



Improvement the barrier and mechanical properties of whey protein isolate film using fructan obtained from Serish (*Eremurus spectabilis*) root as a novel polysaccharide

Rassoul Mozafarpour¹ · Nasser Sedaghat¹ · Faezeh Alhasan¹

Received: 24 September 2020 / Accepted: 12 December 2020

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract

In this study, we investigated the effect of fructan obtained from Serish root (*Eremurus spectabilis*) as a new film-base material in order to improve the barrier and mechanical properties of whey protein isolate (WPI) film. The effects of fructan (0, 50, 60, 70% v/v) and glycerol (Gly) (40 and 50% v/v) ratios on the mechanical, chemical, thermal, surface wetting, water vapor permeability (WVP), and morphological properties of WPI film were investigated. According to scanning electron microscope (SEM), the substitution of fructan to WPI significantly modified the surface properties of composite films, which had fewer cracks and pores compared to WPI films. The substitution of fructan decreased the water vapor permeability (6.64×10^{-6} – 1.85×10^{-6} g / m.s.pa) and increased the tensile strength (6.91–16.25 Mpa) and Young's modulus (30.84–42.84 Mpa) of the WPI film. Increasing the fructan content improved the mechanical properties and reduced the water vapor permeability of composite films. Fourier transform infrared (FTIR) spectra confirmed the formation of hydrogen bonding interactions between WPI and fructan. Differential scanning calorimetry (DSC) analysis exhibited a slight increase in the enthalpy values (81.43–154.3 J/g) with increasing fructan ratio of composite films, so that the thermal stability of WPI film improved due to establishing more hydrogen bonds which also evidenced by FTIR. These results suggest that WPI 30 / Fructan 70 (40% Gly) can be considered as the best ratio for preparation of the composite film.

Keywords Fructan · Composite film · Water vapor permeability · Tensile strength

Introduction

One of the major issues in food industry is using petroleum-based polymers packaging that derived from fossil fuel materials. Therefore, a major emphasis has been focused on developing natural products which have less adverse effect on the environment [1]. Generally, edible and biodegradable polymers could be considered as safe material to preserve the quality of fresh foods, extend the shelf life of the food products and have less deleterious effect on the environment [2, 3]. The edibility and biodegradability of biopolymers are considered as the most important characteristics which make them good alternative for production of biopolymer films [3]. Edible films can be produced from many biopolymer

resources including polysaccharides, lipids, and proteins [4]. Although various bio-films exhibit different advantages and disadvantages, it is possible that using a combination of these biopolymers would improve the mechanical and barrier properties of the final product. Furthermore, the resultant films may have the best functional properties of each component [5]. Generally, polysaccharide-based films are considered as non-toxic, renewable and low-cost polymers and they reduce food packaging waste streams [6], furthermore, they exhibit desirable gas barrier properties compared to protein films which means they can delay the respiration and ripening of many fruits and vegetables [7]. However, poor mechanical properties and high water vapor permeability restrict the use of resultant polysaccharide film as packaging material in high moisture foods [8].

In recent years, protein-based film and coatings have also gained a lot of attention due to good combination of functional, biodegradable and nutritional properties. Whey protein isolate is considered as a valuable and low-cost by-product which forms a colorless, odorless and flexible

✉ Nasser Sedaghat
sedaghat@um.ac.ir

¹ Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran

film [1]. However, WPI film exhibits a poor mechanical and water barrier properties [9, 10].

Numerous researches have been carried out related to development of biopolymer composite films using protein and polysaccharide blends [11–13]. The properties of Composite films strongly depend on many factors like film-forming methods, molecular structure of the components, interactions between them, and the ratio of crystalline to amorphous zones [14]. Composite films normally consist of two or more hydrocolloids which offer good barrier properties to gases and provide a selective barrier to oxygen and carbon dioxide [15]. Blending polysaccharides and proteins in composite films can be considered as a successful method to avoid the poor mechanical and water barrier properties of individual polymers films which improves their physical properties, but this process largely depends on the intermolecular and intramolecular interactions between components [16]. Fructan is a soluble carbohydrates which can accumulate temporarily in the stem, leaf and root of a plant during the growth of the stem to the early stages of fruit production [17]. *Eremurus spectabilis*, which is named as Serish constitutes a 10,000 different species, this plant naturally grows as an indigenous plant in the spring in the south, east and west of Iran and other countries like west Pakistan, Afghanistan, Iraq, Turkey, Palestine, Lebanon, Syria, and the Caucasus [18]. Generally, it contains about 14 to 22% sugar in which fructan is considered to be the predominant component [17]. Fructan or polyfructosylsucrose has been used widely in the food industry as a functional fiber because of its low caloric value (1.5 kcal/g) [19] and physiological and biochemical effects in metabolism process [20]. Fructan is also considered as an important prebiotics product which has many applications in the food formulations because of its techno-functional properties [21].

It has been reported that polysaccharides can be used to reinforce the properties of protein-based films [9, 11]. Therefore, blending of WPI and fructan might reinforce the characteristics of single WPI and fructan film for application in food packaging. According to the information the incorporation of fructan extracted from Serish as a component in composite films hasn't investigated yet. Therefore, the aim of this study was to fabricate composite films in which aqueous extract of fructan was utilized as a new film base material and blended with WPI solution to prepare composite films. For this purpose, fructan solution at different ratios (0, 50, 60, 70% v/v) and glycerol (Gly) (40 and 50% v/v) were added to WPI solution in order to fabricate composite WPI / fructan film. Then we investigated the effect of WPI / fructan and glycerol ratios on the mechanical (including tensile strength (TS), percent of elongation at break (EAB %) and Young's modulus), microstructural, thermal and water vapor barrier properties of resultant films.

Materials and methods

Materials

Whey protein isolate (WPI) (92.1% protein dry basis) was purchased from German 107 Prot, Sachsenmilch Lepperrsdorf GmbH (Saxony, Germany). (According to the manufacturer's specifications, this product consisted of 92.1% protein with a fat content < 1% on a dry weight basis.). Glycerol (Gly) and all other chemical agents were of analytical grade.

Preparation of Serish roots powder

The Serish root powders were purchased from the local medical herb store, Mashhad, Iran. Then, the prepared samples were passed through a 50 µm sieve and stored in a dry container for further applications.

Aqueous extraction of fructan

The Aqueous extraction procedure of fructan was carried out according to the method of Pour farzad et al. (2015a). Serish root powder was suspended in distilled water while stirring at a ratio of 1:50 (W/V). Then the prepared suspension was heated in a water bath (Memmert water bath, model WB/0B7-45, Schwabach, Germany) at 85 °C for 30 min. The solution then filtered through a muslin cloth to remove the insoluble residues. The resulting extracted slurry was centrifuged (Sigma centrifuge, model 4-16KS, Osterede, Germany) at 6000 RPM for 10 min to collect the supernatant. In order to remove colored materials, activated carbon powder (150 µm) was added to the supernatant at 60 °C and mixed for 15 min. The treated syrup was filtered (Whatman No. 1). The resulting solution was dried for 24 h at 45 °C in a hot air oven (model 100–800; Memmert, Schwabach, Germany). The dried samples were milled and packed in the air-tight containers before the next step.

Preparation of WPI -fructan film

WPI-fructan films were prepared using the casting technique. A 5% (w/v) solution of WPI (solution1) was prepared by adding the powder in deionized water. In order to unfold the compact structure of WPI, the solution was heated at 80 °C for 30 min while being stirred continuously and then transferred to a cold water bath in order to prevent aggregation. 2 wt% fructan powder was dissolved in deionized water (solution 2) and heated with stirring for 30 min at 60 °C to complete dessolvation. Mixtures of solutions 1 and 2 were mixed together while stirring at WPI to fructan volume ratios of (100:0), (50:50), (40:60), (30:70), and (0:100).

Glycerol was added to the blend solution as a plasticizer at two ratios of 40 and 50% based on the dry weight. Pure solutions of WPI and fructan were prepared at just a 50% ratio of glycerol. The pH of mixture solutions was adjusted to 8 using 2 M NaOH and heated for 15 min at 60 °C. The film solutions were degassed for 15 min using a sonicator (Sonincs & Materials, USA). The resulting solutions (20 g) were poured onto Teflon-coated plates and allowed to dry in a hot air oven at 45 °C for 24 h. Then peeled off and stored in a desiccator before testing.

Scanning electron microscope (SEM)

Scanning electron microscope (FESEM, XL30 ESEM-TMP, Philips FEI, Netherland) was used to study the surface and cross-section morphologies of the films. Samples were prepared by dropping a film into liquid nitrogen followed by fracturing with a razor. Then the specimens were stuck onto a cylindrical aluminum stub with conducting resin. Afterward, the stub with the film was sputtered with gold in an ion sputter coater (K-450X, EMITECH, England) for 2 min. All samples were viewed and photographed at a voltage of 15 kV [22].

FTIR analysis

The Fourier transform infrared spectroscopy analyses (Nigaoli 360 intelligent, USA) were carried out to observe the structural interaction between fructan and WPI. The scans were carried out in a spectral range varying from 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1} [23].

Water vapor permeability measurement

Water vapor permeability of edible films was measured according to ASTM standard E96-05 with some modification [24, 25]. In this way, calcium chloride anhydrous (0% RH) was used as a moisture absorbing material, it was placed in special vials (Diameter 2 cm and height 4 cm), and its rapper bungs have 0.5 cm air gap, the samples were cut and placed between the vials' mouth and bungs. Then, they were stored in environmental chambers controlled at 25 ± 1 °C / $95 \pm 3\%$ RH. The weight variation curve is plotted against time, and the slope of the line in the linear curve is calculated. The permeability to the vapor was obtained by using Formula 2:

$$WVP = \frac{\text{slope} \times X}{A \times S \times \Delta R}$$

where slope \times is the weight gain of the cup per hour (g/h) (i.e. slope of the linear behavior), X is the average film thickness (mm), and A is the area of the exposed film surface

(m^2), S , saturated vapor pressure at test temperature (Pa), $\Delta R = R_2 - R_1$; R_1 is the relative humidity in the measuring medium and R_2 is the relative humidity inside the vials.

Contact angle measurement

The contact angle of the film was determined by the sessile-drop method using contact angle measurement [26]. the sessile drop method is based on the optical contact angle method. A 20 μl drop of distilled water was put on the surface of a film sample with a dimension of 5.0×5.0 Cm, and images were taken right after using a digital camera (Canon Powershot, Model A520, Taiwan) and then the angle between the baseline of the drop and the tangent at the drop boundary was measured using Image J software.

Film thickness measurement

To determine the thickness of the films, the digital micrometer (QLR digit-IP54, China) was used with an accuracy of 1 μm . Thickness at five different points of the film was measured, and then the average thickness corresponds to five measurements was calculated [1].

Mechanical properties

Films were conditioned for two days in a desiccator at 50% relative humidity (RH) and 25° C and then cut into strips (50×10 mm) before testing. The mechanical properties were determined using a TA-XT PlusTM, Texture Analyzer (Stable Micro Systems, England). Tensile strength (TS), percentage of elongation at break (% E) and elastic modulus (EM) were determined according to a modified ASTM method D882 [27]. The crosshead speed was set at 20 mm. min^{-1} and initial grip separation were set at 50 mm. Five samples of each film were tested, and the average of five results was reported. Young's modulus (YM) was evaluated as the slope of the initial linear portion of stress–strain curves. The tensile strength (TS) and elongation at break (E) were calculated according to the following equations:

$$TS = \frac{F}{L \cdot X}$$

where F is the maximum force at the rupture of the film, L is the film width (mm), X is the film thickness (mm).

$$E (\%) = \frac{L_1 - L_0}{L_0} \times 100$$

where L_1 is the fracture length (mm), L_0 is the initial length of film (mm).

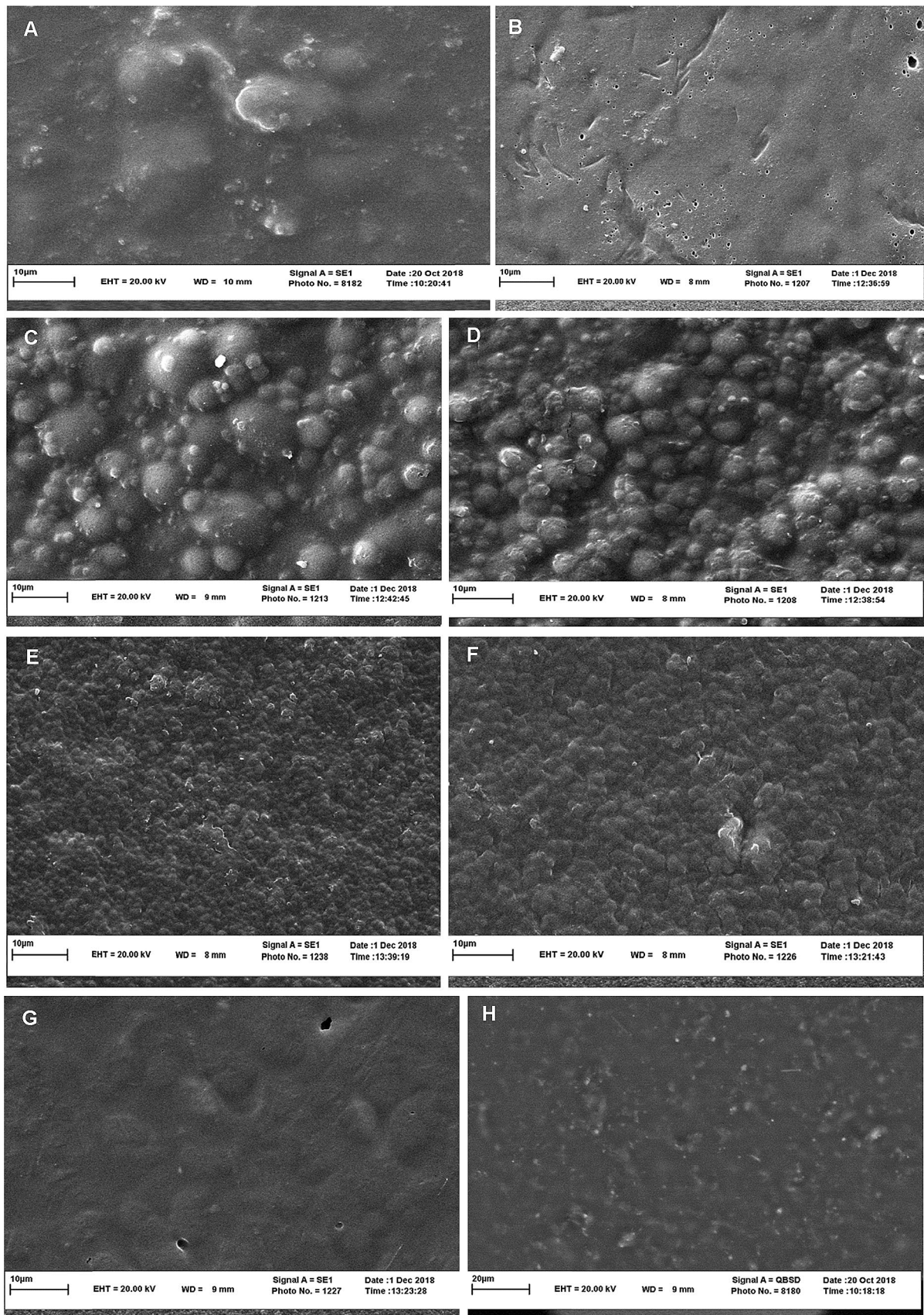


Fig. 1 SEM surface micrograph of Fructan **a**, WPI **b**, and composite WPI-Fructan films (WPI50/ fructan 50 (40% Gly) **c**, WPI50/ fructan 50 (50% Gly) **d**, WPI40/ fructan 60 (40% Gly) **e**, WPI40/ fructan 60 (50% Gly) **f**, WPI30/ fructan 70 (40% Gly) **g**, WPI30/ fructan 70 (50% Gly) **h**)

Differential scanning calorimetry (DSC)

DSC analysis was performed using a DSC calorimeter (Spico, SL 800, China), equipped with a thermal analyzer. The samples (18 mg) were hermetically sealed within aluminum pans and heated at a rate of 10 °C/min from 25 to 200 °C [28]. The degradation temperature (T_d), melting temperature (T_m) and ΔH values of different samples were calculated.

Statistical analysis

Data analyses were accomplished using SPSS 20. A one-way analysis of variance (ANOVA) using Duncan's test was applied. The measurements were conducted in triplicate with confidence level at $p < 0.05$.

Results and discussion

Scanning electron microscope (SEM)

SEM was used to visualize the surface and cross-sectional area of each film. The SEM micrographs for the surface and the cross-section of WPI and WPI-fructan composite film with different volumes are shown in Fig. 1. The incorporation of fructan resulted in a significant change in the structure and appearance of the WPI film. Furthermore, the heterogeneity of the film structure was decreased when fructan incorporated in different ratios (Fig. 1). Silva et al. (2018) also observed that the heterogeneity of WPI film decreased with polysaccharide addition. The rough surface of WPI-fructan films were mainly related to the presence of aggregated protein molecules, which were arranged and aggregated in the solution, resulting in the formation of spherulites. As the fructan incorporated, the surface of the WPI films became more compact and denser. This could be responsible for the less WVP values of composite WPI- fructan films compared to WPI film which had a large number of holes and pores (Fig. 1). The resulting structure may be due to intermolecular interaction between biopolymers, which facilitate the miscibility of the two phases. In addition, the WVP values are consistent with these results, indicating more resistance to moisture transmission. WPI at the highest ratio exhibited the most heterogeneous and uneven surface with a high amount of blisters in the composite film (Fig. 1c and d). This may be attributed to less miscibility of the component at a higher

ratio of WPI [29]. The cross-section of WPI film had numerous small pinholes and exhibited a sponge-like structure while those of films containing fructan were compact and smooth (Fig. 2). It was observed that at the highest ratio of fructan the number of pores and degree of cracks were substantially decreased, which was an indication of good compatibility and interaction between the WPI and fructan [30]. However, the size and number of holes and pores decreased with increasing fructan concentration to 70%, which in turn improved the WVP values of composite films. So, the addition of fructan significantly modifies the surface properties and vapor permeability of WPI film.

FTIR analysis

The FTIR spectra of WPI and WPI-fructan composite films with different concentrations of Gly are shown in Fig. 3. For Fructan and WPI-fructan composite films spectra, the peaks at the vicinity of 800–1200 cm^{-1} were observed, which are characteristic for polysaccharides. In addition, the absorption peaks in the region of 800–1200 cm^{-1} in the WPI spectra could be attributed to the presence of Gly [31]. The absorption peak at the region of 600–700 cm^{-1} in the fructan spectra representing the absorption of C-H aliphatic bending [32]. The bands at 811 and 867 cm^{-1} of fructan spectra are probably related to out of plane bending of C-H bonds in the fructopyranose units of inulin [21, 33]. The strongest absorption peak at the vicinity of 950–1150 cm^{-1} (fingerprint region) was attributed to the fructan spectrum. Goodfellow and Wilson (1990), also stated that this region is sensitive to the conformation of carbohydrates. The characteristic bands of fructan were located at 1034, 1056, and 1150 cm^{-1} in this region, which are attributed to C-O bond asymmetric stretching [23]. All the spectra showed an absorption peak at 1546 cm^{-1} except the spectra corresponding to fructan. This is attributed to the amide II region in the protein IR spectrum. Generally, in the amid I region, WPI and WPI-fructan composite films had more intense signals compared to fructan, suggesting the absorption of C-O and C-N stretching [21]. As can be seen in Fig. 3, all the spectra exhibited more absorption intensity at a wavenumber of 2881, 2930, and 3394 cm^{-1} compared to the WPI spectrum. The intensity enhancement of these bonds is related to the presence of fructan in composite films. It should be mentioned that the fructan spectrum exhibited the strongest absorption peaks in these regions, which affected by CH₂ stretching and bending vibration and also hydrogen bonding of the hydroxyl group [21]. The broad absorption band ranging between 3000 and 3500 cm^{-1} in the IR spectra of fructan film is attributable to O-H stretching vibration of CH-OH groups from a fructo-furanose units [21], which can form intermolecular hydrogen bonds with the carbonyl groups of the protein. Whereas the band observed at 2929 cm^{-1}

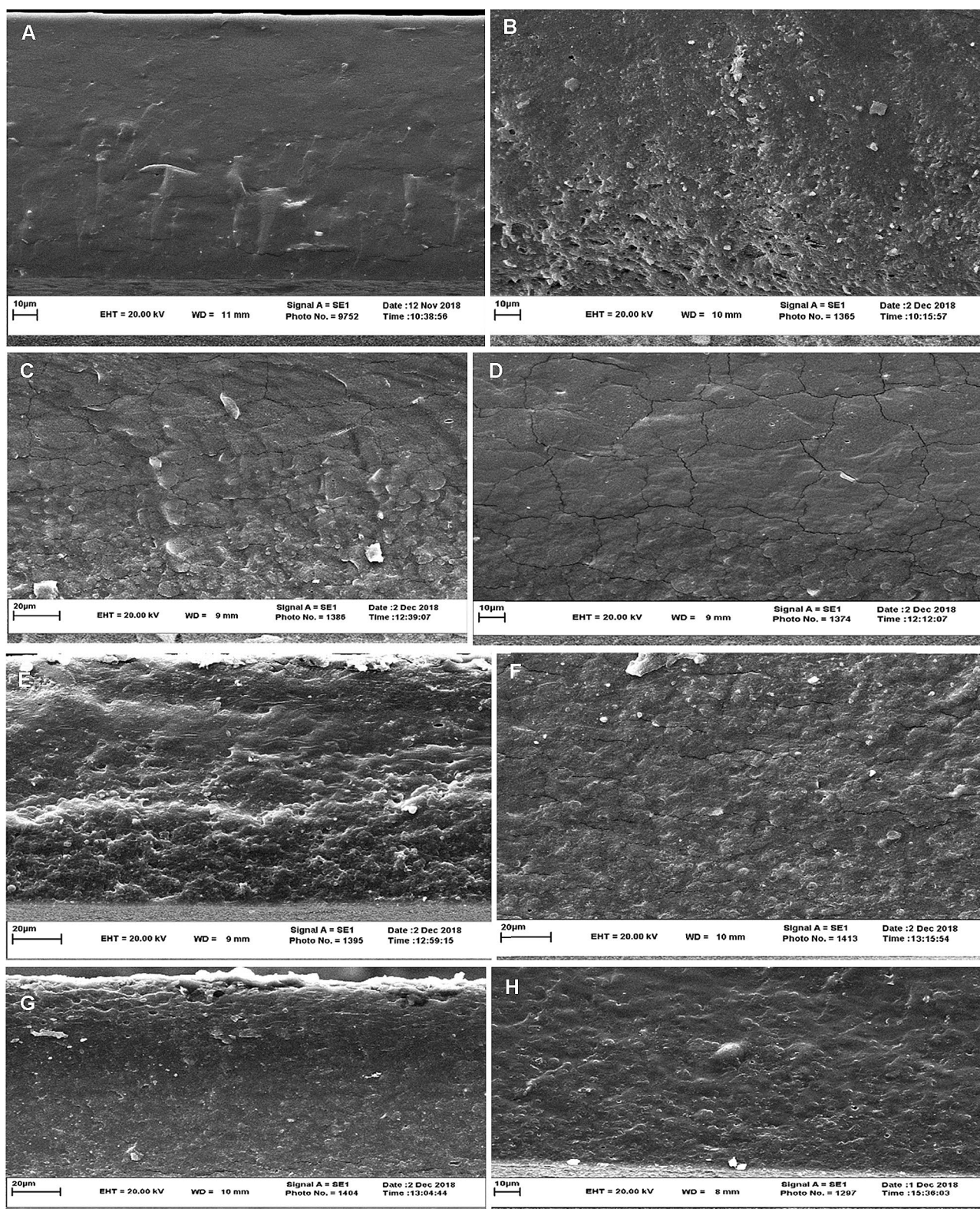


Fig. 2 SEM cross-section micrograph of Fructan **a**, WPI **b**, and composite WPI-Fructan films (WPI50/ fructan 50 (40% Gly) **c**, WPI50/ fructan 50 (50% Gly) **d**, WPI40/ fructan 60 (40% Gly) **e**, WPI40/

fructan 60 (50% Gly) **f**, WPI30/ fructan 70 (40% Gly) **g**, WPI30/ fructan 70 (50% Gly) **h**)

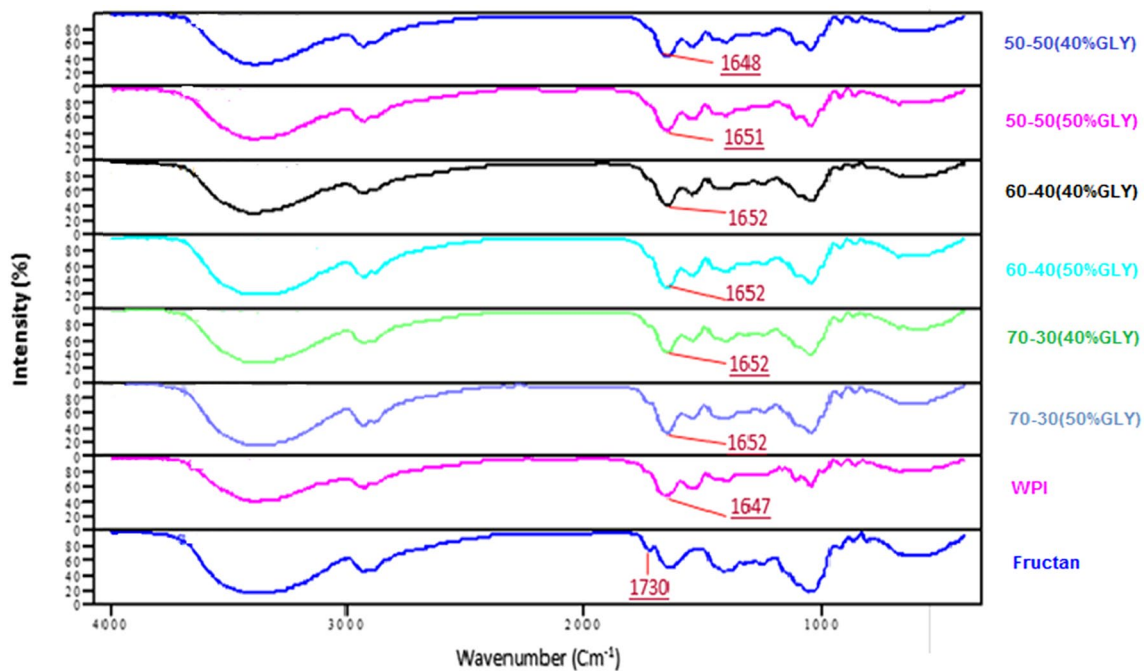


Fig. 3 FTIR spectroscopy of Fructan, WPI and Fructan-WPI composite films

for all the spectra is related to the C-H stretching region. The enhancement of intensity in this region is attributed to the increasing ratio of fructan in composite films. The most considerable effect created by the incorporation of fructan was a relative increase in the intensity of the amid I signal, and also the absorption band at 1647 cm^{-1} in WPI film (in the amid I region) moved to 1652 cm^{-1} . This results confirming the existence of hydrogen bonding interaction between WPI and fructan [34]. It can be seen that the band related to C=O group of polysaccharide (1730 cm^{-1}) was not observed in the composite films, suggesting the formation of intermolecular hydrogen bonding between components. This observation also highlights the lower capacity of carbohydrates conformations to form hydrogen bonding interaction between themselves at the presence of protein [35].

Water vapor permeability (WVP)

It was observed that the WVP increased with increasing WPI concentration Table 1). The higher water vapor permeability of WPI film may be related to protein hydrophilicity, which favors water absorption. Besides, the presence of large numbers of holes in the surface of composite films with a higher concentration of WPI (Fig. 1) leads to a loss of the film integrity, which favors water vapor transfer through hydrophilic films [36]. Permeability depends on the composition and the microscopic structure of the matrix [37]. After substitution with fructan, WVP of WPI film decreased approximately

3.6 times with a 70% aqueous fructan addition. WPI 30 / fructan 70 (40% Gly) indicated the best results in terms of water vapor permeability. Similar results were reported by Gounga et al. (2007), who observed that the WVP values of WPI films increased with increasing WPI concentration from 5 to 9% (w/w). This could be explained by the fact that fructan molecules increase internal hydrogen bonding of the protein network and reduce intermolecular spacing which consequently improve the WVP of composite films [38]. As we discussed earlier this observation also evidenced by FTIR. An increase of plasticizer concentration from 40 to 50% caused an increase in WVP values of composite films owing to a consequent increase in free volume. Furthermore, the hydrophilic nature of Gly significantly affects the water diffusion in the film matrix [39]. Kokoszka et al. (2010) also reported the same results when the Gly concentration increased from 50 to 60% in WPI based edible films.

Contact angle measurement

The contact angle of water droplets upon the film surface is an indication of surface hydrophobicity of films. Generally, films with higher contact angle values possess higher surface hydrophobicity [40, 41]. The contact angle of different films is shown in Table 1. Compared with the pure WPI film, the water contact angle of all composite films substantially increased with an increasing fructan ratio. This phenomenon may arise from the relatively high hydrophobic character of

Table 1 Values (average \pm standard deviation) of thickness, WVP and contact angle of whey protein isolate (WPI), fructan and WPI-fructan composite films at two ratios of glycerol

Samples	Thickness (mm)	WVP ($\times 10^{-6}$ g / m.s.pa)	Contact angle ($^{\circ}$)
Fructan (control)	0.071 ± 0.003^b	2.55 ± 0.04^e	73.96 ± 4.31^a
WPI (control)	0.099 ± 0.001^a	6.64 ± 0.02^a	36.17 ± 2.75^f
WPI 30/ Fructan 70 (40% Gly)	0.039 ± 0.004^e	1.85 ± 0.05^g	65.24 ± 2.73^b
WPI 30/ Fructan 70 (50% Gly)	0.041 ± 0.005^c	2.035 ± 0.02^f	64.13 ± 6.11^d
WPI 40/ Fructan 60 (40% Gly)	0.046 ± 0.003^d	2.56 ± 0.02^e	65.85 ± 5.45^b
WPI 40/ Fructan 60 (50% Gly)	0.049 ± 0.001^c	3.08 ± 0.01^d	62.56 ± 8.33^e
WPI 50/ Fructan 50 (40% Gly)	0.063 ± 0.003^c	3.74 ± 0.01^c	64.9 ± 2.51^c
WPI 50/ Fructan 50 (50% Gly)	0.071 ± 0.002^b	4.11 ± 0.01^b	61.27 ± 4.86^f

Different letters in the same column indicate significant differences ($P < 0.05$)

fructan (contact angle of 73.96°), which demonstrated high performance on the surface hydrophobicity of the WPI film. A contact angle of about 61 – 65° was observed for three different composite films (Table 1). It can be stated that the presence of high content of fructan could cause conformational change due to intermolecular interaction between protein and polysaccharide compounds. This statement is also confirmed by FTIR analysis. It is also observed in Table 1 that the contact angle of composite films decreased with increasing in Gly, corresponding to the high hygroscopic nature of Gly, which increases the polymer chain mobility and subsequently enhances the moisture sensitivity of the films [41]. A similar observation has been recently reported by Ramos et al. (2013) for films made from whey protein with three levels of glycerol. So, it can be concluded that the incorporation of fructan into WPI films provided a more hydrophobic character (water repellent). These results are also consistent with the promotion of water vapor barrier properties of composite films, as well as with the results from the surface morphology studies of the films which showed that composite films were more compact (Sect. 3.1), and thus entertained lower diffusion rates to water molecules because of obstruction to transform through the more compact biopolymer network.

Film thickness

Table 1 represents the thickness values of the resulting films. The film thickness decreased slightly from 0.071 to 0.039 mm as the ratio of fructan increased. This phenomenon may be due to the reduction of protein concentration when higher ratio of fructan was incorporated into composite films [22]. The incorporation of higher amount of glycerol (i.e., 50%) slightly increased the thickness value

of composite films because a higher amount of glycerol can increase the intermolecular spacing between polymer chains [39, 42]. It should be noted that a high film thickness increases WVP so, it must be controlled carefully [4].

Mechanical properties

The mechanical properties of WPI, fructan, and composite films including tensile strength, elongation and Young's modulus (elastic modulus) are represented in Table 2. There

Table 2 Mechanical properties (Tensile strength (TS), percent of elongation at break (EAB %) and Young's modulus (average \pm standard deviation)) of whey protein isolate (WPI), fructan and WPI-fructan composite films at two ratios of glycerol

Samples	TS (Mpa)	EAB (%)	Young's modulus (Mpa)
Fructan (control)	29.53 ± 0.01^a	29.2 ± 0.29^c	43.77 ± 7.23^a
WPI (control)	6.91 ± 0.87^f	2.02 ± 1.32^h	30.84 ± 2.63^g
WPI 50/ Fructan 50(40% Gly)	6.13 ± 1.02^g	14.62 ± 0.51^g	32.13 ± 4.32^f
WPI 50/ Fructan 50(50% Gly)	4.32 ± 0.56^h	15.16 ± 0.42^f	30.9 ± 2.33^g
WPI 40/ Fructan 60(40% Gly)	10.35 ± 0.91^d	26.96 ± 1.36^e	41.1 ± 5.42^d
WPI 40/ Fructan 60(50% Gly)	8.19 ± 0.02^e	29.4 ± 0.12^d	40.69 ± 1.86^e
WPI 30/ Fructan 70 (40% Gly)	16.25 ± 0.48^b	36.08 ± 1.11^b	42.84 ± 3.11^b
WPI 30/ Fructan 70 (50% Gly)	11.82 ± 0.83^c	35.46 ± 2.23^a	41.39 ± 4.22^c

Different letters in the same column indicate significant differences ($P < 0.05$)

Table 3 Thermal properties of whey protein isolate (WPI), fructan and WPI-fructan composite films at two ratios of glycerol: degradation temperature (Td), melting temperature (Tm) and ΔH values (average \pm standard deviation)

Films	Tm (°C)	Td (°C)	ΔH (J/g)
Fructan (control)	58.4 \pm 1.2 ^g	76.5 \pm 0.8 ^g	114.9 \pm 1.5 ^g
WPI (control)	56.12 \pm 1.3 ^h	74.1 \pm 1.1 ^h	81.43 \pm 1.7 ^h
WPI 30/ Fructan 70 (40% Gly)	187.4 \pm 2.6 ^a	191.4 \pm 2.3 ^b	154.3 \pm 2.3 ^a
WPI 30/ Fructan 70 (50% Gly)	179.9 \pm 2.6 ^c	192.3 \pm 0.6 ^a	152.8 \pm 0.6 ^b
WPI 40/ Fructan 60(40% Gly)	180.2 \pm 4.1 ^b	132.6 \pm 1.3 ^d	141.3 \pm 1.8 ^c
WPI 40/ Fructan 60(50% Gly)	178.6 \pm 2.1 ^d	134.1 \pm 1.6 ^c	139.8 \pm 2.6 ^e
WPI 50/ Fructan 50(40% Gly)	121.6 \pm 1.1 ^e	123.8 \pm 2.2 ^e	140.6 \pm 1.5 ^d
WPI 50/ Fructan 50(50% Gly)	117.5 \pm 1.8 ^f	121.1 \pm 0.9 ^f	138.9 \pm 1.3 ^f

Different letters in the same column indicate significant differences ($P < 0.05$)

was a significant decrease in tensile strength when the plasticizer content increased. So the films with a higher content of glycerol became more flexible and stretchable due to the plasticizing effect of glycerol, which increases the mobility of polymer chains. Generally, formulations with a higher ratio of fructan exhibited higher tensile strength than WPI film. This effect was more considerable in formulation with the highest ratio of fructan (i.e. 70%). Pranoto et al. (2007) reported that cross-linking of long-chain carbohydrates macromolecules with gelatin increased TS. Fonkwe et al. (2003) also observed that interaction between polysaccharides and gelatin molecules resulted in strengthening of the film structure. WPI film showed less resistance to deformation compared to fructan and composite films. In this regard, Silva et al. (2016) stated that the combination of disulfide bonds, hydrophobic interactions, and hydrogen bonds in the structure of whey protein films make them very brittle. Generally, when the fructan content increased to 60%, a considerable change was observed in the Young's modulus. The values of Young's modulus in WPI / fructan composite film were increased from 32.13 to 41.1 Mpa and 30.9–40.69 Mpa with increasing fructan ratio from 50 to 60% v/v with 40 and 50% Gly respectively. Further incorporation of fructan slightly increased the Young's modulus. As a matter of fact, the incorporation of fructan into WPI resulted in stronger and more elastic films. This phenomenon could arise from a good interaction between blended polymers. These results are consistent with WPI films [43] in which elongation at break increased as the concentration of polysaccharide (konjac galactomannan) increased. As shown in Table 2, WPI film exhibited the lowest TS of 6.91 Mpa, while the fructan film had the highest TS of 29.53 Mpa. The TS of composite films increased as the fructan ratio increased, suggesting that fructan substantially strengthened the WPI-fructan films. The greatest TS value (16.25 Mpa) at the highest fructan ratio (70% with 40% Gly) in the WPI-fructan composite film suggests that intermolecular interactions between protein and polysaccharide are considered as the main responsible factor for the increasing film strength [14]. This statement also confirmed by FTIR results. WPI film had an EAB value

of 2.02%. A significant difference was observed between EAB of all samples ($p < 0.05$). The addition of fructan substantially increased the EAB%, so that the WPI film with the highest ratio of fructan exhibited the highest EAB%. The increasing ratio of fructan could increase the cross-linking reaction of fructan with WPI which in turn resulted in the increasing EAB% of the composite films due to the extending of macromolecular relaxations along with polymer chain mobility [39]. The glycerol content of the composite films also caused a significant difference in EAB% values, but it was not considerable. It can be stated that the interaction between WPI and fructan improved the film crosslinking, causing an increasing cohesion between polymer chain, which resulted in higher tensile strength and elongation of WPI film.

Differential scanning calorimetry (DSC)

The degradation temperature (Td), melting temperature (Tm), and ΔH values of different films are shown in Table 3. A slight increase in the enthalpy values of composite films with increasing the amount of fructan might arise from the protecting role of polysaccharides molecules which behave as steric spacers between protein molecules and consequently reduce the extent of aggregation [44]. In addition, the formation of hydrogen bonds between protein and polysaccharide in composite films, as evidenced by FTIR, could be another explanation for increasing enthalpy values. The substitution of fructan caused changes in the Tm and degradation temperature (Td) of WPI films. The degradation temperature of composite films was observed at the range of 191.4–134.1 °C (Table 3). The increase in the denaturation temperature of WPI at the presence of fructan may be attributed to hydrogen bond formation, leading to increasing the thermal stability of the WPI. Ibanoglu (2005) also reported a continuous increase in the thermal stability of different proteins in the presence of hydrocolloids and noted that hydrocolloids could influence the degradation temperature by establishing more hydrogen bonds. As shown in Table 2, Tm of composite films was dependent on the fructan ratio. It

can be concluded that increasing the fructan ratio decreased the mobility of the biopolymer chains, which indicated that the substitution of fructan could enhance the Tm of WPI film [28]. All composite films showed that Tm and ΔH decreased when the glycerol content increased ($P < 0.05$). This phenomenon is a consequence of the plasticizing effect of glycerol, which reduces the interaction between biopolymers due to increasing the free volume of polymer network and the mobility of the polymer chains [38].

Conclusion

The water vapor permeability, thermal, and mechanical properties of WPI-based films were improved markedly with the incorporation of different ratios of fructan. Results demonstrated that fructan effectively interacted with WPI in the composite film by hydrogen bonding interactions, which evidenced by FTIR and DSC analysis. Pure fructan and WPI-fructan composite films had higher TS and EAB% than pure WPI films. With increasing glycerol concentration, WVP increased while TS and Young's modulus decreased. SEM revealed the formation of more homogenous film surfaces at the highest ratio of fructan. Based on the results the WPI 30 / Fructan 70 (40% Gly) is suggested as the best ratio for preparation of the composite film. It can, therefore, be concluded that the incorporation of WPI by fructan made it possible to obtain polymer composites with improved barrier and mechanical properties and represents an innovation for application as a packaging material. Therefore, the WPI-fructan composite film is suggested as a good packaging material with suitable barrier and mechanical properties depending on applications.

Acknowledgment The authors would like to acknowledge the financial support of the Ferdowsi University of Mashhad.

Compliance with ethical standards

Conflict of interest The author declare that they have no conflict of interest.

References

- S. Sukhija, S. Singh, C.S. Riar, *Food Hydrocoll.* **60**, 128 (2016)
- W.S. Lim, S.Y. Ock, G.D. Park, I.W. Lee, M.H. Lee, H.J. Park, *Food Packag. Shelf. Life* **26**, 100556 (2020)
- R. Thakur, P. Pristijono, C.J. Scarlett, M. Bowyer, S.P. Singh, Q.V. Vuong, *Int. J. Biol. Macromol.* (2019).
- M. E. Gounga, S. Y. Xu, Z. Wang (2007) *J Food Eng.*
- T. Bourtoom, *Int. Food Res. J.* (2008).
- L.Z. Wang, L. Liu, J. Holmes, J.F. Kerry, J.P. Kerry, *Int. J. Food Sci. Technol.* (2007).
- M.E. Embuscado, K.C. Huber, *Edible Films and Coatings for Food Applications* (Springer, New York, 2009).
- K. Khwaldia, C. Ferez, S. Banon, S. Desobry, J. Hardy, *Crit. Rev. Food Sci. Nutr.* (2004).
- X. Zhang, Y. Zhao, Y. Li, L. Zhu, Z. Fang, Q. Shi, *Int. J. Biol. Macromol.* (2020).
- S. Jiang, T. Zhang, Y. Song, F. Qian, Y. Tuo, G. Mu, *Int. J. Biol. Macromol.* **126**, 1266 (2019)
- E. Basiak, S. Galus, A. Lenart, *Int. J. Food Sci. Technol.* **50**, 372 (2015)
- S.M. Jafari, M. Khanzadi, H. Mirzaei, D. Dehnad, F.K. Chegini, Y. Maghsoudlou, *Int. J. Biol. Macromol.* **80**, 506 (2015)
- S. Sukhija, S. Singh, C.S. Riar, *Polym. Compos.* **39**, E407 (2018)
- J. Osés, M. Fabregat-Vázquez, R. Pedroza-Islas, S.A. Tomás, A. Cruz-Orea, J.I. Maté, *J. Food Eng.* **92**, 56 (2009)
- M. A. García, A. Pinotti, M. N. Martino, and N. E. Zaritzky, in *Edible Film. Coatings Food Appl.* (2009).
- S.F. Sabato, N. Nakamurakare, P.J.A. Sobral, *Radiat. Phys. Chem.* **76**, 1862 (2007)
- A. Pourfarzad, M. B. Habibi Najafi, M. H. Haddad Khodaparast, and M. Hassanzadeh Khayyat, *J. Food Sci. Technol.* (2015).
- R. Gaggeri, D. Rossi, K. Mahmood, D. Gozzini, B. Mannucci, F. Corana, M. Daglia, A. Avanzini, M. Mantelli, E. Martino, *J. Med. Plants Res.* **9**, 1038 (2015)
- H. Hosoya, *Discret. Appl. Math.* **19**, 239 (1988)
- N. Kaur and A. K. Gupta, *J. Biosci.* (2002).
- A. Pourfarzad, M. B. Habibi Najafi, M. H. Haddad Khodaparast, and M. Hassanzadeh Khayyat, *Qual. Assur. Saf. Crop. Foods* (2015).
- K.S. Silva, T.M.R. Fonseca, L.R. Amado, M.A. Mauro, *Food Packag. Shelf. Life* **16**, 122 (2018)
- R. V. de B. Fernandes, S. V. Borges, D. A. Botrel, and C. R. de Oliveira, *Int. J. Food Sci. Technol.* (2014).
- L.X. Mei, A.M. Nafchi, F. Ghasemipour, A.M. Easa, S. Jafarzadeh, A.A. Al-Hassan, *Int. J. Biol. Macromol.* **164**, 4603 (2020)
- S. Jiang, X. Zhang, Y. Ma, Y. Tuo, F. Qian, W. Fu, G. Mu, *Carbohydr. Polym.* **153**, 153 (2016)
- C. Ramírez, I. Gallegos, M. Ihl, V. Bifani, *J. Food Eng.* **109**, 424 (2012)
- S. Ekramian, H. Abbaspour, B. Roudi, L. Amjad, and A. M. Nafchi, *J. Polym. Environ.* **1** (2020).
- M. Zolfi, F. Khodaiyan, M. Mousavi, M. Hashemi, *J. Food Sci. Technol.* **52**, 3485 (2015)
- L. M. Pérez, C. E. Balagué, A. C. Rubiolo, and R. A. Verdini, *Procedia Food Sci.* (2011).
- F. Askari, E. Sadeghi, R. Mohammadi, M. Rouhi, M. Taghizadeh, M. Hosein Shirgahoun, and M. Kariminejad, *J. Food Process. Preserv.* **42**, e13715 (2018).
- P. Guerrero, A. Retegi, N. Gabilondo, and K. De La Caba, *J. Food Eng.* (2010).
- A. Pourfarzad, M. B. Habibi Najafi, M. H. Haddad Khodaparast, and M. H. Khayyat, *J. Food Sci. Technol.* (2015).
- S. Bambang Widjanarko, A. Nugroho, and T. Estiasih, *African J. Food Sci.* (2011).
- P. Guerrero, T. Garrido, I. Leceta, and K. De La Caba, *Eur. Polym. J.* (2013).
- P. Guerrero, J. P. Kerry, and K. De La Caba, *Carbohydr. Polym.* (2014).
- J.F. Rubilar, R.N. Zúñiga, F. Osorio, F. Pedreschi, *Carbohydr. Polym.* **123**, 27 (2015)
- C. Li, W. Zhu, H. Xue, Z. Chen, Y. Chen, and X. Wang, *Food Hydrocoll.* (2015).
- Ó. L. Ramos, I. Reinas, S. I. Silva, J. C. Fernandes, M. A. Cerqueira, R. N. Pereira, A. A. Vicente, M. F. Poças, M. E. Pintado, and F. X. Malcata, *Food Hydrocoll.* (2013).
- A. Abedinia, F. Ariffin, N. Huda, A.M. Nafchi, *Int. J. Biol. Macromol.* **109**, 855 (2018)

40. S. Kokoszka, F. Debeaufort, A. Lenart, and A. Voilley, *Int. Dairy J.* (2010).
41. J.-F. Su, Z. Huang, X.-Y. Yuan, X.-Y. Wang, M. Li, *Carbohydr. Polym.* **79**, 145 (2010)
42. L.M. Pérez, G.N. Piccirilli, N.J. Delorenzi, R.A. Verdini, *Food Hydrocoll.* **56**, 352 (2016)
43. S. Yoo and J. M. Krochta, *J. Sci. Food. Agric.* (2011).
44. E. Ibanoglu, *Food. Chem.* (2005).

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.