Research Article

The Effect of Mixed Hydrocolloids and Protein Isolate Film on the Physical-Mechanical Properties of Film and the Survivability of *Lacticaseibacillus paracasei* and *Bifidobacterium animalis*

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ABSTRACT

The effect of mixed hydrocolloids and protein isolate film on its physical-mechanical properties and the survival rate of *Lacticaseibacillus paracasei* and *Bifidobacterium animalis* that were added into the films were studied. The results showed the shear thinning behavior of the film solution. The agar mixed solution exhibited the highest apparent viscosity. The thickness and transparency of the film were varied from 0.0547±0.005 to 0.102±0.007 mm and 9.03±0.93 to 32.65±4.55, respectively. The films that consisted of soy protein isolate (SPI) had the highest thickness and the lowest transparency. The high mechanical properties were observed in the agar-incorporated film. Using glycerol as a plasticizer had higher elongation at break than using sorbitol. The survivability of each probiotic bacteria was found ranging from 41.84±0.46% to 92.58±2.20%, and the highest survivability was found in the film-incorporated protein isolate, while it was not found any probiotic bacteria in the agar film without protein. However, *B. animalis* had a greater survival rate in mixed film than *L. paracasei*. In summary, the application of probiotic film would depend on the component of mixed films and probiotic strains that affected physical-mechanical properties of the film and probiotic survivability.

Keywords: Hydrolysate konjac glucomannan film, Probiotic film, Mechanical properties, *Lacticaseibacillus paracasei, Bifidobacterium animalis*

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Introduction

Hydrocolloid is one of popular materials used for edible film such as polysaccharides and protein [1]. Glucomannan, a linear polymer chain composed of mannopyranose and glucopyranose units by β-(1-4) linkages [2], was studied to incorporate with other polymers such as methylcellulose, pectin, cellulose nanofibril, cassava starch, maltodextrin, and gelatin [3-5] to develop the film-forming matrix. The konjac glucomannan hydrolysate, also has ability as prebiotic material that promotes growing of probiotic, which might be applied in functional products such as foods, feeds, and healthcare products for improvement of human health, and can be an effective probiotic delivery tool [6,7]. Carrageenan, agar, and CMC are polysaccharides that are widely used in industrial biopolymers with high potential activities for film-forming materials, such as good mechanical strength, transparency, and flexible [1,8].

Protein isolate is one of the raw materials used in film formation due to the excellent gas barrier properties, water vapor permeability, mechanical weakness, and lower elongation of protein film [9]. Whey protein isolate (WPI) can be formed to film and sheet via using a thermoplastic process [10], and can produce the film with interrelated sensorial, optical, and mechanical barrier properties with the lacking of taste and odor [11]. Soy protein isolate (SPI) has a preferable film forming ability and great oxygen permeability [9].

Lactobacillus species are well-known as probiotic bacteria, especially *Lacticaseibacillus paracasei* species that have been used in probiotic dairy products, such as yogurt, cheese, fermented milk, and ice cream [12,13]. *Bifidobacterium animalis* is a gram-positive, rod-shaped bacterium which is found in the human large intestine [14]. Some research works showed that *B. animalis* incorporated with edible films consisting of either alginate or whey protein that affected the properties of the film [15]. Additionally, probiotics can be incorporated with a biopolymer matrix to develop the bioactive food packaging to be an alternative pathogenic microorganism controlling method, and improve consumers' health by food stability and safety through probiotic edible film [7].

Non-degradable packaging such as polyethylene became a main problem for the environment [16]. Alternative materials used as biodegradable or edible were a selected choice for reducing this environmental problem [17]. Several materials were interesting for a composite edible film, biodegradable film, or coating material such as polysaccharides, proteins, or lipids [18]. From the previous study, the mixture of konjac glucomannan hydrolysate, agar, and carrageenan could be able to produce the edible film [19]. This research would continue to keep on the study of film properties after probiotic addition. Hence, the objective of this research was to study the effect of protein isolate and mixed hydrocolloids incorporated with probiotics, such as *L. paracasei* and *B. animalis*, on the physical-mechanical properties of film and the survival rate of probiotic bacteria.

Materials and Methods

Material

Konjac glucomannan, agar, carboxy methyl cellulose (CMC), carrageenan, glycerol, sorbitol, whey protein isolate (WPI), and soy protein isolate (SPI) were purchased from Bkkchemi (Krungthepchemi Co., Ltd., Thailand). Because konjac glucomannan (KG) (purity 95%) has high viscosity when dissolved in water, the application of KG in food is restricted. Consequently, enzyme-hydrolyzed KG was chosen in this study. Konjac glucomannan hydrolysate (HKG) was prepared by enzyme treatment and then was spray dried using Buchi Mini Spray Dryer B-290 (Buchi (Thailand) Ltd., Thailand) into powder form [19]. The hydrolysate konjac glucomannan (HKG) powder (MW <900: DP3-6) was kept in air tight package before using in further experiments.

L. paracasei (TISTR 2593) and *B. animalis* (TISTR1925) from TISTR probiotic bank (Thailand institute of scientific and technological research, Thailand) were used in the experiment. To prepare culture, 1% v/v probiotic strain was inoculated into de Man Rogosa and Sharpe (MRS) broth (Merck Millipore, Germany) supplemented with 0.05% L-cysteine (Merck Millipore, Germany) for 48 hr, and then was incubated under anaerobic condition using an anaerobic jar Anaerocult A GasPac system (Merck, Darmstadt, Germany) at 37°C for 24 hr. Cell pellets were obtained by centrifugation at 8,500 rpm for 5 min at 25°C and washed 3 times with 0.85% NaCl. After washing, they were adjusted their turbidity to the cell concentration approximate 10^{10} CFU/mL in 0.85% NaCl. Cell suspension was kept in bottle tightly and stored at 4°C, no longer than 48 hr, before using [20].

Film Preparation

The film solution was prepared by mixing ingredients according to the ratio shown in Table 1 with distilled water at a concentration of 2% (w/w). After that, the solutions were heated at 120°C for polysaccharide and 60°C for protein on a hot plate stirrer for 1 hr. Glycerol or sorbitol were added as a plasticizer at concentration of 0.6% (w/w). The samples were then heated at 60°C for 1 hr and autoclaved before the solution temperature dropped below 50°C but still in the liquid form. The film solution samples were incorporated with probiotic bacteria (*L. paracasei* and *B. animalis*) solution at a concentration of 2 mL/100 g each of the film solution. Then, 15 mL of the film solution sample was poured onto 7.7 cm-diameter Petri dishes before drying in a hot air dryer at controlled temperature of 45°C for 14 hr. Dried probiotic films were kept at 4°C before further testing for other properties [21].

Table 1 The mixture concentration ratio of the film solutions.

Treatment	CMC (%)	HKG (%)	Carrageenan (%)	Agar (%)	WPI (%)	SPI (%)	Glycerol (%)	Sorbitol (%)
C-G	50	25	25	-	-	-	0.6	-
C-S	50	25	25	-	-	-	-	0.6
C-WPI-G	25	12.5	12.5	-	50	-	0.6	-

Table 1 ***(cont.)*** The mixture concentration ratio of the film solutions.

Treatment	CMC (%)	HKG (%)	Carrageenan (%)	Agar (%)	WPI (%)	SPI (%)	Glycerol (%)	Sorbitol (%)
C-WPI-S	25	12.5	12.5	-	50	-	-	0.6
C-SPI-G	25	12.5	12.5	-	-	50	0.6	-
C-SPI-S	25	12.5	12.5	-	-	50	-	0.6
A-G	-	25	25	50	-	-	0.6	-
A-S	-	25	25	50	-	-	-	0.6

*C = CMC; A = agar; WPI = Whey protein, SPI = Soy protein isolate; G = Glycerol and S = Sorbitol

Rheological properties of film solutions

The viscosity of film solution was measured by HAAKE Viscotester iQ Air rheometer (Thermo Fisher Scientific, Tewksbury, USA). The film solutions were kept in water bath at 50°C. Then, the sample solutions were measured using spindle CC25DIN and shear rate 100-300 s^{-1}. The flow behavior of the film suspensions was calculated with using the Power Law model following equation:

$$\tau = k\gamma^n \quad (1)$$

Where τ the shear stress (Pa), k is the consistency index, n is the flow behavior index, and γ is the shear rate/s [22].

Film Thickness

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

Transparency

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

$$\text{Film transparency (TR)} = \log \frac{T}{x} \quad (2)$$

Where T is transmittance (%) and x is the thickness of the film (mm) [23].

Mechanical properties

Mechanical properties of the probiotic 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 cut into 60x5 mm. Mechanical properties were recorded during extension at 50 mm/min, with an initial distance between the grips of 50 mm [24].

Determination of cell numbers and cell survival rate

Either film solution (1 mL) or dried film (0.5 g) samples were taken and serially diluted in peptone water buffered (Merck Millipore, Germany), and cells were counted by the drop plate technique using Transgalctosylated oligiosaccharide supplement Lithium-Mupirocin (TOS-MUP) agar (Merck Millipore, Germany) for *B. animalis* and MRS-L-cysteine agar for *L. paracasei*. The number of viable cells was expressed in CFU/g sample and the percentage of probiotic survival rate was calculated as follows:

$$\%\text{Survival} = \frac{N}{N_0} \text{x}100 \quad (3)$$

Where N is cell number of bacteria after rehydration in treatment solution (CFU/g dried sample), N_0 is cell number of bacteria before spray drying (CFU/g dried sample).

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

Rheological properties of film solution

The rheological properties of hydrocolloids solution could be classified with the applied shear rate as 1; non-Newtonian with shear thinning (pseudoplastic) in which apparent viscosity was decreased, 2; non-Newtonian with shear thickening (dilatant) in which apparent viscosity was increased, and 3; Newtonian flow behavior, in which apparent viscosity was constant [25]. The flow behaviors of the film solution in the present study were calculated using the Power Law model and showed a non-Newtonian fluid with shear-thinning behavior (Figure 1 and Table 2), especially A-G and A-S. Moreover, the significant low flow behavior of high agar concentrations such as A-G and A-S (Table 2) would be resulting in high apparent viscosity (Figure 1). These results might be due to compose of high concentration of agar solution that was similar to Zhang et al. [26] who reported the viscosity of agar solution exhibited shear-thinning behavior and decreased with a rise of shear rate. The flow behavior of the film solution might be different due to the component used, the concentration used, and the shear rate applied. Hentati et al. [27] reported the polysaccharide from seaweed that exhibited a non-Newtonian shear-thinning behavior at low shear rates and a Newtonian behavior when the shear rate was increased above a critical threshold at a concentration below 2% (w/w), and the flow curves reported shear-thinning behavior with a total absence of the Newtonian plateau region at the whole range of shear rates at concentration above 2% (w/w).

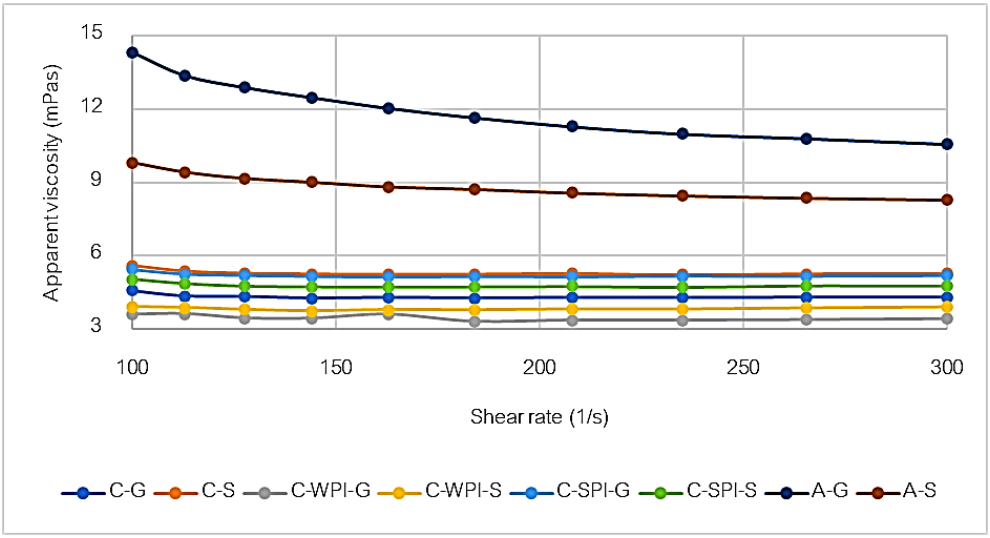


Figure 1 Apparent viscosity of film solution.

Table 2 Flow behavior of film solution.

Sample	Flow behavior index	Consistency index	R^2
C-G	0.967±0.022[a]	0.005±0.001[b]	0.997
C-S	0.965±0.042[a]	0.007±0.001[b]	0.997
C-WPI-G	1.023±0.022[a]	0.003±0.001[b]	0.997
C-WPI-S	1.000±0.020[a]	0.004±0.000[b]	0.998
C-SPI-G	0.972±0.024[a]	0.006±0.001[b]	0.998
C-SPI-S	0.968±0.020[a]	0.006±0.001[b]	0.998
A-G	0.755±0.107[c]	0.054±0.044[a]	0.995
A-S	0.858±0.062[b]	0.020±0.007[b]	0.998

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

Film thickness and transparency

The thickness of the probiotic film was different with the use of different components with controlling the volume in the preparation process. From the results, the thickness of the film increased while transparency decreased. The film incorporated with SPI, such as C-SPI-G and C-SPI-S, showed the highest thickness and the lowest transparency (Table 3). In this study, the edible film increased thickness with an increased SPI concentration which might be due to either a higher mass of the same solution or a higher concentration of the film-forming solution [24].

Protein-incorporated film in this study, both WPI and SPI, could have higher thicknesses and lower transparency than incorporated CMC, HKG, and carrageenan without the protein (C-G and C-S) which might be due to the matrix not having enough compatibility. The transparency would explain the compatibility of matrix system according to good light transmittance, and high optical transmittance

would be also good compatibility [5]. The polymer compatibility and polymer chain ordering would prefer to the conjugation and stabilization of polymer bound interaction [28]. The polymers were not miscible ability or incompatibility. Phase separation would be appeared as nucleation and growth by forming droplets irregularly spaced at different times and size distributions, and during solvent evaporation [29]**.** The different thicknesses of the films would also depend on the concentration of the component, the amount of initial film solution, and the rate of pouring on the surface [30].

Table 3 Thickness and transparency of the probiotic film.

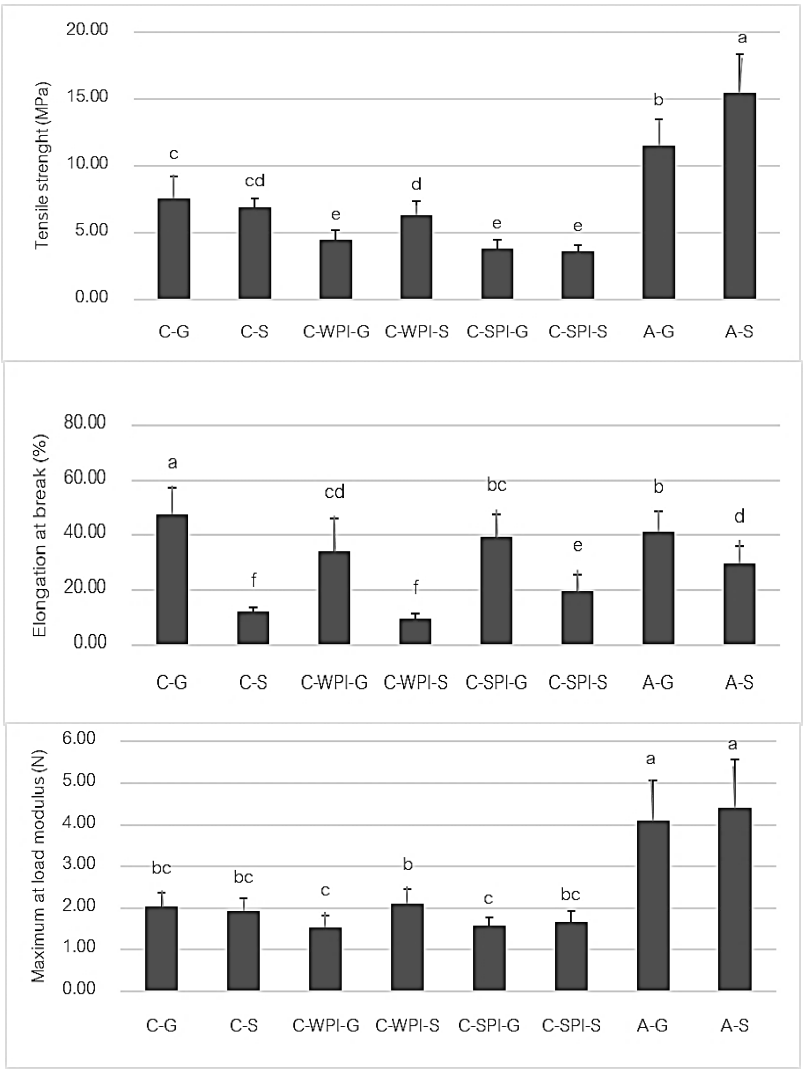
Sample	Thickness (mm)	Transparency
C-G	0.0549 ± 0.008^{d}	32.65 ± 4.55^{a}
C-S	0.0576 ± 0.006^{d}	30.82 ± 1.21^{b}
C-WPI-G	0.0680 ± 0.006^{c}	13.27 ± 1.06^{e}
C-WPI-S	0.0654 ± 0.007^{c}	11.43 ± 0.88^{f}
C-SPI-G	0.0959 ± 0.005^{b}	9.44 ± 1.27^{g}
C-SPI-S	0.1020 ± 0.007^{a}	9.03 ± 0.93^{g}
A-G	0.0677 ± 0.005^{c}	21.37 ± 0.37^{d}
A-S	0.0547 ± 0.005^{d}	27.62 ± 0.46^{c}

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

Mechanical properties of the probiotic films

The results of the mechanical properties of the probiotic films are shown in Figure 2. Tensile strength and load modulus were shown in the same trend, such as the agar incorporated film with the highest tensile strength and load modulus, and these results were similar to the previous study [19]. The mechanical properties of the probiotic film might relate to the viscosity of the film solution that tensile strength increased with an increase in the viscosity of the film solution [4]. The elongation at break of the probiotic film using glycerol as a plasticizer could be higher than that of using sorbitol as a plasticizer. The smaller molecule of plasticizer as glycerol (92 g/mol) might be a result of more easily created intermolecular spaces between the polymer chains than sorbitol (182 g/mol). The small molecule of plasticizer could decrease the number of hydrogen bonds attached to the polymer chains and could interrupt the polymer structure. Then, the film would turn into the flexible structure and great mobility [31].

The protein as WPI and SPI incorporated film plasticized with glycerol in this study seemed to lower tensile strength than that of only polysaccharide incorporated film. The strong bonds interaction of polysaccharide-protein composites could be created by hydrophobic-hydrophobic interaction and electrostatic interaction depending on the material properties of protein blended with polysaccharides such as their size [29].



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

Figure 2 Mechanical properties of the probiotic film.

Survivability of probiotics in the film

After the film solution was heated and cooled down to 45°C, probiotic cell pellets were mixed into the film solution and poured to form a film. The results showed that the probiotic survival rate had a significant difference among the types of film solution. The survivability of probiotic in the film was around 41.84±0.46 to 92.58±2.20% which was similar to the previous study reported that hydrocolloids containing probiotic film had a survival of *Lactobacillus casei* in the range of 47.6% to 87.5% depending on different types of hydrocolloids [32]. High levels of probiotic survival could be observed in samples incorporated with protein isolates (Table 4). The film samples consisted of CMC, carrageenan, and HKG showed lower level of probiotic survival rate, whereas probiotics was not found when the film contained agar, carrageenan, and HKG. The protein isolate could increase the survivability of probiotics similar to Ghasemi et al. [1], in which the CMC-protein hydrolysate could accelerate *Lactobacillus casei* cells to reproduction after 30-day storage. Moreover, the protein addition could promote or

maintain the stability of probiotics due to the nutrients provided to the cells, the redox potential of the medium reduced, and the buffering capacity of the medium increased [11].

Table 4 Survivability of probiotics in the film.

Samples	%Survival		
	L. paracasei	***B. animalis***	**Total**
C-G	0.00±0.00^{e}	0.00±0.00^{e}	49.86±1.11^{d}
C-S	0.00±0.00^{e}	41.84±0.46^{d}	55.33±1.73^{c}
C-WPI-G	0.00±0.00^{e}	76.51±1.03^{c}	92.90±4.99^{ab}
C-WPI-S	86.05±5.49^{a}	92.58±2.20^{a}	94.31±4.84^{ab}
C-SPI-G	60.48±1.28^{b}	88.10±4.84^{b}	90.76±1.90^{b}
C-SPI-S	88.34±1.15^{a}	91.88±2.23^{a}	96.02±4.45^{a}
A-G	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}
A-S	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}

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

These indicated that both *L. paracasei* and *B. animalis* were the most survived in the film contained protein, especially the film that used sorbitol as a plasticizer (C-WPI-S and C-WPI-S film). Although probiotics entrapped on A-G and A-S film solutions before counted cells around 10.41±0.21 to 10.77±0.19 log CFU/g, the probiotics could not survive after the solution drying to film. Probiotic cells in the film without protein might be injured by the heating and drying environment, while the viability of *B. animalis subsp. lactis* BB-12 in the protein and alginate film had not significantly decreased at low drying temperature [11]. From this study, protein could interact with polysaccharide and strengthen the cell that could survive in the harsh conditions. From the results, *B. animalis* would have survivability more than *L. paracasei* due to many factors affecting the survival of probiotics, such as the type of bacterial strains which has different cell wall characteristic, water activity, water vapor, and oxygen permeability of film and storage temperature [1]. Moreover, the total survivability of probiotic film showed the highest with C-SPI-S film at 96.02±4.45 % (Table 4). Thus, the C-SPI-S film might be the suitable condition for the probiotic film in this experiment. Since this experiment determined the survival of probiotic cell after encapsulating in various kinds of film, it would be recommended that the survival rate in simulated gastric juice and bile conditions should be further studied in order to compare the protection of film formulation when probiotic cells pass to the intestines.

Conclusion

The hydrolysate konjac glucomannan mixed hydrocolloids and protein isolate film solution showed the flow behavior as shear-thinning. The agar mixed solution could be higher apparent viscosity than the other solutions. After the film solution formed, the mix of SPI had the highest thickness and the lowest transparency. For the mechanical properties, agar mixed film had the highest tensile strength. Using plasticizer as glycerol could produce higher elongation at break of the film than using sorbitol. However, the soy protein isolate incorporated in the film could maintain the survivability of probiotic bacteria more than the film without protein isolate. The highest total survivability could be seen in the C-SPI-S film. Thus, the C-SPI-S film might be the suitable condition for the probiotic film in this experiment.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. Ghasemi Z, Alizadeh Khaled-Abad M, Almasi H, Nikoo M. Carboxymethyl cellulose based bioactive edible films with *Lactobacillus casei* and fish protein hydrolysates. Iranian Food Sci Technol Res J. 2022;17(6):85–102.
2. Nešić A, Cabrera-Barjas G, Dimitrijević-Branković S, Davidović S, Radovanović N, Delattre C. Prospect of polysaccharide-based materials as advanced food packaging. Molecules. 2020;25(1):135.
3. Chambi HNM, Grosso CRF. Mechanical and water vapor permeability properties of biodegradables films based on methylcellulose glucomannan pectin and gelatin. Food Sci Technol. 2011;31(3):739–46.
4. Ma S, Zheng Y, Zhou R, Ma M. Characterization of chitosan films incorporated with different substances of konjac glucomannan cassava starch maltodextrin and gelatin and application in mongolian cheese packaging. Coatings. 2021;11(1):84.
5. Wang J, Chen X, Zhang C, Akbar AR, Shi Z, Yang Q, et al. Transparent konjac glucomannan/cellulose nanofibril composite films with improved mechanical properties and thermal stability. Cellulose. 2019;26(5):3155–65.
6. Al-Ghazzewi FH, Khanna S, Tester RF, Piggott J. The potential use of hydrolysed konjac glucomannan as a prebiotic. J Sci Food Agric. 2007;87(9):1758–66.
7. Pavli F, Tassou C, Nychas GJE, Chorianopoulos N. Probiotic incorporation in edible films and coatings: bioactive solution for functional foods. Int J Mol Sci. 2018;19(1):150.
8. Kumar N, Neeraj. Polysaccharide-based component and their relevance in edible film/coating: a review. Nutr Food Sci. 2019;49(5):793–823.

9. Wang L, Xiao M, Dai S, Song J, Ni X, Fang Y, et al. Interactions between carboxymethyl konjac glucomannan and soy protein isolate in blended films. Carbohydr Polym. 2014;101:136–45.
10. Leuangsukrerk M, Phupoksakul T, Tananuwong K, Borompichaichartkul C, Janjarasskul T. Properties of konjac glucomannan–whey protein isolate blend films. LWT-Food Sci Technol. 2014;59:94–100.
11. Pereira J, Soares J, Costa E, Silva S, Gomes A, Pintado M. Characterization of edible films based on alginate or whey protein incorporated with *Bifidobacterium animalis* subsp. *Lactis* BB-12 and Prebiotics. Coatings. 2019;9(8):493.
12. Guerra AF, Lemos Junior WJF, dos Santos GO, Andrighetto C, Gianomini A, Corich V, et al. *Lactobacillus paracasei* probiotic properties and survivability under stress-induced by processing and storage of ice cream bar or ice-lolly. Ciênc Rural. 2018;48(9):e20170601.
13. Xu Y, Tian Y, Cao Y, Li J, Guo H, Su Y, et al. Probiotic properties of *Lactobacillus paracasei* subsp. *Paracasei* L1 and its growth performance-promotion in chicken by improving the intestinal microflora. Front Physiol. 2019;10:937.
14. Bozkurt K, Denktas C, Ozdemir O, Altındal A, Avdan Z, Bozkurt HS. Charge transport in *Bifidobacterium animalis subsp.lactis BB*-12 under various atmospheres. Open J Appl Sci. 2019;9(6):506-14.
15. Hashemi SS, Khorshidian N, Mohammadi M. An insight to potential application of synbiotic edible films and coatings in food products. Front Nutr. 2022;9:875368.
16. Debiagi F, Kobayashi RKT, Nakazato G, Panagio LA, Mali S. Biodegradable active packaging based on cassava bagasse polyvinyl alcohol and essential oils. Ind Crops Prod. 2014;52:664-70.
17. Noiduang P, Thawla L, Pa-ai O. Study on edible film production from Chinese water chestnuts starch. Agric Sci J. 2015;46(3):665-8.
18. Tassavil P, Kerdchoechen O, Laohakunjit N. Effects of edible coatings on weight loss vitamin C loss and appearance of minimally processed pummelo. Agric Sci J. 2009;40(1):261-4.
19. Ploypetchara T, Srimongkoluk U, Borompichaichartkul C Lekhavat S. The physical and mechanical properties of agar and carrageenan film incorporated with hydrolysate-konjac glucomannan. SWU Sci J. 2022;38(1):70-82.
20. Azmi AFMN, Mustafa S, Hashim D, Md, Manap YA. Prebiotic activity of polysaccharides extracted from *Gigantochloa Levis* (Buluh beting) shoots. Molecules. 2012;17(2):1635-51.
21. Soukoulis C, Singh P, Macnaughtan W, Parmenter C, Fisk ID. Compositional and physicochemical factors governing the viability of *Lactobacillus rhamnosus* GG embedded in starch-protein based edible films. Food Hydrocoll. 2016;52:876-87.
22. Abdou ES, & Sorour MA. Preparation and characterization of starch/carrageenan edible film. Int Food Res J. 2014;21(1):189-93.

23. Saberi B, Vuong QV, Chockchaisawasdee S, Golding JB, Scarlett CJ, Stathopoulos CE. Mechanical and physical properties of pea starch edible films in the presence of glycerol. J Food Process Preserv. 2016;40(6):1339–51.
24. Nandane AS, Jain R. Study of mechanical properties of soy protein based edible film as affected by its composition and process parameters by using RSM. J Food Sci Technol. 2015;52(6):3645–50.
25. Goff HD, Guo Q. The role of hydrocolloids in the development of food structure. In: Fotis S, Aris L, Ian N, editors. Handbook of food structure development. CPI Group (UK) Ltd. 2019.
26. Zhang L, Che L, Zhou W, Chen X. Rheological behavior of agar solution in relation to the making of instant edible bird's nest products. Int J Food Eng. 2012;8(3):1-20.
27. Hentati F, Pierre G, Ursu AV, Vial C, Delattre C, Abdelkafi S, et al. Rheological investigations of water-soluble polysaccharides from the Tunisian brown seaweed *Cystoseira compressa*. Food Hydrocoll. 2020;103:105631.
28. Hashemi SMB, Jafarpour D. Bioactive edible film based on Konjac glucomannan and probiotic *Lactobacillus plantarum* strains: physicochemical properties and shelf life of fresh-cut kiwis. J Food Sci. 2021;86(2):513–22.
29. Bealer EJ, Onissema-Karimu S, Rivera-Galletti A, Francis M, Wilkowski J, Salas-de la Cruz D, et al. Protein-polysaccharide composite materials: fabrication and applications. Polymers (Basel). 2020;12(2):464.
30. Hashemi SMB, Jafarpour D. The efficacy of edible film from Konjac glucomannan and saffron petal extract to improve shelf life of fresh-cut cucumber. Food Sci Nutr. 2020;8(7):3128-37.
31. Ballesteros-Mártinez L, Pérez-Cervera C, Andrade-Pizarro R. Effect of glycerol and sorbitol concentrations on mechanical optical and barrier properties of sweet potato starch film. NFS J. 2020;20:1-9.
32. Phovisay S, Siriwoharn T, Surawang S. Effect of drying process and storage temperature on probiotic *Lactobacillus casei* in edible films containing prebiotics. Food Appl Biosci J. 2018;6(Special):105-16.