.* 22% and 17% respectively for the SRC/NC/PLC compared with SRC/NC films. The PCL layer also increased the WCA values of the SRC and SRC/NC films to a value greater than 95° (see **Table 5-2**). The enhancement of the water barrier properties of the films would be beneficial in protecting packaged foods affected by moisture content or high water activity (Labuza & Hyman, 1998). The overall moisture barrier and water resistance improvement provided by the PCL layer can be associated with its hydrophobic characteristics that protects the SRC or SRC/NC film surface from direct contact with any surrounding water. Moreover, the PCL layer may reduce the number of hydroxyl functional groups per unit volume or area in the SRC films that are able to interact with water molecules from the environment (Fabunmi et al., 2011). The results of the present study have been confirmed by the negligible moisture or water sorption reported for other PCL-based films (Mahieu et al., 2017; Myllymaki et al., 1998).

5.4.3 Mechanical Properties

The mechanical properties of the film samples namely the TS, EM, and EB results, are shown in **Fig. 5-2**. The incorporation of NC into the matrix of the SRC matrix had no significant effect on the TS but increased the EM with a corresponding decrease in the EB value. The incorporation of NC in the SRC increased the EM of the film by 18.6% whereas the EB was decreased by 17.2%. The increased value of EM can be associated with the interactions between the polymer matrix and intercalated clay particles resulting in a high aspect ratio and high surface area through hydrogen bonds (Alexandre & Dubois, 2000; Almasi et al., 2010). The reduction in EB is attributed to the decreasing moisture content in the SRC/NC film. Since water molecules plasticize the SRC polymer matrix, decreasing the water content will consequently increase the stiffness, and thus decrease the EB (El Halal et al., 2018; Müller et al., 2011; Nafchi et al., 2013).

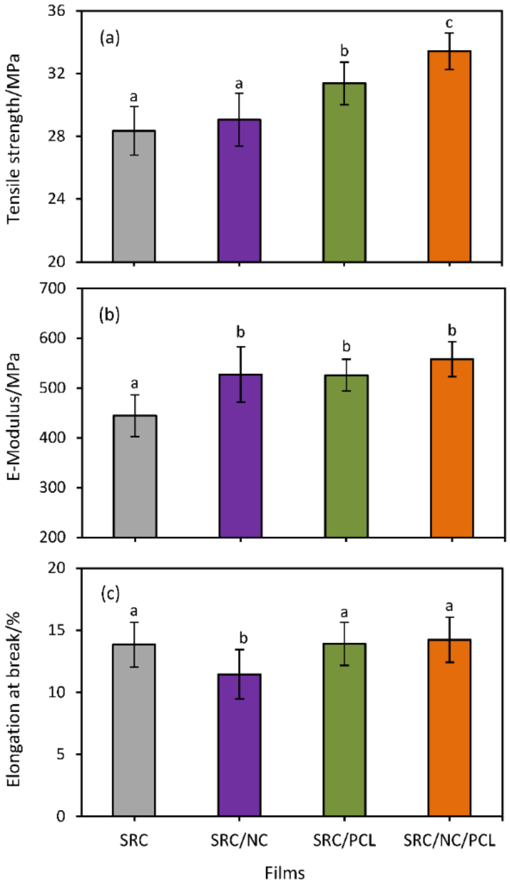


Figure 5-2. Tensile strength (TS), elongation at break (EB) and elastic modulus (EM) of SRC/NC/PCL film formulations measured in accordance with ASTM Method D882. Error bars represent the standard deviation calculated at the 95% level of confidence. The same letters above data bars indicate no significant difference in the values as determined by a Duncan’s test (i.e. $p > 0.05$). Color image available online.

The PCL lamination of the SRC film increased the TS and EM by 10.7% and 18.3% respectively but had no significant effect on the EB. Moreover, the combination of both NC reinforcement and PCL lamination ultimately contributed to the highest enhancement in both the TS and EM of the SRC film with respective increases in these mechanical properties of 17.9% and 25.6%. There appears to be a slight (2.8%) increase in the EB value however this difference was found to be statistically insignificant compared to the SRC control sample.

5.4.4 Thermal Properties

The thermogravimetric analysis (TGA) provides information about the effect of NC incorporation and PCL lamination on the thermal stability of the films. As shown in **Fig. 5-3**, the mass loss during thermal degradation for each of the films exhibited similar behavior with three major stages of decomposition. The initial decomposition occurred between 50 and 120 °C with small, broad peaks (see the derivative, dTGA curves) that corresponds to the evaporation of bonded water within the films. The second mass loss step occurred between 170 and 240 °C and corresponds to the volatilization of the glycerol plasticizer from the polymer (Rhim, 2013). The third and final step occurred at *ca.* 260 °C and is attributed to the decomposition of the SRC polymer chains as well as the commencement of the PCL layer decomposition which begins at 265 °C.

As displayed in **Fig. 5-3**, both NC inclusion and PCL lamination resulted in an increase in the thermal stability of the SRC films with smaller mass losses observed than those of the SRC control film. Furthermore, the incorporation of NC imparted a slightly higher stability than PCL lamination, particularly during the first and second stages of degradation. The migration of water, plasticizer, and/or other volatilized compounds may be retarded by the dispersed clay within the matrix due to the thermal insulation properties of the clay, as well as the tortuous pathway introduced by the NC platelets that can inhibit the exudation of the molecules from the matrix (Aouada et al., 2011). Similar results have also been reported by Kumar et al. (2010) who found a significant delay in the mass loss of soy protein isolate films reinforced with NC during thermal degradation.

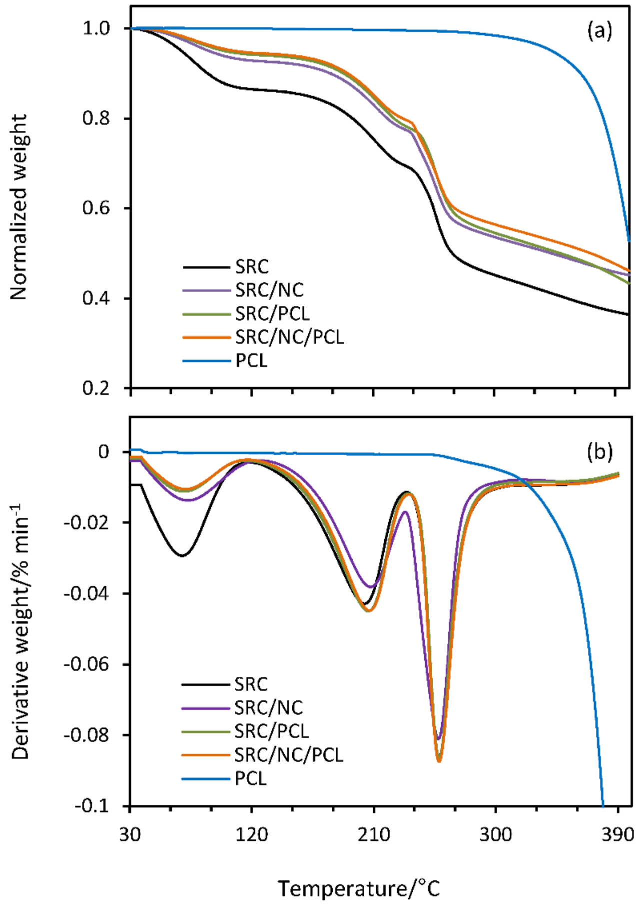


Figure 5-3. Thermograms of SRC/NC/PCL film formulations: (a) TGA thermogram and (b) derivative dTGA thermogram. Samples were heated under nitrogen (flow rate 20 mL min^{-1}) at a heating rate of 10°C min^{-1}. Color image available online.

The DSC thermograms in **Fig. 5-4** demonstrate that the incorporation of NC increased the glass transition temperature (T_g) of the SRC from 133 to 163 °C that is also associated with the melting temperature of the polymer, whereas the presence of the PCL layer had little effect on the T_g of the SRC substrate. The relatively sharp exothermic peak at 222 °C observed in **Figures 5-4(a)**, **(b)** and **(d)** is attributed to the decomposition temperature of the SRC substrate and/or glycerol component (Sedayu et al., 2018) which is less intense in the case of the PCL-laminated SRC sample (**Fig. 5-4(c)**. At temperatures greater than 222 °C, convoluted peaks attributed to the incipient stage of the decomposition of the main SRC polymers are visible with a more intense peak observable at 265 °C which is consistent with the TGA data (see **Fig. 5-3**). The intensity of the peak at 265 °C is reduced in the presence of the NC confirming the results of the TGA experiments that suggest the NC may stabilize the substrate to some extent. Moreover, the PCL layer began to melt at *ca*. 60 °C and this molten polymer may subsequently protect the SRC films during further heating that indirectly leads to an increase in the thermal stability of the SRC film.

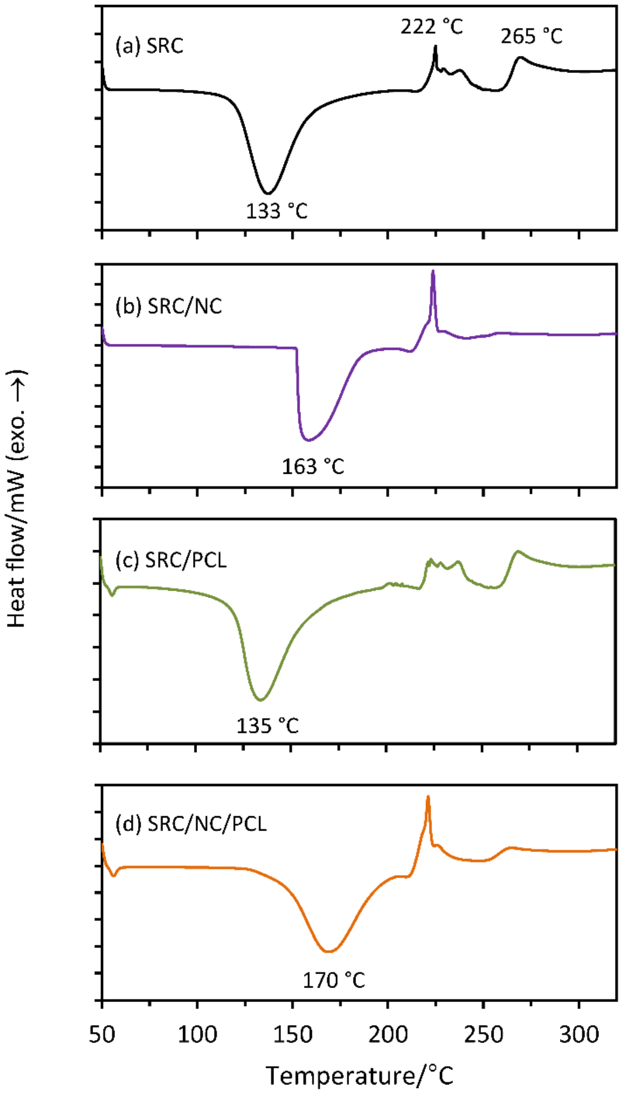


Figure 5-4. The DSC thermograms of SRC/NC/PCL film formulations: (a) SRC, (b) SRC/NC, (c) SRC/PCL and (d) SRC/NC/PCL. Samples were heated under nitrogen (flow rate 20 mL min^{-1}) at a heating rate of 10°C min^{-1}. Color image available online.

5.4.5 Structural Properties

Fourier transform infrared spectra were obtained to investigate the interaction between the SRC film, NC particles and the PCL layer. **Figure 5-5** shows the spectrum of each of the film samples where the laminated PCL film spectra were obtained for the PCL layer only.

The spectra of the SRC and SRC/NC films are similar. Broad peaks between 3650 and 3200 cm^{-1} correspond to the hydroxyl groups of the SRC and bentonite clay, and the peaks between 3000 and 2700 cm^{-1} are due to the alkane bonds of the SRC. Peaks at 844, 930, 1035 and 1218 cm^{-1} are typical of the SRC functional groups of the C–O–SO_3 bonds of the d-galactose-4-sulfate, C–O of the 3,6-anhydro-d-galactose, the glycosidic linkage (C–O) of 3,6-anhydro-d-galactose, and the S=O bond of the sulfate ester respectively (Sedayu et al., 2018).

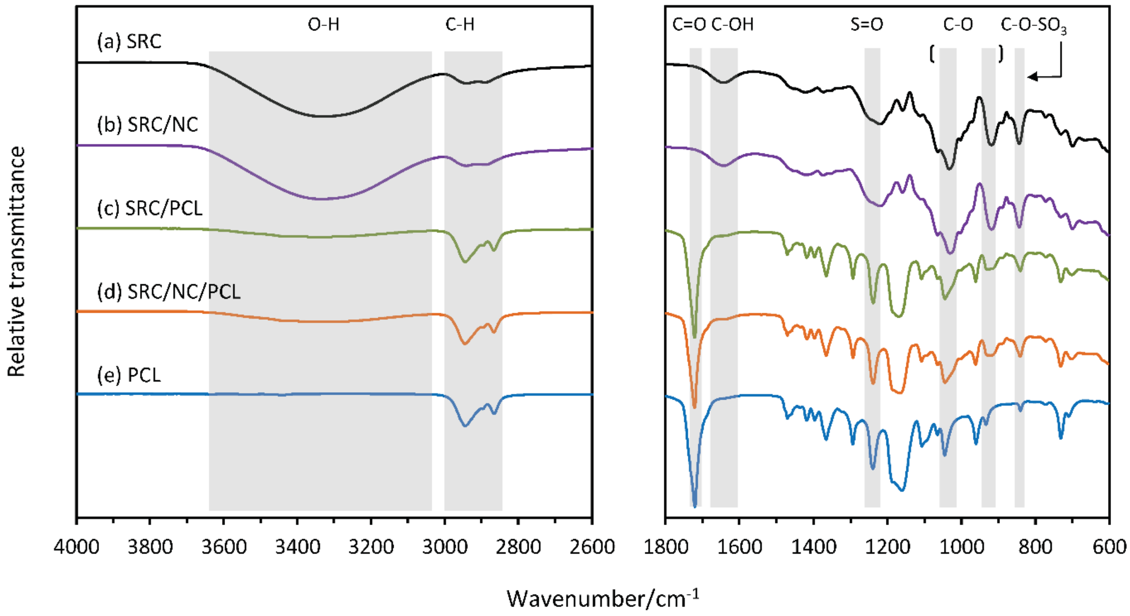


Figure 5-5. FTIR spectra of SRC/NC/PCL film formulations: (a) SRC, (b) SRC/NC, (c) SRC/PCL, (d) SRC/NC/PCL, and (e) PCL. Spectra were recorded using an average of 64 scans at 4 cm^{-1} resolution. For the laminated film specimens, the surface of the PCL layer was placed in contact with the ATR crystal. Color image available online.

The typical bentonite clay peaks that are expected to appear at 660, 1450, and 1100-800 cm^{-1} and are usually attributed to the Si-O-Si, Si-OH and Al-O-Si bonds respectively (Almeida et al., 2017) are overlapped by the SRC bands. The PCL-laminated SRC and SRC/NC films exhibit almost exclusively the PCL vibrational peaks with the exception of there being a slight band corresponding to hydroxyl group absorption between 3650 and 3200 cm^{-1} when compared with the neat PCL film. This may indicate there is no significant, or very little, diffusion between the SRC and PCL polymer molecules at the interface even though an interfacial interaction exists as suggested by the SEM cross-section analysis (see **Fig. 5-1(a)**).

A very small band in the 3650-3200 cm^{-1} region is noticeable in the spectrum of the PCL film that is associated with the hydroxyl group and which may be due to the hydrophobic character of the PCL layer. The other peaks occurring at 2949, 2865, 1720, 1293, 1240, and 1170 cm^{-1} are attributed to common PCL characteristic stretching for asymmetric CH_2, symmetric CH_2, C=O, C-O and C-C, asymmetric C-O-C, and symmetric C-O-C modes respectively (Janarthanan et al., 2019).

5.5 Conclusions

Incorporating hydrophilic bentonite NC into SRC polymer film resulted in a general increase the mechanical properties, but with little or no improvement to the barrier properties. The latter is attributed to possible agglomeration of NC in the SRC matrix but more likely its hydrophilic nature that consequently impacts the water vapor barrier and water resistance. Laminating the SRC and SRC/NC films with PCL, however, increased the barrier properties of the film, compensating for the loss of these properties due to NC incorporation as well as further improved the mechanical properties. The thermal stability of the SRC film was improved by NC incorporation and PCL lamination with the combination of these modifications resulting in the greatest improvement in the overall properties of the SRC film.

CHAPTER 6

Effects of Surface Photo-Crosslinking on Film Properties

6.1 Overview

This chapter presents an investigation of the modification of the surface of semi-refined carrageenan film by photo-crosslinking using sodium benzoate as sensitizer and irradiation under UV light for various exposure times. The overall properties and chemical structure changes as well as the crystallinity changes in the film are presented and discussed. The manuscript entitled: “Effects of Surface Photo-Crosslinking on the Properties of Semi-Refined Carrageenan Film”, Sedayu, B. B., Cran, M. J., Bigger, S. W. has been published in *Food Hydrocolloids*, doi: https://doi.org/10.1016/j.foodhyd.2020.106196

6.2 Introduction

Even though the reinforcement with nanocellulose and nanoclay into the SRC polymer matrix has enhanced the film properties, there may be further improvements made possible by other means. Thus, alternative methods, or combinations of methods, need to be investigated to optimally enhance the properties of SRC film. One such method is crosslinking which results in the modification of the chemical structure of biopolymer films. In principle, crosslinking can be described as the interconnection among the polymer molecules within matrix through some form of bonding such as covalent, ionic or hydrogen bonding. This can subsequently change the network dynamics and molecular mobility within the polymer, facilitate improved structural integrity of the film, and limit the interaction of the polymer with water molecules (Detduangchan et al., 2014). For example, crosslinking has been reported to successfully improve the mechanical properties as well as water vapour barrier of cellulose/starch biocomposite films (Kumar & Singh, 2008).

In general, there are two techniques to obtain a crosslinked polymer in bio-based films, namely physical or photo-crosslinking, and chemical crosslinking. Photo-crosslinking involves the use of photo-radiation and ultraviolet (UV) radiation is most commonly used for this purpose as it is inexpensive, offers easy processing, and can be applied to large-scale processing, particularly in the food industry (Zhou et al., 2009). In the case of chemical crosslinking, bulk-crosslinking is the most common method as it can effectively improve the functional properties of bio-based composites (Mahdiyar Shahbazi et al., 2016). However, bulk-crosslinking is relatively expensive as it requires considerable amounts of chemical crosslinking agents, and it also changes the bulk chemical composition of the biopolymer (Zhou et al., 2009).

Crosslinking that is confined to the surface of the material is an effective means of reducing the amount of crosslinking agent required in the process and also maintaining the chemical composition of the bulk polymer (Zhou et al., 2009). Surface photo-crosslinking of biodegradable films such as thermoplastic starch, polyvinyl alcohol and corn starch, has been shown to successfully reduce the surface hydrophilicity of the films and increase their mechanical strength (Niazi & Broekhuis, 2015; Villarruel et al., 2015; Zhou et al., 2008). Like carrageenan, these materials have a large number of hydroxyl groups in their structure that are capable of undergoing free-radical crosslinking reactions initiated by photosensitizers such as sodium benzoate (Bhat & Karim, 2009). The sensitizer, sodium benzoate, is known to be photo-decomposed under UV irradiation to produce radicals that initiate crosslinking reactions through a hydrogen abstraction mechanism (Delville et al., 2002; Ghosh & Gangopadhyay, 2000). Moreover, sodium benzoate is reported to impart desirable antimicrobial properties to material formulations used in food packaging (Birck et al., 2016; Mondal et al., 2015) which is an added advantage of its choice as a suitable crosslinking agent in such materials.

A study of SRC food packaging film formulations was undertaken to assess the potential improvements to the barrier and mechanical properties of SRC food packaging films that may be imparted by surface crosslinking using sodium benzoate. Semi-refined carrageenan was of particular interest as the base polymer for the formulations in this study in view of its potential use as a cheaper alternative to refined carrageenan as well as its environmental advantages over synthetic polymeric packaging materials.

6.3 Materials and Methods

6.3.1 Materials

Semi-refined carrageenan used in this investigation was the same as the material described in **Sec. 3.3.1**. Glycerol and sodium benzoate were used in the preparation of films as the plasticizer and crosslinking agent respectively and were purchased from Sigma-Aldrich (Australia). Milli-Q water was used as the solvent for the film preparations.

6.3.2 Film Preparation

The SRC film samples were prepared according to the procedure described in **Sec. 4.3.1**.

The crosslinking was achieved by pouring a 30 mL of 6% (w/v) aqueous sodium benzoate solution evenly onto the upper surface of the dried SRC films (200 × 150 mm) where it remained for 2 min before the excess solution was drained away, and the films left to dry for 24 h at 22°C. After that, the samples of film were exposed to UV light in a laminar flow cabinet (Laftech, Australia) equipped with a Philips UV light TUV 30W/G30T8 (USA) for different times *i.e.* 0, 5, 10, 20 and 40 min. The sides of the films containing the photosensitizer coating were directly exposed to the UV source that had a total photon count intensity (integrated between 250 and 400 nm) of 1.4×10^{-6} mol m^{-2} s^{-1}. A sample of SRC film that had not been coated with sodium benzoate or exposed to UV light was used as the control in each of the experiments.

Prior to testing, the films were cut into specimens of the following dimensions: square samples of 20 × 20 mm for the moisture content and water solubility (WS) measurements; rectangular strips of 60 × 12.5 mm for opacity measurements, 50 × 25 mm rectangles for water uptake measurements, 100 × 15 mm rectangles for mechanical property measurements; and circular disks of 11.3 mm diameter for the water vapour permeability (WVP) measurements. All of the tests were performed according to the methods outlined in a previous study (Sedayu et al., 2018) and prior to testing, all samples were preconditioned at 53% RH in a desiccator containing saturated $Mg(NO_3)_2$ solution (Ajax Finechem, Australia) and stored in the dark for at least 24 h.

6.3.3 Physical and Optical Properties

Film thickness, color and opacity were measured following the method described in **Sec. 3.3.2**.

6.3.4 Structural and Thermal Properties

Fourier transform infrared (FTIR) spectra of the film samples were recorded using the method described in **Sec. 3.3.2**. Changes in the crystallinity of the samples brought about by the crosslinking were assessed by X-ray diffraction (XRD) following the method described in **Sec 4.3.9**. The thermal properties of the films were observed using TGA and DSC according to the methods described in **Sec. 4.3.8**.

6.3.5 Water Sensitivity and Moisture Barrier

The moisture content and WVP were measured using the methods described in **Sec. 3.3.2**. The water uptake, water solubility, and WCA were measured in accordance with the methods described in **Sec. 4.3.4**, **Sec. 4.3.5** and **Sec. 4.3.5**, respectively.

6.3.6 Mechanical Testing

The tensile strength (TS), elastic modulus (EM) and elongation at break (EAB) were determined following the methods described in **Sec. 4.3.7**.

All collected experimental data were statistically analysed following the procedure described in **Sec. 3.3.3**.

6.4 Results and Discussion

6.4.1 Surface Colour

The colour parameters pertaining to the surfaces of the SRC films at different times of exposure to the UV source are listed in **Table 6-1** along with the corresponding parameters for the control sample for comparison. Also listed in the table are the colour differences (ΔE values) between the samples.

Table 6-1. Colour parameters of the surface of UV-crosslinked SRC films at different times of UV exposure

UV exposure time /min	L^*	a^*	b^*	ΔE
5	86.93 ± 0.20^{a}	-0.03 ± 0.03^{a}	8.55 ± 0.51^{a}	57.05 ± 0.21^{ab}
10	86.91 ± 0.20^{a}	-0.11 ± 0.05^{b}	8.82 ± 0.39^{ab}	57.14 ± 0.17^{a}
20	86.97 ± 0.23^{a}	-0.18 ± 0.05^{c}	8.78 ± 0.39^{ab}	57.06 ± 0.23^{ab}
40	86.72 ± 0.24^{a}	-0.20 ± 0.05^{c}	9.20 ± 0.31^{b}	56.81 ± 0.23^{b}
control	86.94 ± 0.28^{a}	-0.01 ± 0.01^{a}	8.62 ± 0.42^{a}	57.06 ± 0.28^{ab}

Values are given as mean with a standard deviation. Any two means in the same column with a similar letter are not significantly different ($p > 0.05$) as determined by a Duncan test. The control sample was not treated with sodium benzoate and not exposed to UV irradiation.

Upon inspection of the data in the table as well as visual observations made of the exposed films, it is apparent that the UV-crosslinking treatment has had a minor effect on the colour of the films. All SRC films retained their yellowish translucent appearance with the lightness (L^*) and overall colour difference (ΔE) remaining almost the same as the respective values of the original SRC film. Nonetheless, a reduction in the redness (a^*) possibly compensated for by a slight increase in the yellowness (b^*) with the longer UV irradiation times, can be observed amongst the data in particular for exposure times greater than 10 min. These colour changes may indicate slight photodegradation of the film has occurred under prolonged exposure to the UV source, as has been observed in other bio-based films such as in soy protein (Gennadios et al., 1998) and gelatine-chitosan films (Salami et al., 2019).

6.4.2 Chemical Structure and Morphology

Fourier transform infrared (FTIR) spectroscopy was used to observe any noticeable changes in the chemical structure of the SRC films that were brought about by the crosslinking. The FTIR spectrum of an SRC film crosslinked at 10 min exposure to the UV source is shown in **Fig. 6-1** along with a spectrum of a control film for comparison. The spectrum of sodium benzoate is also shown. The complete spectrum for each film sample can be seen in the **App.3-S1** in the Appendix.

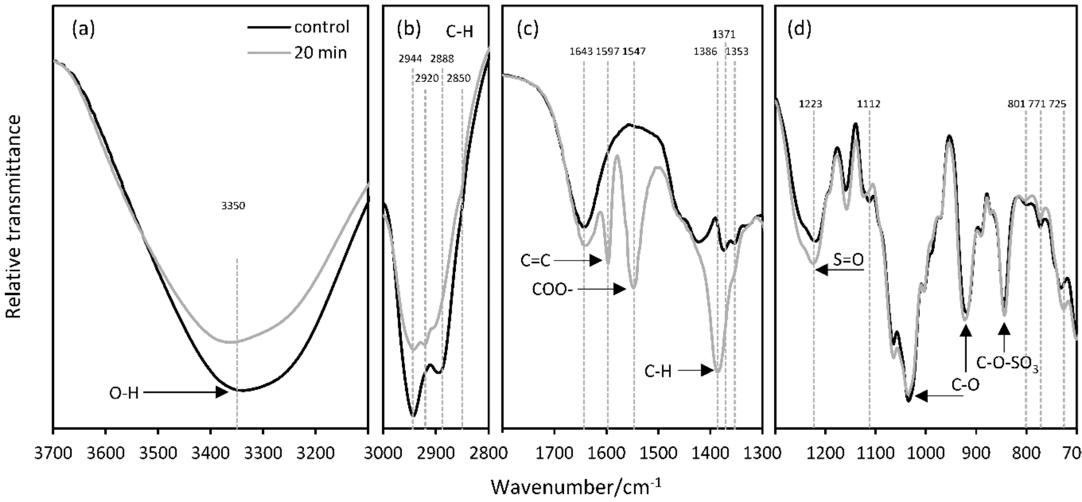


Figure 6-1. FTIR spectra of the control SRC film and SRC film crosslinked at 20 min exposure to UV irradiation over the wavenumber ranges: (a) 3700-3000 cm^{-1}, (b) 3000-2800 cm^{-1}, (c) 1800-1300 cm^{-1}, and (d) 1300-700 cm^{-1}. Spectra are the average of 64 scans.

The crosslinking resulted in a less intense vibrational stretching band at 3700–3000 cm^{-1} that corresponds to free, inter- and intra-hydroxyl group bonding as well as a slightly less intense band at 2990–2830 cm^{-1} attributed to the alkyl C–H stretch associated with aliphatic chains of the SRC polymer (Karbowiak et al., 2011). Additional peaks are also identified in the crosslinked SRC film at 1597, 1548 and 1385 cm^{-1} (see **Fig. 6-1**) and presumably originate from the sodium benzoate. These are attributed respectively to the C=C bonds, asymmetric carboxylate anion (COO^{-}), and C–H bonds of the benzoate ring (Miranda et al., 2001). The remaining vibrational stretches observed at wavenumbers 1225, 1035, 922 and 840 cm^{-1} are respectively attributed to the S=O bonds of sulfate esters, C–O bonds of the glycosidic linkage of 3,6-anhydro-D-galactose, the C–O bond of 3,6-anhydro-D-galactose, and the C–O–SO$_3$ bonds of D-galactose-4-sulfate all of which represent the typical vibrational stretching found in SRC film (Sedayu et al., 2018).

The significant reduction in the hydroxyl band at 3700–3000 cm^{-1} that occurs upon crosslinking of the SRC confirms the notion that crosslinking occurs mainly through hydroxyl groups (Farhan & Hani, 2017) as is observed in other comparable polymer systems (Bhat & Karim, 2009; Sonker et al., 2018). In particular, the UV production of reactive bezoate free radicals induces the abstraction of tertiary hydrogen atoms of the SRC polymer rendering a polymeric radical (Zain et al., 2018) that may further interact with hydroxyl groups within the SRC structure, thus facilitating the crosslinking of the carrageenan polymer chains. Moreover, the reactions of free radicals produced from the sodium benzoate may also contribute to a decrease in the number of C–H bonds of alkanes from aliphatic chains and an increase in the number of the C–O bonds of the glycosidic linkage of 3,6-anhydro-D-galactose from the original SRC polymer.

Shown in **Fig. 6-2** are the X-ray diffraction (XRD) spectra that were collected to observe any changes to the crystallization resulting from the crosslinking. The control SRC film has a semi-crystaline structure with a remarkably broad shoulder at the band between 2θ = 17.8–24.8° due to its amorphous regions along with crystalline peaks identified at 2θ = 14.3°, 17.2°, 25.6° and 29.8°, all of which resembles closely the diffraction spectrum of the Type I cellulose (El Achaby et al., 2018). The peak at 36.4°

corresponds to minerals or unknown impurities typically found in seaweed (Corvaglia et al., 2016; El Achaby et al., 2018).

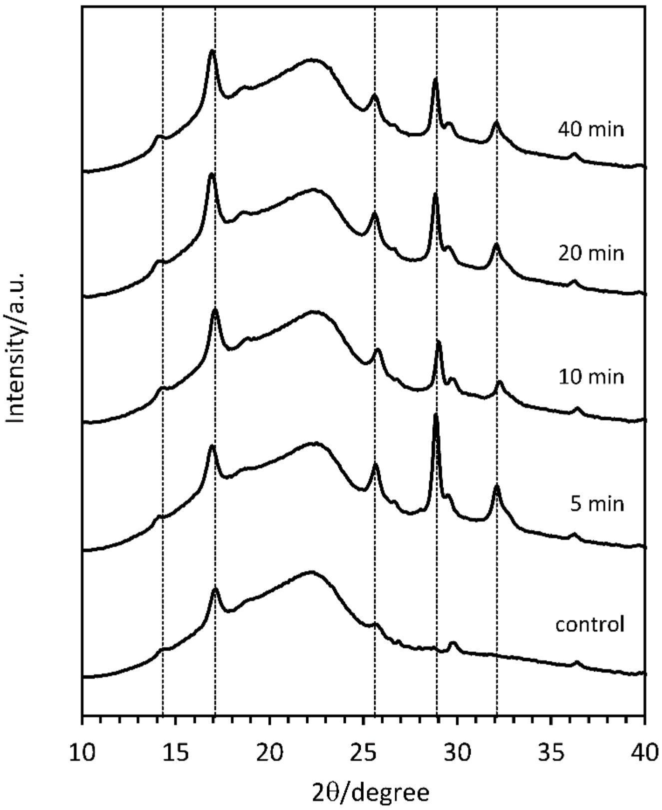


Figure 6-2. X-ray diffraction spectra of the SRC films crosslinked with sodium benzoate at UV exposure times of: (a) 5, (b), 10, (c) 20 and (d) 40 min. The spectrum of the SRC control sample is also shown. The spectra were recorded using Cu Kα radiation (λ = 0.154 nm) at 40 kV and 20 mA.

Relative to the control sample, the intensity of the peaks at 2θ = 14.3°, 17.2° and 25.6° increased with the longer exposure times and additional, prominent, sharp diffraction peaks also appeared at 2θ = 28.9° and 32.1°. Exposure to the light source for 5 min increased the crystallinity of the control film by *ca.* 32% as shown in **Fig. 6-3**. However, it seems that exposure of the samples to the UV source for longer than 5 to 10 min does not produce any significant change (see also **App.3-S2** in the Appendix) which is also consistent with the observations made above on the other properties. This possibly confirms that the crosslinking is confined primarily to the surface regions of the samples and the resulting increase in the crystallinity of the SRC film has occurred due to an increase in the macromolecular chain network (Zhou et al., 2009). The increased crystallinity observed upon crosslinking is also consistent with observations made on crosslinked carboxymethyl cellulose film (Mahdiyar Shahbazi et al., 2016).

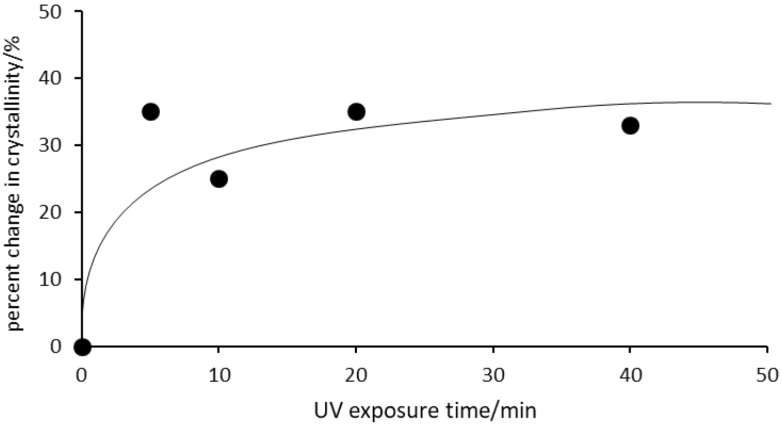


Figure 6-3. Plot of the percentage change in crystallinity of the SRC film at different UV exposure times. The data were determined by measuring the percentage change in the area under the crystalline diffraction peaks of the XRD diffraction spectrum relative to the unexposed (control) sample.

6.4.3 Thermal Properties

The thermal degradation behaviour of crosslinked films in comparison with the control SRC film was evaluated by thermogravimetric analysis (TGA) and the results are shown in **Fig. 6-4** in terms of the normalized mass loss as a function of temperature and the corresponding derivative plots (d*m*/d*t*) of the normalized mass loss. The TGA profile of the control sample is markedly different to those of the crosslinked samples which in turn, are almost superimposable on one another with only slight differences able to be seen in the overlayed TGA profiles.

The initial mass loss that occurs at *ca*. 100°C in the control sample is attributed to the loss of water in the sample. The corresponding mass losses in each of the crosslinked samples are significantly less suggesting that these samples have an inherently lower moisture content than the control. The second stage in the decomposition of the control sample that is clearly visible between *ca*. 170–225°C is attributed to the volatilization of plasticizer (glycerol) from the polymer matrix (Rhim, 2013). Interestingly, this second stage of decomposition appears to be almost absent in the case of the crosslinked films, an observation that is supported more readily by the differential plot of the data shown also in **Fig. 6-4**.

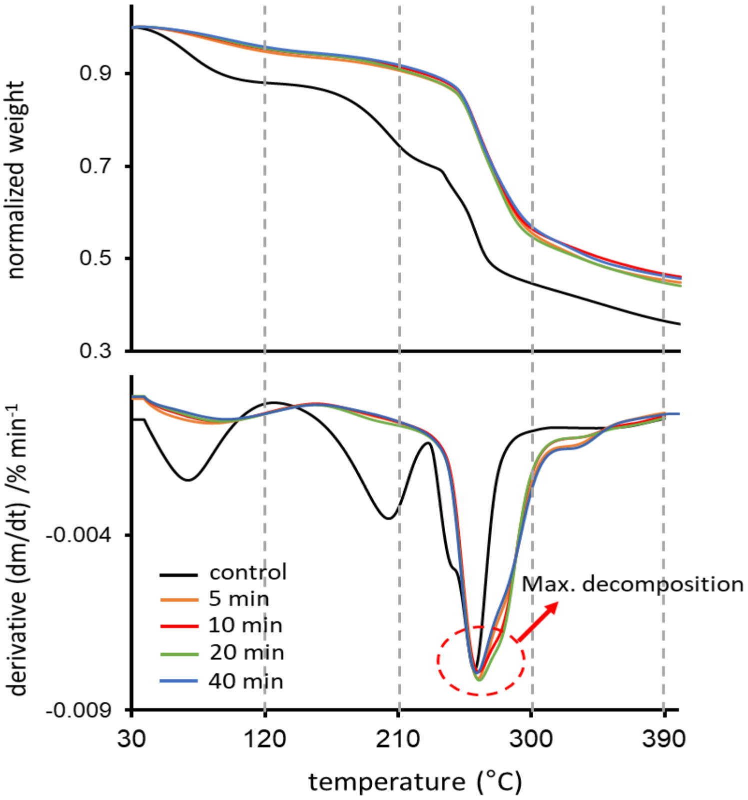


Figure 6-4. Plots of the normalized mass loss and its derivative as a function of temperature for SRC films crosslinked with sodium benzoate at UV exposure times of: (a) 5, (b), 10, (c) 20 and (d) 40 min. The plots for the control sample are also shown. Samples were heated from 30 to 400°C at a heating rate of 10°C min^{-1} under a nitrogen atmosphere (20 mL min^{-1} flow rate).

It is possible that the volatilization of the plasticizer in the crosslinked films may be shifted to higher temperatures due to the egress of the plasticizer having been inhibited by the crosslinking. In particular, such delayed volatilization may be ascribed to stronger hydrogen bonding and covalent bonding near or at the surface of the polymer brought about by the crosslinking (Zain et al., 2018). As a result, the plasticizer may have been degraded along with the carrageenan at a later stage in the profile. The third stage shown in the TGA profiles corresponds to the thermal degradation of the carrageenan polymer and occurs within the temperature range of *ca*. 240–275°C for the control SRC film and *ca*. 240–300°C for the crosslinked films. The slightly broader temperature range observed in the case of the crosslinked films might also be explained similarly by there being a stronger polymer chain network, as already suggested.

Table 6-2 lists numerical data extracted from the thermograms shown in **Fig. 6-4**. These data indicate the crosslinking slightly shifted the onset temperature of the decomposition of carrageenan from *ca*. 249°C to an average of *ca*. 253°C and slightly shifted the temperature at which the maximum rate of decomposition occurred from *ca*. 263°C to an average of *ca*. 266°C thereby consistent with there being enhanced thermal stability of the crosslinked films. At UV exposure times longer than 5 min, however, there appears to be no further increase in the thermal stability of the SRC.

The mass loss data at 100°C in the table suggest that the inherent moisture content in the crosslinked films is on average *ca*. 69% less than the control and that there is a steadily decreasing trend in the moisture content with an increasing period of UV exposure. Such a trend is not observed in the data for 275°C and 400°C, however, the average mass loss of the crosslinked samples at 275°C is *ca*. 41% less than the control confirming the notion that the crosslinking has thermally stabilized the SRC to some extent. Similarly, at the extreme temperature of 400°C the average mass loss of the samples is *ca*. 15% less than the control thereby leading to the same conclusion.

Table 6-2. Decomposition temperatures and mass loss data of UV-crosslinked SRC films at different times of UV exposure

UV exposure time /min	Decomposition Temperature /°C		Mass loss /%		
	T_{onset}	$T_{max.}$	100°C	275°C	400°C
5	253	266	3.94	32.27	55.19
10	254	266	3.39	31.10	53.96
20	253	267	3.25	32.71	55.93
40	253	265	2.95	31.07	54.34
Average	253	266	3.38	31,79	54.74
control	249	263	11.06	54.32	64.20
% difference			69	41	15

The control sample was not treated with sodium benzoate and not exposed to UV irradiation.

The effects of crosslinking on the thermal properties of the SRC films are also depicted in the DSC thermograms shown in **Fig. 6-5** where the broad, endothermic peak assigned to the glass transition temperature (T_g) of the SRC is observed to shift from *ca*. 163°C to *ca*. 174° in the case of the sample crosslinked at 10 min exposure to the UV source. The downward shift in the T_g values for samples exposed to the UV source for 20 and 40 min may be due to photodecomposition having occurred in these samples. The elevated T_g value of all crosslinked samples compared with that of the control is consistent with the increased crystallinity indicated by the XRD results (see

Fig. 6-2) and the overall increased thermal stability indicated by the TGA results (see **Fig. 6-3**). The latter is also supported by the significant shift in the exothermic peaks towards higher temperatures (see **Fig. 6-4**) that are attributed to the decomposition of the SRC film.

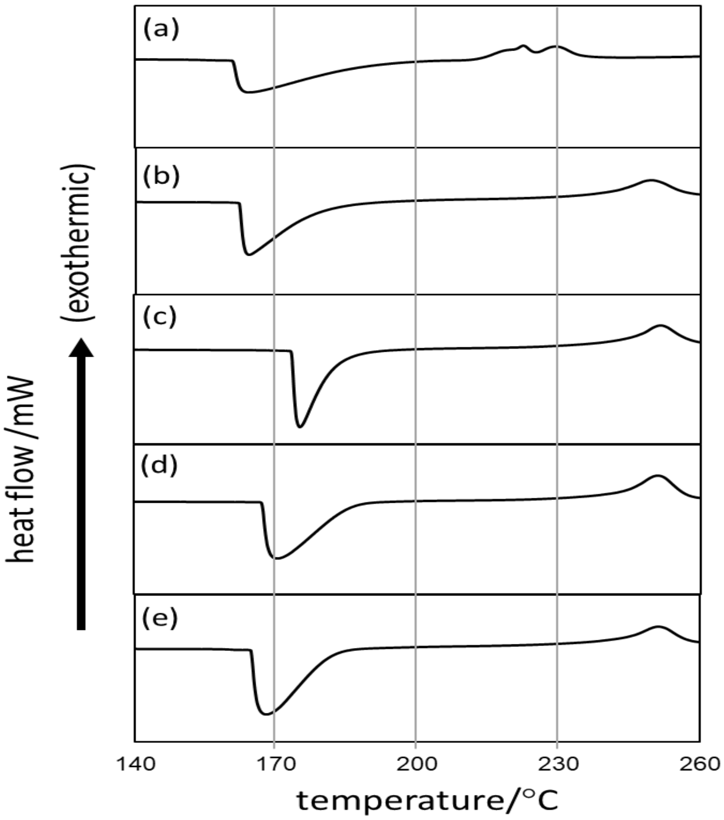


Figure 6-5. The DSC thermograms of SRC films crosslinked with sodium benzoate at UV exposure times of: (b) 5, (c) 10, (d) 20 and (d) 20 min. The thermogram of the control sample is also shown (a). Samples were heated under nitrogen (flow rate 20 mL min^{-1}) from 50 to 320°C at a heating rate of 10°C min^{-1}.

6.4.4 Mechanical Properties

Inter- and intra-molecular bonding of polymer chains in the matrix are the major factors contributing to the final mechanical properties of a polymeric film (Mahdiyar Shahbazi et al., 2016). The effects of crosslinking of SRC films on the TS, EM and EAB are shown in **Fig. 6-6**. The values of the mechanical properties for the control are also listed for comparison.

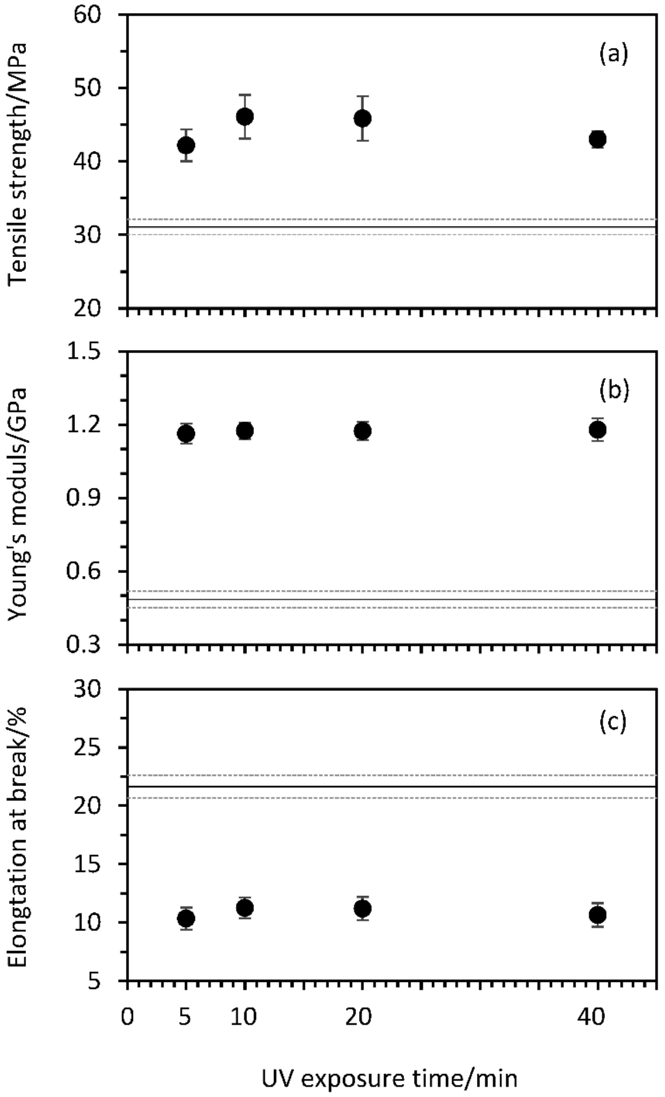


Figure 6-6. Plots of: (a) tensile strength, (b) elastic modulus, and (c) elongation at break of the UV-crosslinked SRC films at different times of UV exposure in comparison with the control. The control sample was not treated with sodium benzoate and not exposed to UV irradiation.

The TS values of the crosslinked films were on average *ca*. 42% greater than the control. These values increased with an increasing time of exposure to the UV source with a *ca*. 55% increase compared to the control reached at 20 min after which the TS dropped significantly at 40 min of UV exposure presumably due to degradation. The EM values were on average *ca*. 142% greater than the control and the EM was found to increase most dramatically after 10 min exposure, attaining a *ca*. 144% increase over the control after which there appears to be a levelling of the data. The increase in the TS and EM is consistent with the increased crystallinity revealed in the XRD data (see **Fig. 6-3**) and DSC data (see **Fig. 6-5**). As expected, the EAB values of the crosslinked films were considerably less than that of the control (on average *ca*. 50% lower than the control) and these values appear to follow a similar trend to the TS values in that a maximum was reached at 20 min UV exposure after which a decrease occurred.

The observed changes to the tensile properties upon crosslinking are due to intermolecular bridges formed among the carrageenan chains in the matrix which contribute to a prominent, firmer and stiffer structure (Mahdiyar Shahbazi et al., 2016). The increase in the TS corresponds to an increased level of crosslinking within the SRC matrix which, in turn, is determined by the duration of exposure to the UV source (Decker & Moussa, 1988). However, a sufficiently long period of UV exposure (or a higher UV intensity) may also promote rapid chain cleavage of the SRC that may equally degrade the polymer structure (Khan et al., 2006) evidence of which can be seen in the colour parameters listed in **Table 6-1** corresponding to the higher levels of exposure. Hence, there is a maximum time for which samples should be exposed to the UV source in order to optimize the mechanical properties such as the TS that are imparted by crosslinking. The results in **Table 6-2** suggest that such a time is *ca*. 20 min for the SRC films under the conditions used in this study.

6.4.5 Water Sensitivity and Barrier Properties

Figure 6-7 shows the water sensitivity and water barrier properties of UV-crosslinked SRC samples along with the corresponding values of the control for comparison. The crosslinking resulted in a significant decrease in the moisture content of the SRC samples, the average moisture content of the crosslinked samples being *ca*. 50% less than the control (see **Fig. 6-7a**). This arises presumably due to the reduced number of hydroxyl groups present in the crosslinked polymer that are available to interact with and retain water molecules as well as the increased crystallinity (see **Fig. 6-2**) that reduces the extent of the amorphous regions where water can be accommodated. The result is also consistent with the TGA analysis (see **Table 6-2**) in so far as crosslinking reduces the inherent moisture content of SRC and there appears to be a downward trend in the moisture content with increasing time of UV exposure. However, the result is inconsistent with the magnitude of the difference with respect to the control as determined in the TGA experiments, where the result was found to be an average *ca*. 69% less than the control. The discrepancy may be due to limitations in the accuracy to which the onset and completion of convoluted transitions in thermograms derived from TGA experiments can be resolved with the software currently available.

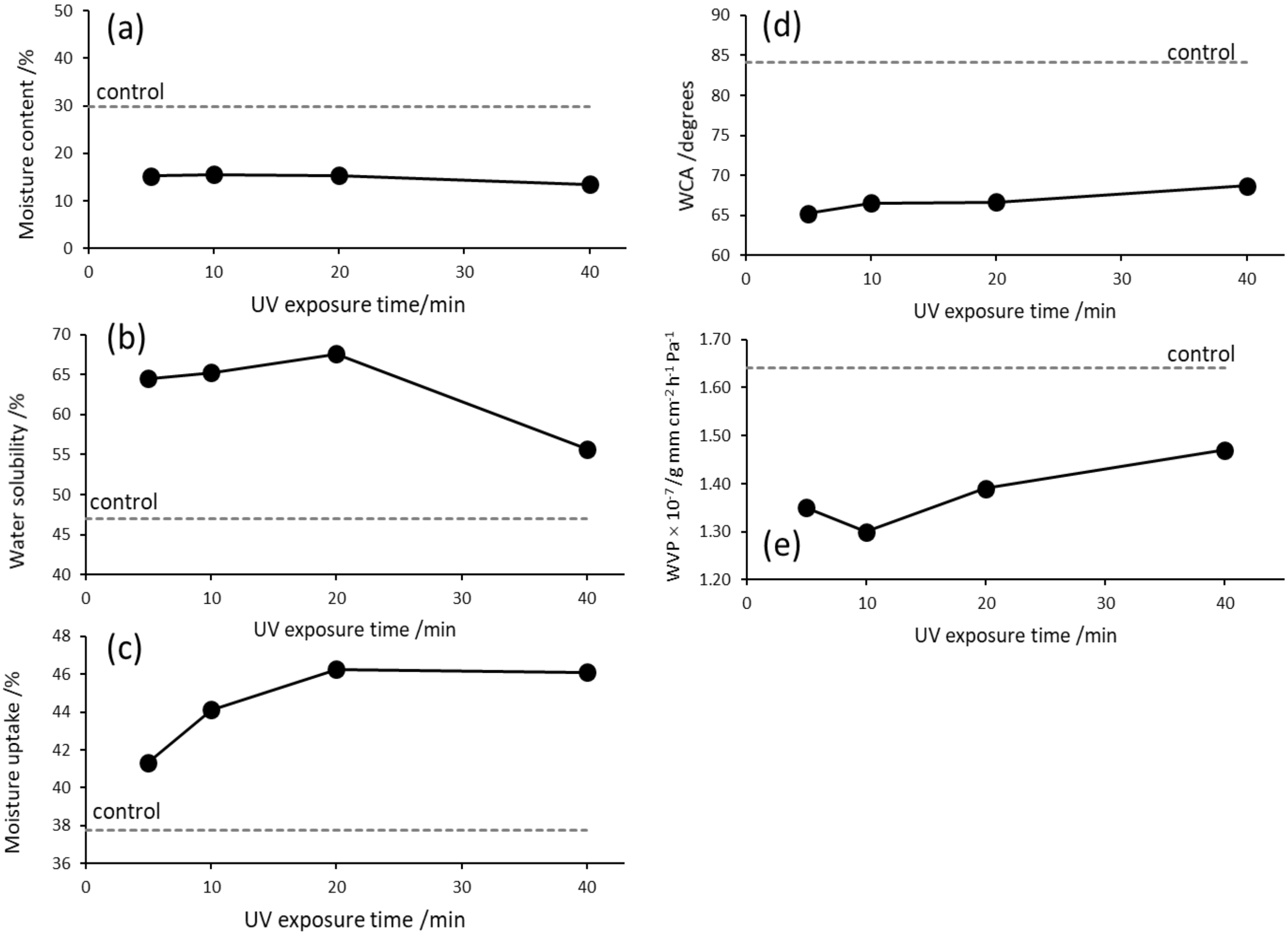


Figure 6-7. Plots of: (a) moisture content, (b) WS, (c) moisture uptake, (d) WCA, and (e) WVP of the UV-crosslinked SRC films at different times of UV exposure in comparison with the control. The control sample was not treated with sodium benzoate and not exposed to UV irradiation.

The WS values are on average *ca*. 35% higher than the control which is an unexpected result (see **Fig. 6-7b**). Nonetheless, the WS value seems to be decreased at 40 min exposure suggesting a possible downward trend in the value at much longer exposure times. Similarly, the moisture uptake is unexpectedly higher than the control showing an upward trend with exposure time that levels off at *ca*. 46% (i.e. about 22% higher than the control) (see **Fig. 6-7c**). Furthermore, the WS and moisture uptake results are consistent with the WCA results which suggests the crosslinked samples have a higher affinity for water than the control with the WCA for the samples being on average *ca*. 21% lower than the control (see **Fig. 6-7d**).

Collectively, the WS, moisture uptake and WCA results all suggest that crosslinking has sensitized the material to water. This apparent increase in the water sensitivity of the SRC may be due to the presence of residual sodium benzoate present on or near the surface of the crosslinked SRC films. Sodium benzoate is a hygroscopic compound that is readily soluble in water (solubility 62.7 g per 100 mL at 20°C) and so the presence of any residual sodium benzoate in the sample will render it more hygroscopic which, in turn, enables it to absorb more water. The FTIR spectra in **Fig. 6-1**, as well as the downward trend in the WS value after an exposure time of 20 min suggesting further reaction of the photosensitizer is possible, for example, provide evidence of the existence of residual sodium benzoate and thus support the previous contention. The results might be explained further by the photodegradation of the polymer during exposure to the UV source during which pores and microcracks in the polymer structure may be created (Zhou et al., 2008) that consequently deteriorate the water sensitivity properties of the film. Evidence of photodegradation can be seen in the results of the surface colour measurements (**Table 6-1**) and the shifts in T_g values revealed by the DSC data (see **Fig. 6-5**).

The WVP values are favourable in comparison with the control as these are on average *ca*. 16% less than the control with an apparent minimum that is *ca*. 21 % less than the control occurring at an exposure time of 10 min (see **Fig. 6-7e**). A similar enhancement of the water vapour barrier properties by crosslinking as seen in the current study have also been reported in the case of starch and chitosan films (Delville et al., 2002; Kumar & Singh, 2008; Liang et al., 2019). There is an overall upward trend in the WVP values with increasing time of exposure which is consistent with the notion that photodegradation at higher levels of exposure may lead to pores, microcracks, etc., that render the material more permeable to water. Nonetheless, the overall decrease in the transmission rate of water molecules through the film that has occurred due to crosslinking, and subsequently the enhanced WVP values, can be explained by: (i) the decrease in hydroxyl groups (see **Fig. 6-1**) that are capable of facilitating the transfer of water molecules (Müller et al., 2011), (ii) the formation of intermolecular bridges (Zhou et al., 2008) within the matrix and (iii) increased crystallinity (see **Fig. 6-2**) causing there to be less amorphous regions in the polymer and thereby increasing the tortuosity within the polymer matrix (Mahdiyar Shahbazi et al., 2016).

6.5 Conclusions

The photo-crosslinking of SRC film at or near the surface using UV irradiation and sodium benzoate as the photosensitizer appears to have only a minor effect on the colour of the film but there is evidence of photodegradation at longer exposure times. The crosslinking increases the crystallinity and the number of hydroxyl groups in the substrate due to the crosslinking reactions and possible other reactions initiated by the photosensitizer. The morphological and chemical changes brought about by crosslinking, particularly at the lower levels of UV exposure, impart thermal stability to the bulk of the material but perhaps more importantly, enhance the tensile strength and modulus with an expected concomitant decrease in the elongation at break. The crosslinking also decreases the transmission of water through the film as well as decreases the inherent moisture content. However, it appears that the presence of residual photosensitizer in SRC film increases the water sensitivity.

This study has demonstrated the potential to enhance the mechanical and barrier properties of SRC films by photo-crosslinking these with a view to producing cheaper food packaging materials with enhanced properties from renewable resources and which have little impact on the land environment. However, in order to fully realize the benefits that SRC films may deliver in the future, further work needs to be performed on optimizing the exposure conditions (time, intensity, etc.) as well as the concentration of photosensitizer used in the coating. These measures are needed to overcome the shortcomings identified in this study associated with the photodegradation of the substrate and the presence of residual photosensitizer. Work is currently continuing in our laboratory to address these.

CHAPTER 7

CONCLUSIONS

7.1 General Conclusions

Semi-refined carrageenan has been processed into a biodegradable plastic film by using a solution casting method resulting in a visually yellowish-translucent film that was inherently brittle and highly sensitive to water. The poor clarity and flexibility of the resultant SRC film in comparison with its counterpart, refined-carrageenan film, are mainly due to the presence of natural impurities that are mostly residual cellulose, whereby the poor water sensitivity is attributed to the large number of hydroxyl groups in the polymer chains. Such properties thus constrain the SRC film usage for real food packaging application in particular for high moisture-containing food products. The incorporation of glycerol as a plasticizer improved the mechanical properties of the SRC film in so far as it reduced brittleness and increased the tensile strength and stretchability. It also enhanced the optical properties by creating a smoother film surface as well as increased the thermal stability of the film. Nonetheless, the glycerol addition increased the hydroxyl group content in the SRC matrix which, in turn, deteriorated its moisture barrier properties.

The impurities in SRC were also observed to affect the efficiency of NCF reinforcement in the polymer film. Even though NCF reinforcement improved the tensile properties overall, the water sensitivity and moisture barrier of the SRC film did not reach a similar level of the improvement to that observed in the case of RC film. The threshold amount of NCF was higher than that observed for RC film, whereas NCF loadings above the threshold brought about a levelling off or a decrease in the water sensitivity, moisture barrier, and tensile properties that was caused by possible self-agglomeration of the NCF particles. On the other hand, the presence of solid impurities such as cellulose and minerals in SRC film matrix resulted in the SRC film formulation having a more hydrophobic surface than the RC films. In addition, the impurities were also observed to interact with NCF particles contributing a delayed self-agglomeration of the NCF particles within the SRC polymer matrix. The incorporation of glycerol in SRC reinforced with NCF resulted in a higher thermal stability of the films which was attributed to the increased crystalline regions in the polymer matrix.

Similar to the results obtained by NCF inclusion, enhanced mechanical and thermal properties of SRC films were also achieved by hydrophilic bentonite NC reinforcement with effects on the polymer matrix similar to those observed in the case of NCF reinforcement. Whilst the PCL lamination of the SRC and SRC/NC film surface considerably decreased the water sensitivity and moisture barrier of the resultant film as it creates a hydrophobic surface area of the films. The PCL lamination was found to enhance the overall mechanical properties and thermal stability of the SRC film, with the greatest enhancement of properties being generally achieved by the combination of NC reinforcement and PCL lamination.

The modification of the surface of SRC film by photo-crosslinking using sodium benzoate prominently increased the rigidity of the SRC film. In addition to the crosslinking itself, this result is attributed to the increase in crystallinity of the SRC polymer. Crosslinking subsequently increased the thermal stability of the films which was reflected by a higher melting temperature and delayed polymer decomposition during heating. The crosslinked SRC film exhibited lower water vapour permeability than the control film, however the overall water sensitivity of the crosslinked-SRC film was increased by the presence of residual sodium benzoate on or near the surface of the film as well as photodegradation that had occurred during prolonged UV exposure.

The current investigations have clearly demonstrated the potential to improve the mechanical, barrier, and thermal properties of the SRC films by the incorporation of plasticizer, reinforcement of nanomaterials, surface lamination, and photo-crosslinking. Such systems show great promise for the future production of cheaper food packaging materials from renewable resources and that have little impact on the land environment.

7.2 Recommendation for Future Work

Success in delivering from laboratory-scale work a real applicable food packaging product on a commercial scale that that has been derived from a marine bio-based film will bring huge benefits to people, nature, and also wildlife in the future. The public concern on reducing synthetic plastic has recently arisen globally however it is almost impossible to fully remove the use of synthetic plastics in human daily life.

The present study is an early stage in development of a relatively new biomaterial derived from a marine resource namely semi-refined carrageenan, which shows great potential as an alternative material among other bio-material sources. This study has demonstrated the overall improvement of properties including the mechanical, physical, thermal, and moisture barrier properties that position SRC film production in readiness to be explored for scale-up with a view to implementation perhaps in commercial production. Rigid food packaging applications could also be explored in this regard.

To obtain SRC film properties comparable to petroleum-based films, further experimental work is needed particularly aimed at improving the water resistance and stretchability of the SRC film formulations. Film modification by blending with water-resistant polymers including chemical modification to obtain a miscible blend can be potentially investigated.

In the current investigation, the enhanced SRC film properties was successfully prepared using a solvent casting method. However, in order to fully realize the benefits that SRC films may deliver in the future, further investigations need to be performed particularly with regard to other film formation techniques. To this end, it is recommended that a continuous production system for SRC film be investigated in the future by using a polymer extruder. Such an investigation will need to determine the optimum processing conditions such as the possibility of any pre-treatment processing, the processing temperature, as well as the incorporation of additives such as stabilizers that may be required.

Furthermore, the transformation SRC into thick-section packaging by injection molding may also be investigated along with the testing of the effectiveness of SRC films and thick-section packaging for food preservation are exciting possibilities for this hitherto little explored resource.

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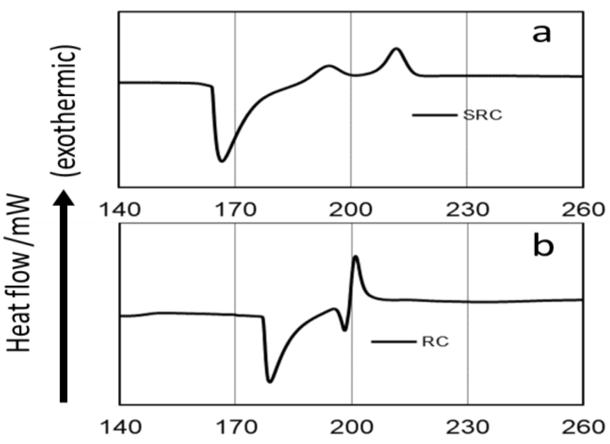
Appendix 1. Supplementary Material Chapter 4

App. 1-S1. Figure of DSC thermographs of (a) SRC and (b) RC powder



App. 1-S2. Film thickness and moisture content of the SRC/NCF and RC/NCF films

Film samples	Film thickness/mm		Moisture content/%	
	SRC	RC	SRC	RC
NCF 0%	0.066 ± 0.004^{a}	0.039 ± 0.006^{A}	24.54 ± 0.92^{a}	24.86 ± 1.22^{A}
NCF 1%	0.064 ± 0.002^{a}	0.042 ± 0.003^{ABC}	24.21 ± 0.10^{a}	24.93 ± 0.81^{A}
NCF 3%	0.064 ± 0.003^{a}	0.042 ± 0.006^{AB}	23.79 ± 0.30^{ab}	25.21 ± 0.90^{A}
NCF 5%	0.065 ± 0.003^{a}	0.047 ± 0.003^{C}	$23.82 \pm 0\ .96^{ab}$	25.51 ± 0.51^{A}
NCF 7%	0.060 ± 0.002^{b}	0.043 ± 0.001^{BC}	22.56 ± 1.49^{b}	25.41 ± 1.16^{A}

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

App. 1-S3. Opacity of the SRC and RC films

Film samples	Opacity	
	SRC	RC
NCF 0%	12.45 ± 0.37^{a}	1.12 ± 0.04^{A}
NCF 1%	12.55 ± 0.32^{a}	1.16 ± 0.12^{AB}
NCF 3%	12.83 ± 0.51^{ab}	1.20 ± 0.07^{B}
NCF 5%	13.06 ± 0.52^{b}	1.49 ± 0.11^{C}
NCF 7%	14.21 ± 0.49^{c}	1.58 ± 0.06^{D}

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

App. 1-S4. Table of decomposition profiles of the SRC and RC films

Film samples	2nd decomposition peak /°C	Onset temp. /°C	Endset temp. /°C	Residue at 225°C /%	3rd (the max.) decomposition peak /°C	Onset temp. /°C	Endset temp. /°C	residue at 400°C /%
SRC	200.11	170.77	214.21	78.73	257.87	245.6	268.99	44.11
SRC/NCF1%	193.5	164.46	208.51	78.8	257.61	245.16	269.71	44.54
SRC/NCF3%	195.48	166.89	210.14	78.68	257.67	245.6	268.53	43.76
SRC/NCF5%	195.21	166.07	209.69	78.35	256.71	244.7	269.27	44.27
SRC/NCF7%	192.85	164.09	207.74	78.27	257.46	245.28	270.19	43.02
RC	219.58	197.42	224.67	71.63	235.81	243.34	239.76	38.68
RC/NCF1%	219.73	200.54	223.97	77.88	235.38	242.01	238.61	44.27
RC/NCF3%	219.8	199.71	224.07	79.49	234.95	244.19	239.06	45.74
RC/NCF5%	221.1	201.13	225.35	80.07	236.82	244.7	240.23	45.76
RC/NCF7%	220.21	193.03	225.17	78.04	236.15	244.98	240.16	45.37
NCF					335.83	303.14	353.48	32.68

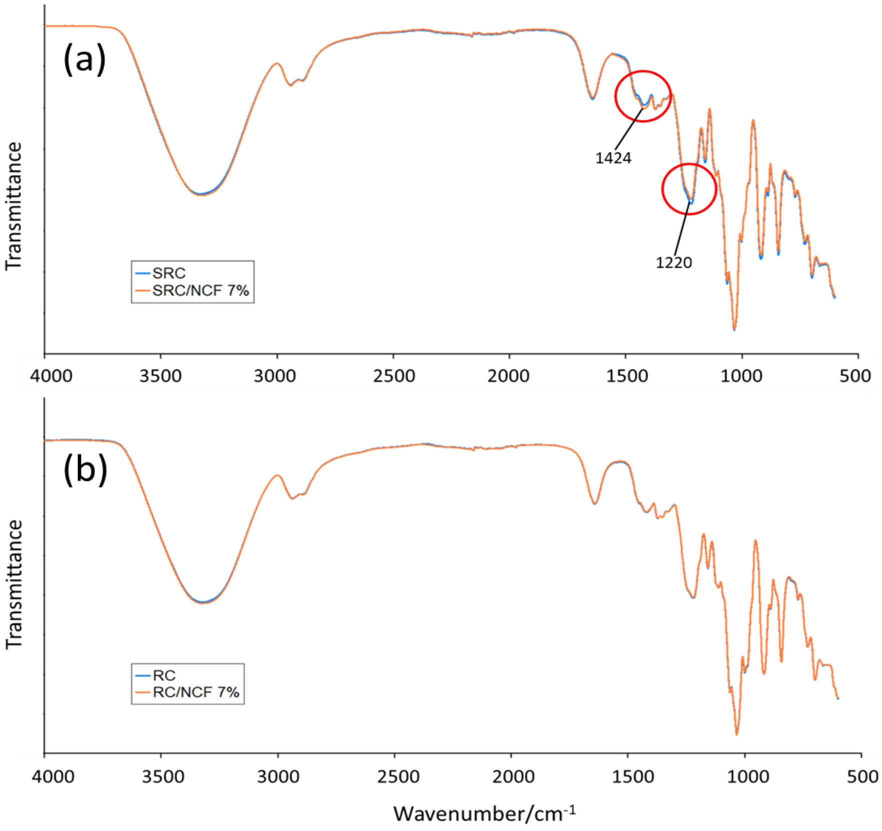
App. 1-S5. Table of DSC profiles of SRC/NCF and RC/NCF Films

Samples	**T_g /°C**	**Enthalpy /mJ**	**Decomposition peaks/°C**
SRC/NCF 0%	172	-2516.09	
SRC/NCF 1%	176	-447.07	
SRC/NCF 3%	178	-450.05	
SRC/NCF 5%	172	-492.34	
SRC/NCF 7%	175	-902.07	
RC/NCF 0%	162	-336.34	224.72
RC/NCF 1%	178	-295.79	223.77
RC/NCF 3%	176	-174.52	224.76
RC/NCF 5%	171	-316.78	223.14
RC/NCF 7%	178	-398.13	230.61
NCF	153.16	219.38	>260

App. 1-S6. Table of Degree of Crystallinity (DOC) of SRC/NCF and RC/NCF Films

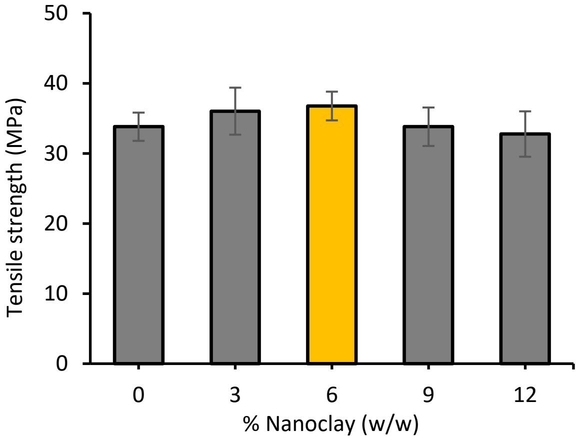
Samples	**% Crystallinity**
SRC	56.05
SRC/NCF 1%	63.82
SRC/NCF 3%	71.20
SRC/NCF 5%	70.20
SRC/NCF 7%	55.09
RC	60.53
RC/NCF 1%	66.18
RC/NCF 3%	64.63
RC/NCF 5%	60.05
RC/NCF 7%	60.92
NCF	78.06

App. 1-S7. FTIR spectra of the (a) SRC/NCF films and (b) RC/NCF films formulations

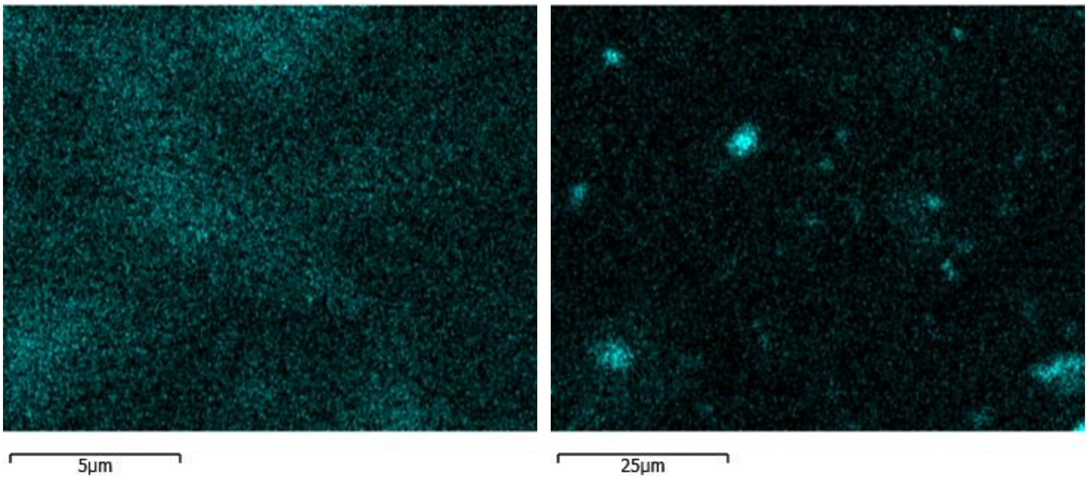


Appendix 2. Supplementary Material Chapter 5

App. 2-S1. Tensile strength of SRC incorporated with different levels of hydrophilic bentonite nanoclay. The level of 6% (w/w) highlighted in yellow demonstrated the optimum value of TS.



App. 2-S2. EDS images showing silica dispersion with different types of nanoclays incorporated in SRC films. Hydrophilic nanoclay (left) shows greater dispersion with the less hydrophilic nanoclay (right) showing silica agglomeration.

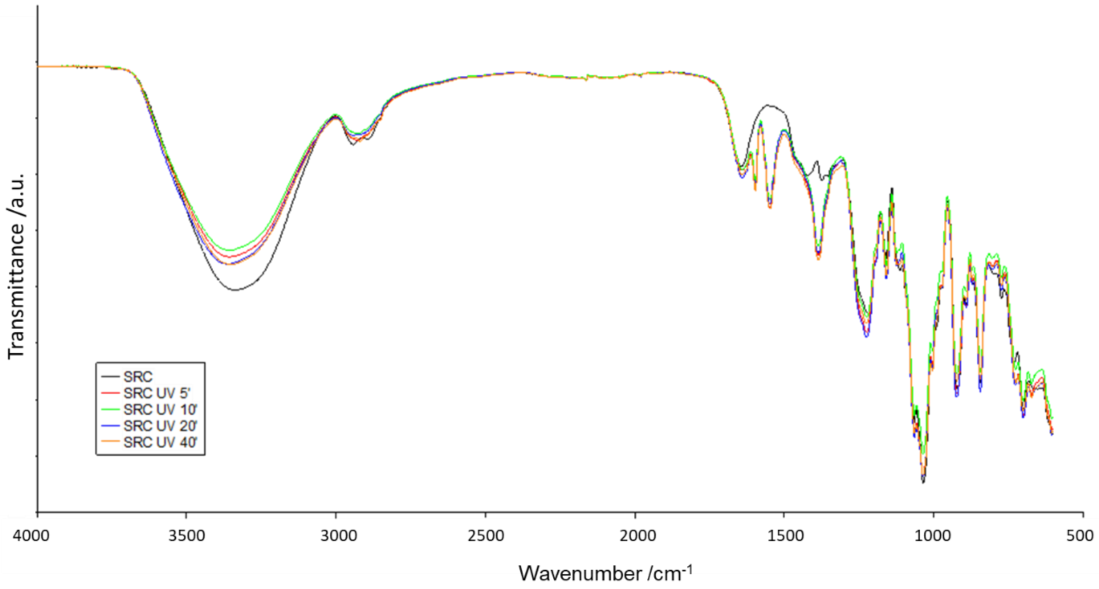


SRC film incorporated with hydrophilic bentonite nanoclay

SRC film incorporated with less hydrophilic nanoclay (Cloisite 30b)

Appendix 3. Supplementary Material Chapter 6

App. 3-S1. FTIR spectra of crosslinked SRC films recorded after: 0, 5, 10, 20 and 40 min exposure to the UV source. Spectra are the average of 64 scans.



App. 3-S2. Percentage crystallinity of the crosslinked SRC films at different times of UV exposure

UV Irradiated SRC Films /min	% Crystallinity (abs. unit)
Control	34
5	69
10	59
20	69
40	67

App. 3-S3. Tensile strength, elastic modulus and elongation at break of UV-crosslinked SRC films at different times of UV exposure

UV exposure time /min	TS /MPa	EM /MPa	EAB /%
5	42.20 ± 2.15^{b}	1163 ± 41^{b}	10.34 ± 0.94^{b}
10	43.53 ± 1.86^{b}	1180 ± 36^{b}	10.78 ± 0.85^{bc}
20	48.24 ± 1.66^{c}	1170 ± 34^{b}	11.66 ± 0.77^{c}
40	43.02 ± 1.14^{b}	1179 ± 46^{b}	10.64 ± 1.01^{bc}
control	31.10 ± 1.04^{a}	484 ± 34^{a}	21.65 ± 0.97^{a}

Values are given as mean with a standard deviation. Any two means in the same column with a similar letter are not significantly different ($p > 0.05$) as determined by a Duncan test. The control sample was not treated with sodium benzoate and not exposed to UV irradiation.

App. 3-S4. Water sensitivity and water barrier properties of UV-crosslinked SRC films at different times of UV exposure

UV exposure time /min	Moisture content /%	WS /%	Moisture uptake /%	WCA /°	WVP × 10^{-7} /g mm cm^{-2} h^{-1} Pa^{-1}
5	15.23 ± 0.64^{b}	64.52 ± 2.14^{b}	41.34 ± 1.56^{b}	65.26 ± 2.67^{b}	1.35 ± 0.09^{bc}
10	15.57 ± 0.22^{b}	65.25 ± 1.48^{bc}	44.13 ± 1.45^{bc}	66.55 ± 1.60^{bc}	1.30 ± 0.05^{b}
20	15.36 ± 1.06^{b}	67.56 ± 1.61^{c}	46.27 ± 1.57^{c}	66.67 ± 1.14^{bc}	1.39 ± 0.09^{bc}
40	13.51 ± 1.45^{c}	55.68 ± 1.55^{d}	46.10 ± 1.63^{c}	68.71 ± 2.07^{c}	1.47 ± 0.10^{c}
control	29.81 ± 1.60^{a}	46.99 ± 0.37^{a}	37.77 ± 1.93^{a}	84.06 ± 3.37^{a}	1.64 ± 0.04^{a}

Values are given as mean with a standard deviation. Any two means in the same column with a similar letter are not significantly different ($p > 0.05$) as determined by a Duncan test. The control sample was not treated with sodium benzoate and not exposed to UV irradiation.