Research Article

The Physical and Mechanical Properties of Agar and Carrageenan Film Incorporated with Hydrolysate-Konjac Glucomannan

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ABSTRACT

The effect of combine-polysaccharides ratio on the properties of edible film was studied. The objective of this research is to investigate the physical and mechanical properties of polysaccharide film that incorporated with hydrolysate-konjac glucomannan. Film samples containing agar (0-100%), carrageenan (0-50%), or hydrolysate-konjac glucomannan (HKG) (0-50%) at different calculated ratios of mixing using mixture design and adding glycerol as a plasticizer were prepared. The results showed that viscosity of the film solution was decreased while adding HKG. The addition of HKG could increase thickness and moisture content but could decrease the transparency of the film. The result showed that the highest water vapor permeability (WVP) came from film which contained mixture of HKG and carrageenan (1.22×10^{-5} g mm m^{-2} s^{-1} Pa^{-1}). The lowest WVP came from agar, while the mixture of HKG and carrageenan had the lowest oxygen permeability (OP) (5.84×10^{-8} mm cm^3 m^{-2} s^{-1} Pa^{-1}). The mechanical properties in terms of tensile strength and load at the maximum of the film were decreased as addition of HKG but were increased with agar adding. The elongation at break was increased with the mixture of HKG and carrageenan, and the mixture HKG, carrageenan, and agar (32.42%), respectively. In conclusion, the calculated mixture ratio from mixture design contributed to different physical and mechanical properties of the polysaccharide film, depending on their concentrations and type of polysaccharide in the film solution. The mixture of HKG, carrageenan, and agar could be applied for food packaging. However, the barrier behavior of the film need to be improved in future research.

Keywords: Hydrolysate-konjac glucomannan, Film, Physical Properties, Mechanical Properties

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Introduction

Nowadays, environmental problems are seriously concerned by several organizations as well as consumers. Plastic packaging materials such as polyethylene are commonly used in the food industry due to low cost, high strength, and convenience for the production process. However, polyethylene is non-degradable packaging and becomes the main problem for the environment [1]. Therefore, using biodegradable or edible film is an alternative material to reduce environmental problems [2], especially the food packaging from the synthetic materials being substituted by the biodegradable materials is interesting. Edible film, biodegradable film, and coating material might be composed of several materials such as polysaccharides, proteins, or lipids [3]. Carrageenan is a polysaccharide extracted from the red seaweed cell wall, which can be soluble in water, and has high potential activities for film-forming materials [4]. Agar, an unbranched and hydrocolloid consisting polysaccharide derivative of agarose and agaropectin, is used in food processing industries due to its gelling property, and the agar based edible film/coating has been reported to have good mechanical strength, physical properties, transparent, homogenous, and flexible [4]. Glucomannan, a constituent of bacterial and yeast cell walls, is present in minor amounts in the wood and can be recovered as a by-product from various processes of the forest and agroindustry [5]. The structure of glucomannan is mainly a linear-chain polymer composed of mannopyranose and glucopyranose units connected with β-(1, 4) linkages [5]. Glucomannan is studied as a biopolymer matrix to incorporate with other materials such as methylcellulose, pectin, cellulose nanofibril, cassava starch, maltodextrin, and gelatin [6-8] to improve the properties of the film-forming matrix.

Konjac glucomannan was studied as an alternative material edible-based coating for maintaining post-harvest food such as guava, kiwi, and cucumber [9-11]. Moreover, glucomannan was used to apply for loading or entrapping active compounds such as saffron petal extract, thyme essential oil, or probiotics [10-12]. However, the use of the high molecular weight of konjac glucomannans was limited due to its high water solubility that contributes to viscous solutions and even at low concentrations, and this is resulting in low mechanical resistance in glucomannan films [5, 13]. Thus, the application of konjac glucomannan in food is restricted. Consequently, the enzymatic hydrolysate technique to reduce the molecular size of konjac glucomannan will be used in this experiment to reduce its molecular size. Wattanaprasert et al. reported that konjac glucomannan hydrolyzed with mannanase into suitable viscosity could have the ability to be a coating material in the encapsulation process [13]. Moreover, the hydrolysate-konjac glucomannan could be valuable as a prebiotic due to promoted probiotic growth as the lactobacilli and bifidobacterial growth profile which hydrolysate-konjac glucomannan might apply in a wide range of foods, feeds, and healthcare products [14]. Also, the probiotic edible film had become relatively popular interesting research according to improving the functional products for promoting human health and becoming a tool for effective probiotic delivery to the consumers [15]. Hence, the hydrolysate-konjac glucomannan is an interest for edible film or coating application.

From the above information, to create a stable functional edible film, the physical and mechanical properties of biopolymer film which is fabricated from various polysaccharides were

examined. Therefore, the objective of this research is to investigate the physical and mechanical properties of agar and carrageenan film incorporated with hydrolysate-konjac glucomannan.

Materials and Methods

Materials

Konjac glucomannan, agar, carrageenan, and glycerol were purchased from Bkkchemi (Krungthepchemi Co., Ltd., Thailand). Konjac glucomannan (KG) (purity 95%) has high viscosity when dissolved in water because of its high molecular weight, which ranges from 200 to 2000 KDa. Thus, the application of KG in food is restricted. Consequently, enzymatic hydrolysate technique is employed to reduce molecular size of KG in this study.

From a preliminary experiment, in order to obtain Konjac glucomannan hydrolysate (HKG) (Mw <900: DP3-DP6), Konjac glucomannan (KG) was hydrolyzed using mannanase enzyme (Food grade, Amano Enzyme Inc, Japan).

Konjac glucomannan hydrolysis

Konjac glucomannan was hydrolyzed by using mannanase enzyme 200 IU/g at 70 ^{O}C for 30 min. After the hydrolysis is over, the hydrolysate solution was heated in a boiled water to stop the enzymatic activity for 10 min. Then, the hydrolysate solution was packed in aluminium laminated pouch and autoclaved at 121 ^{O}C for 15 min for shelf stable before spray drying at outlet temperature at 150 ^{O}C by using Buchi Mini Spray dryer B-290 (Buchi (Thailand) Ltd., Thailand). The Konjac glucomannan hydrosate obtained in powder form (MW <900: DP3-6) was kept in air tight package before using in the further experiment.

Film Preparation

All the polysaccharide solution such as agar, carrageenan and konjac glucomannan hydrolysate, were prepared separately by mixing with distilled water at concentration of 1 % (w/w). After that, the solutions were heated at 120 ^{O}C for 1 h by hot plate stirrer and kept overnight before mixing. Film solutions were obtained by using polysaccharides solution prepared from agar, carrageenan, and konjac glucomannan hydrolysate solution at different ratios of polysaccharides that the experimental were designed by using mixture design according to the preliminary experiment (Figure 1 and Table 1) with the addition of 10% glycerol as plasticizer (w/w of polysaccharides weight). Then, the samples solutions were heated at 90 ^{O}C for 1 h by hot plate stirrer to uniformly dissolve completely before casting film. Film casting were prepared from pouring 15 g of film solution in Petri dishes (7.7 cm diameter) at temperature of 55 ^{O}C. Films were dried at 40 ^{O}C and 50%Rh in a circulated air oven (Binder Model KBF 115, Germany) for 24 h. Dried films were stored within plastic bags under dried atmosphere which controlled by silica gel in a desiccator at 25 ^{O}C until measurement [16].
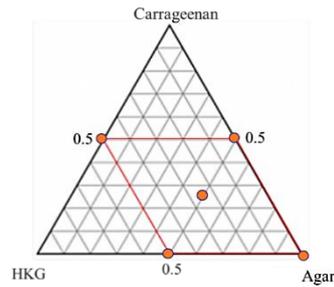
Figure 1 The mixing ratio of polysaccharide solutions for prepared film solutions by Mixture design.

Table 1 The mixture concentration of polysaccharide film solutions

	Treatment*	Agar	Carrageenan	Hydrolysate
1	C-A	50	50	-
2	HKG-C	-	50	50
3	HKG-A	50	-	50
4	Agar	100	-	-
5	Mix	50	25	25

* C = carrageenan; A = agar; HKG = hydrolysate-konjac glucomannan; and Mix = the mixed of carrageenan, agar, and hydrolysate-konjac glucomannan.

Viscosity of film solutions

The viscosity of film solution was measured by Brookfield DV-III (Brookfield AMETEK, USA). The film solutions were kept in water bath until the temperature at 55 ^{O}C. Then the sample solutions were measure using spindle No.01 and 170 rpm shear rate (modified from Ma et al. [7]).

Film Thickness

The thickness of the film was measured at 10 different positions with a Mitutoyo Electronic digital micrometre, Japan). The averaged thickness was calculated [17].

Transparency

The transmittance of the films was determined using the UV-VIS spectrophotometer 200V (Hitachi, Japan) at a wavelength of 660 nm. The transparency of the film was calculated following equation (1):

$$\text{Film transparency (TR)} = \log T/x \qquad\qquad (1)$$

T is transmittance (%) and x is the thickness of the film (mm) [18].

Moisture content of the film

The moisture content of the film was determined by cutting the film into 1.5x1.5 cm. Then, the film was dried at 105 $^{\circ}$C in an oven until constant weight. The moisture content was calculated using the equation (2):

$$\text{Moisture content (\%)} = ((m_0 - m_1)/m_0) \times 100 \tag{2}$$

where m_0 and m_1 were the weight before and after drying, respectively [19].

The water vapor permeability (WVP) and oxygen permeability (OP) of the film

The WVP of the film was determined by following the ASTEM E-96 standard method. Water vapor transmission rate (WVTR) of the film samples was determined gravimetrically at 38 $^{\circ}$C under 90%RH with using water vapor transmission measuring cups. The WVTR was calculated from linear portion of the graph plot between the weight change and the time. The WVP of the film samples was calculated following equation (3):

$$\text{WVP} = (\text{WVTR} \times \text{L})/\Delta p \tag{3}$$

where L was the film thickness (m), and Δp was the partial water vapor pressure difference (Pa) across the two side of the film (modified from Rhim and Wang [20]).

Oxygen permeability (OP) was measured according to Standard Method ASTM D 3985. The film sample testing was performed at 23 $^{\circ}$C in 50% RH. Oxygen transmission rate (OTR) was obtained in cm^3 m^{-2} day^{-1}. Then, multiplying these values by film thickness provided oxygen diffusion coefficients (modified from Caner et al. [21]).

Mechanical properties

Mechanical properties of the film (tensile strength, percentage of elongation at break, E, and load at maximum load) were measured by LLOYD TA plus Material Tester (Lloyds, England). The film of each formulation was cutting into 60X5 mm. Mechanical properties were recorded during extension at 50 mm/min, with an initial distance between the grips of 50 mm [17].

Statistical analysis

All the samples were analyzed statistically using SPSS version 17. Different means were investigated by ANOVA and Duncan's multiple range tests at a level of significance of 0.05.

Results and discussion

Film solution viscosity

The viscosity of the film solutions was measured at 55-60 $^{\circ}$C and the temperature was a point before the solution was set as a gel. The C-A film solution showed the highest viscosity but the HKG-A film solution showed the lowest viscosity (Table 2). From the results, the viscosity of film solution would increase with decreasing HKG solution ratio that the results shown as the 25% of HKG in

mixed solution had a higher viscosity than 50% of HKG in other solutions of this study. The viscosity result of the C-A film solution and the agar film solution might be due to the molecular characterization of the agar solution and carrageenan solution. The viscosity of agar solution could be increased almost linear due to the low molecular weight compound characteristic while the high molecular compound of carrageenan solution could appear to have thixotropic properties [22]. Molecular weight and molecular weight distribution played a critical role in the solubility, viscosity, and gelation of polysaccharides as a high molecular weight molecule usually could have a large excluded volume, could generate rigid formation structure, and could have higher viscosity under the same concentration [23].

Table 2 Viscosity of the film solutions

Treatment	Viscosity (cP)
C-A	51.13 ± 3.79^d
HKG-C	28.83 ± 2.40^b
HKG-A	20.48 ± 1.08^a
Agar	39.90 ± 3.62^c
Mix	33.20 ± 4.16^b

*Different letters in each column indicate a significant difference at $p\leq0.05$.

The viscosity changed with the ratio of HKG solution due to change in concentration of HKG and interaction between HKG, carrageenan and agar. HKG seems to cooperate well with carrageenan but may not make a good interaction with agar when comparing viscosity of HKG-C and HKG-A.

Film thickness and transparency

After the film solution dried, all of the polysaccharide films were smooth and clear appearance (Figure 2). The results were similar to Rhim and Wang [20] that the film would be clear, flexible, and free standing. The thickness of the film was different with the use of different polysaccharides. From the results, The HKG-C and mixed polysaccharides film showed the highest thickness and the C-A film showed the lowest thickness (Figure 3). The high thickness of the film might be due to the greater free volume [24], the higher molecular weight would create a large excluded volume [23] as carrageenan has higher molecular weight (MW 551.8) than agar (MW 336.3). However, the compatibility of mixed polymer and the ordering of chain polymer could affect the conjugation and stabilization of polymer bounds [25]. Moreover, the different thicknesses of the films would depend on the concentration of the ingredients, the amount of initial solution of the film, and the rate of pouring on the surface [10].
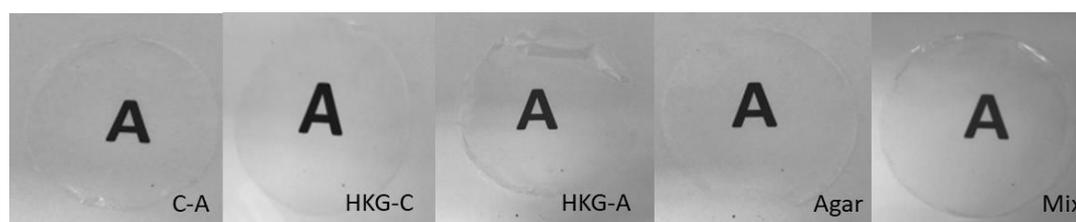

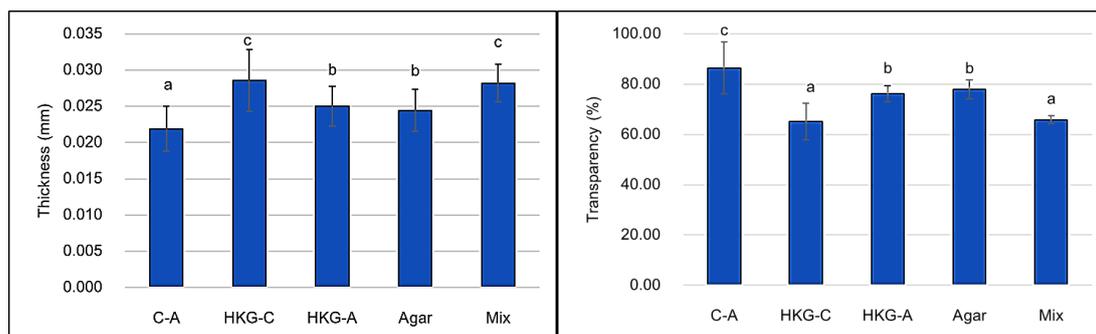**Figure 2** Appearance of the polysaccharide film samples.

Figure 3 Thickness and transparency of the polysaccharide films.

Transparency is one of the important characteristics of film for food packaging. The high transparency of the film could help the food products to be easily visible [26]. However, the lower transparency of the film could protect food from the light and would have higher quality in food packaging film [18]. From the results, the thickness of the film increased while transparency decreased. The thickness of HKG-C film and mixed polysaccharide film showed the highest thickness and the lowest transparency (Figure 3). The highest transparency of C-A film might be the mixture of carrageenan and agar film solution in the system which was similarly reported by Rhim and Wang [20] that the transmittance of the film was the highest in the carrageenan film, followed by the agar and konjac glucomannan films, respectively. Moreover, the transparency could explain the compatibility of material in the system as the films had possessed good light transmittance and high optical transmittance would be also indicated good compatibility [8]. From the result of this experiment (Figure 2 and Figure 3), the transparency of all samples could protect some parts of the food from light but it was still clear and could appear the product inside.

Moisture content of the polysaccharide films

The moisture content of the polysaccharide film increased with an increase in the ratio of the hydrolysate-konjac glucomannan solution, especially the HKG-C film and the mixed polysaccharides film (Figure 4). The moisture content results might be due to the molecular weight of agar, carrageenan, and hydrolysate-konjac glucomannan in this study. The lower molecular weight carbohydrates (<800 effective MW) were more hygroscopic than the higher molecular weight carbohydrates (>800 effective MW) depending on temperature and a_w [27]. However, Rhim and Wang [20] reported that the highest equilibrium moisture content of polysaccharide films was carrageenan film followed by konjac glucomannan and agar films. The results of increased moisture content with an increasing hydrolysate-konjac glucomannan solution in this research might be due to the water-binding capacity of the films increasing progressively with the increasing hydrolysate ratio of glucomannan. The lower molecular weight chains per unit mass would have more active sites for water sorption, and more chain ends with polar (−OH) groups than that of higher molecular weight chains per unit mass [28].
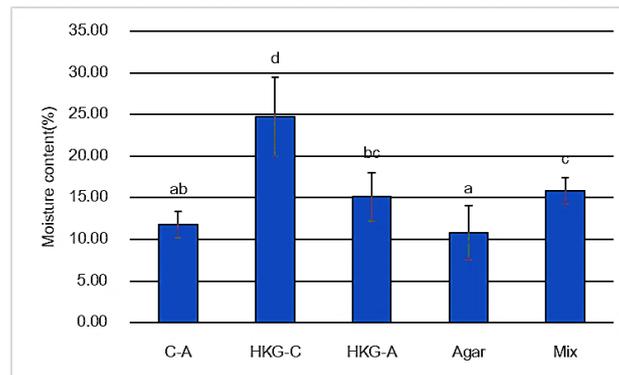
Figure 4 Moisture content of the polysaccharide films.

The water vapor permeability (WVP) and oxygen permeability (OP) of the film

   Water vapor permeability (WVP) was one of the most important properties that relate to wholeness, thickness, and surface behavior of the films due to the application of edible film and coating was mainly objective to maintain food quality and extend shelf life of food products [29-30]. The WVP was the most extensively studied property of biodegradable films due to the deterioration by water reactions resulting in keeping the food freshened, crispy, or preventing dehydration, depending on the food [31]. Thus, a film as low WVP as possible would be required for food packaging industries due to the film would be a reduction of the moisture transfer between the surrounding environment [29-30]. The result of the films showed the highest WVP with using the mixture of HKG and carrageenan and the lowest using agar as the WVP results of the film as 0.90×10^{-5} to 1.22×10^{-5} g mm m^{-2} s^{-1} Pa^{-1}(Table 3). From the result, the WVP of the agar film and C-A film in this study was not significantly different. This might be due to the high concentration of agar and carrageenan while the WVP tended to increase with an increase in the concentration of HKG (HKG-A, HKG-C, and mix film) as a similar resulted trend as Cheng et al. [28] who reported that the WVP of konjac glucomannan film increased with acid hydrolysis increased which WVP of the film was increased in 1.20×10^{14} to 1.64×10^{14} kg/ m^{-1} s^{-1} Pa^{-1} as decreased molecular weight (37.8×10^{-5} to 4.1×10^{-5}). WVP of polymer film basically involved three steps: 1) adsorbed water molecules onto the film surface, 2) diffused the water molecules through the film matrix, and 3) discharged the water molecules from the opposite surface to a low humid environment [28]. The low molecular weight of glucomannan by hydrolysis would be the low tendency to a form junction zone according to water molecule would facilitate adsorption onto the external surface of the film [28]. Thus, the increased HKG portion in the film system would increase WVP.

Table 3 The water vapor permeability (WVP) and oxygen permeability (OP) of the film

Treatment	WVP ($\times 10^{-5}$ g mm m^{-2} s^{-1} Pa^{-1})	OP ($\times 10^{-8}$ mm cm^3 m^{-2} s^{-1} Pa^{-1})
C-A	0.95±0.02^{ab}	13.76±2.54^{c}
HKG-C	1.22±0.00^{c}	5.84±1.24^{a}
HKG-A	1.11±0.02^{bc}	14.07±0.07^{c}
Agar	0.90±0.01^{a}	10.00±0.07^{b}
Mix	1.19±0.13^{c}	9.52±1.04^{b}

*Different letters in each column indicate a significant difference at p≤0.05.

The permeability to oxygen was essential for fresh fruits and vegetable respiration and involved changes in food such as color, odor, taste of food, microorganism growth, enzymatic browning, and vitamin loss [31]. The OP of film samples in this study was 5.84×10^{-8} to 14.07×10^{-8} mm cm^3 m^{-2} s^{-1} Pa^{-1}. The mixture of HKG and carrageenan had the lowest OP but had the highest WVP (Table 3). The opposite behavior between two barrier parameters was similar to [32]. The result might be due to spatial affected dominate oxygen permeability, polarity (and solubility parameter) affected control WVP, and swelling and strong barrier were in relative humidity conditions [32-33]. Moreover, the different OP behavior could be attributed to the modification of the experimental conditions such as relative humidity, temperature, type of polysaccharide in film base, type and addition of plasticizer [34]. However, the appropriate OP was depending on food such as low respiration rate fruits such as apple, carrot, celery, cabbage, and green pepper required oxygen permeability of 8.43×10^{-3} to 1.69×10^{-2} mL mil cm^{-2} hr^{-1} atm^{-1} which would satisfy with LDPE or PVC that had OP around 1.20×10^{-2} to 2.30×10^{-2} mL mil cm^{-2} hr^{-1} atm^{-1} [35]. Thus, the permeability of the films in this study might be suitably developed for the type of food in future research.

Mechanical properties of the polysaccharide films

The results of the mechanical properties of the polysaccharide films were shown in Table 4. Tensile strength and load modulus were shown in the same trend, for example the agar film had the highest tensile strength and load modulus while the mixing of HKG could decrease tensile strength and load modulus, especially in the HKG-A film. However, the mixing of HKG film solution in the system could increase elongation at the break of the film. The mixing of HKG, carrageenan and agar film had higher elongation at break (32.42%) and lower tensile strength (21.47 MPa) than that of the mixing of konjac glucomannan, carrageenan, and agar film in the research from Rhim and Wang [20], which reported the tensile strength and elongation at break as 62.46 MPa and 13.96%, respectively. However, the tensile strength had the same trend as load modulus and reversed with elongation at the break that similar to the mechanical properties of carrageenan, agar, and konjac glucomannan film [20]. The mechanical properties of the film would be related to the viscosity of the film solution due to the viscosity increase with the tensile strength of the film becoming higher [7]. In this experiment, the high

elongation at break of HKG might be attributed to the highly ramified nature of glucomannan that would be leading to the loss of molecular network, more molecular movement, and could be obtaining more flexible films [6]. The higher tensile strength and elongation of the film might be referred to good polysaccharide solubilization and good film solution spreading on the plate [6].

Table 4 Mechanical properties of the polysaccharide films

Treatment	Tensile strength (MPa)	Elongation at break	Load modulus (MPa)
C-A	29.99±3.20[c]	4.79±0.90[a]	3.29±0.78[bc]
HKG-C	14.69±0.42[ab]	30.13±2.39[c]	2.17±0.25[ab]
HKG-A	11.86±3.97[a]	13.39±5.97[b]	1.57±0.59[a]
Agar	32.62±7.22[c]	13.94±0.48[b]	3.81±0.97[c]
Mix	21.47±0.48[b]	32.42±2.67[c]	2.92±0.44[bc]

*Different letters in each column indicate a significant difference at $p \leq 0.05$.

The stability in mixed polysaccharide blend film would depend on physical mixing, the interaction of hydrogen bonds, and polymer chain-to-chain interaction between component polymer [20]. The different mechanical properties of polysaccharides films would be due to the physical and chemical characteristics of the polysaccharides, conditions of film production, the interaction or compatibility between the component polymers, and the different thicknesses of the film [6, 20]. The compatibility of polysaccharide blending would be leading to improvement in mechanical properties, while incompatible blending might lead to inferior mechanical properties [20]. Moreover, physical properties could be also varied in many factors such as chain flexibility and mobility, degree of entanglement, available segmental motion, and plasticizing or antiplasticizing effects exerted by the small molecular weight [6]. Thus, the mixed polysaccharide film (the mixed ratio of 50% agar film solution, 25% carrageenan film solution, and 25% HKG film solution) could be high elongation and middle tensile strength that would be suitable for this experiment and might be applied to use as a food package. However, the mechanical results of the film had a similar mechanical trend to a natural film as Kaewprachu and Rawdkuen [36] who reported soy protein isolate film had the tensile strength of 4.5 MPa and elongation at break of 79.32% that both of the properties would be lower than commercial wrap film or PVC film (tensile strength of 44.93 MPa and elongation at break of 253.71%).

Conclusion

The HKG could be used to produce an edible film by blending with other polysaccharides such as agar and carrageenan. However, the physical properties of the film such as viscosity of film solution, thickness, transparency, moisture content, WVP, OP, and mechanical properties were different depending on the ratio of polysaccharide components. Thus, the calculated mixture ratio from mixture design contributed to the different physical and mechanical properties of the HKG blended with polysaccharides

film which depending on the concentration and type of polysaccharide in film solution.   From the mechanical properties, the mixture of HKG, carrageenan, and agar could be applied for food wrapping. However, the barrier behavior of the film might be improved in future research.

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