Express Polymer Letters Vol.18, No.6 (2024) 592–606
Available online at www.expresspolymlett.com
https://doi.org/10.3144/expresspolymlett.2024.44

Research article

Sustainable packaging: A comparative study on thermoplastic whey protein films with biodegradable plasticizers

*Forough Abbasi Shahir, Zahed Ahmadi**, *Sayed Mahmood Rezaee Darvishi*

Department of Chemistry, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran

Received 29 December 2023; accepted in revised form 12 March 2024

Abstract. Glyceryl lactate (GlyLA) was synthesized through the esterification of glycerol (Gly) and lactic acid (LA) to plasticize whey protein concentrate (WPC) films with three different ratios of WPC to plasticizer (85, 90, and 100% w/w). LA was also introduced as a new plasticizer of whey protein films and compared in similar contents. Gly-plasticized films were also used as control groups. The synthesized GlyLA, including compatible esterification degree with WPC, was characterized by Fourier transform infrared Spectroscopy (FTIR) and hydrogen nuclear magnetic resonance spectroscopy (H-NMR) analyses. Then, the plasticizing effect of different types of plasticizers on the optical, thermal, mechanical, and water resistivity properties of the prepared films was evaluated. FTIR in attenuated total reflectance (ATR), Differential Scanning Calorimetry (DSC), tensile measurements, and cup method were also employed to examine the molecular structure, transition temperatures, mechanical resistivity, and water vapor permeability (WVP) of the modified films. The results showed a lower glass-transition temperature (T_g) and better ductility. By Increasing plasticizer content, solubility and WVP were increased in all groups. Furthermore, WPC-based films plasticized with 80 or 90% w/w of LA and GlyLA exhibited not only higher tensile strength and flexibility but also remarkably lower WVP than Gly plasticized films, turning them into potential alternatives for application as food packaging.

Keywords: *whey protein concentrate, lactic acid, glyceryl lactate, plasticizer, physical properties*

---

1. Introduction

In recent years, considerable efforts have been made to reduce the general concerns related to the detrimental effects of non-biodegradable plastic films on our environment; therefore, edible film formation is of much interest. Edible films must have the necessary properties for food packaging, including a barrier against moisture penetration, improved color appearance, desirable mechanical properties, and non-toxicity [1, 2]. They typically are constructed from proteins, lipids, and polysaccharides alone or in combination with each other [3, 4]. Whey proteins are one type of biopolymer that has good nutritional values and film-forming ability. As compared to polysaccharide-based counterparts, cross-linked protein films are more stable and durable. Lipids are not polymers and usually do not form a continuous independent film [5, 6]. The inherent limitations of whey proteins, such as their brittleness, low water vapor permeability, and poor thermal and physical properties, present a challenge to their widespread application. For instance, brittleness can be caused by hydrogen bonds, electrostatic forces, hydrophobic bonds, or disulfide crosslinking between polymer chains. With the aim of widespread industrial utilization of prepared films, reducing agents, and especially plasticizers, have been applied to whey protein structure in order to impart flexibility, improve

---

*Corresponding author, e-mail: za.ahmadi@gmail.com

toughness, and reduce glass transition temperatures and intermolecular interactions [7, 8].

Plasticizers, as small molecules, reduce the forces holding polymer chains through conducting interactions with the chains, resulting in an elongated and softened matrix [9]. Typical plasticizers used for whey protein films are polyols, including glycerol (Gly), sorbitol, polyethylene glycol, and xylitol [10, 11], a combination of Gly and trehalose [12], and sucrose [13]. Almost all of the mentioned plasticizers have some plastic effects on whey proteins, such as reducing glass-transition temperature (T_g), increasing elongation at break, and improving ductility. Among these, Gly has been chosen as the best plasticizer of whey protein films since it leads to more stable, flexible, and less brittle films. However, the thermal, mechanical, and barrier properties of Gly-plasticized whey protein films are not good enough for widespread industrial application [9, 14, 15].

To develop industrial applications, significant efforts have been made to overcome these challenges of whey protein films, especially in the presence of Gly as a plasticizer. Different parameters, including protein purity and Gly content [15], the proportion of protein and Gly [16], film-forming solution heating time and temperature [17], and different relative humidity of storage [18] on physicochemical properties of whey protein films, were evaluated. Moreover, the plasticizing effect of waxes [19], fatty acids [20], plant oils [21, 22], nanoparticles [23–27], and combination of Gly with other plasticizers [10] or combination of whey with other biopolymers [28–31] on the properties of the formed films was investigated.

It seems that, as compared to Gly, new plasticizers are needed in order to improve the flexibility, strength, elasticity, transparency, mechanical, and barrier properties of whey protein films. In this study lactic acid (LA) and glyceryl lactate (GlyLA) were introduced as two new plasticizer candidates for whey protein concentrate (WPC) films. After GlyLA synthesis and characterization, the molecular structure, thermal, mechanical, barrier, transparency, and solubility of the WPC-based films with different content of plasticizers were studied by Fourier transform infrared spectroscopy (FTIR) analysis under attenuated total reflectance (ATR) mode, differential scanning calorimetry (DSC), tensile measurements, and cup method. The relation of these parameters is essential in optimizing film components in terms of plasticizer type and contents before the film production toward their application as biodegradable packaging.

2. Materials and methods

2.1. Materials

Hilmar 8010 containing 80% WPC instantized powder (Hilmar, California, United States) was purchased and used as the primary material to prepare biodegradable films. Toluene (>99.9%), LA (90%), Gly (99.5%), methanesulfonic acid (<98%), Magnesium Nitrate, Saturated Sodium Chloride, and sodium sulfite were purchased from Merck (Darmstadt, Germany) and used without further purification unless stated otherwise.

2.2. Synthesis of GlyLA

GlyLA, as a bio-based plasticizer of WPC, was synthesized in an esterification reaction with a specific degree according to the following procedure reported in the literature [32]. To this end, 0.31 mol Gly, 0.27 mol LA, and methansulfonic acid (0.1% w/w, diluted in 12.5 g toluene) were transferred into a three-necked flask equipped with an azeotropic column. First, the flask was heated for 2 h at 145 °C and the produced water during the esterification reaction was collected in the azeotropic column. Afterward, the temperature was increased up to 165 °C and then the reaction was left under stirring for 3 h at this temperature. Finally, when the reaction was completed, the residues of toluene and LA were removed through a rotary evaporator.

2.3. Preparation of WPC-based films

WPC films were prepared with various plasticizers, including Gly, LA, and GlyLA, using the solution casting method. First, the WPC powder was dispersed in distilled water (5% w/w) and stirred for 1 h (pH = 7, room temperature). To denaturation of the sulfide bonds of whey protein, the solution was heated up to 90 °C and kept for 30 min and then cooled down to room temperature. After cooling the solutions, Gly, LA, and GlyLA with different contents were added to the whey protein solution as plasticizers (Table 1). It should be noted that the films containing less than 80% w/w of LA or GlyLA and films with 80% w/w of Gly were brittle. Therefore, concentrations of the plasticizers were selected at 80, 90, and 100% w/w for LA and GlyLA, and 85, 90, and 100% w/w for Gly. Moreover, 0.05 g sodium sulfite was added to each solution containing GlyLA.

Table 1. Protein, plasticizer, and water content of the WPC-based edible films.

Film:* WPC a-plasticizer b	Whey protein concentrate [g]	Plasticizer [g]	Water [g]
WPC5-Gly85	5	4.25	90.75
WPC5 -Gly90	5	4.50	90.50
WPC5 -Gly100	5	5.00	90.00
WPC5 -LA80	5	4.00	91.00
WPC5 -LA90	5	4.50	90.50
WPC5 -LA100	5	5.00	90.00
WPC5 -GlyLA80	5	4.00	91.00
WPC5 -GlyLA90	5	4.50	90.50
WPC5 -GlyLA100	5	5.00	90.00

*a: WPC weight [g] in 100 g of total weight
b: The weight percentage % w/w of Gly, LA, and GlyLA compared to the weight of WPC dry powder.

Then, the above solutions were mixed for another 30 min and the ultrasonic probe was applied to obtain a homogenous solution. Finally, Gly and LA Casted protein solutions were dried in ambient humidity and room temperature for at least 24 h.

2.4. Film characterization

2.4.1. FTIR

Bruker Tensor-27 FT-IR Spectrometer (Bruker Corporation, Billerica, Massachusetts, United States) was used to determine the characteristic peaks of whey protein powder and to identify the chemical structure of GlyLA in the range of 400–4000 cm^{-1} wavenumber. Furthermore, the spectra of WPC films were obtained to identify the interactions of whey protein with diverse plasticizers in the ATR mode. Three replicates of each film containing plasticizer were measured.

2.4.2. Hydrogen nuclear magnetic resonance spectroscopy (H-NMR)

The chemical structure confirmation of GlyLA was characterized by applying Bruker nuclear magnetic resonance spectrometer (NMR, 500 MHz, Bruker Corporation, Billerica, Massachusetts, United States), and D_2O at 298k was used as a dissolving agent.

2.4.3. DSC

The T_g of the prepared films was assessed using a Mettler Toledo DSC 823e instrument (Mettler Toledo, Columbus, Ohio, United States), and data was analyzed with STARe SW 9.10 software. The aluminum container was used as a reference for this purpose, then samples with an approximate weight of 7±0.4 g at $5°\cdot cm\cdot min^{-1}$ rate were exposed to temperatures between 50 and 100 °C in the heating cycles.

2.4.4. Film thickness

Prior to property testing, all the WPC-prepared films were conditioned at 35 °C for at least 48 h at a 75% relative humidity (*RH*). The film thickness was also determined using a digital micrometer (ACIMETO, Germany) with an accuracy of ±0.01 mm in at least 10 random points for each film, and the mean data were presented. The average thickness of each film was considered when calculating its water vapor permeability (WVP) and mechanical properties.

2.4.5. Solubility

The solubility of the whey protein films was determined, as reported in the literature [33]. Briefly, small pieces of the prepared films were weighed and dried at 65 °C in an oven to obtain initial dry matter. Afterward, they were immersed in 50 ml of deionized water (0.02% sodium azide to prevent microbial growth) and gently stirred at room temperature for 5 h. Finally, to determine the dry matter that was not solubilized in water, the mixtures, including insoluble and soluble phases, were filtrated to separate the insoluble solids. After leaving filters and insoluble pieces to dry in the oven at 65 °C, the film pieces were weighed. The solubility percentage of films in water was calculated using the Equation (1):

$$\text{Solubility [\%]} = 1 - \frac{\text{weight of dry matter not solubilized}}{\text{weight of dry matter}} \cdot 100 \quad (1)$$

2.4.6. Water vapor permeability (WVP)

The ASTM standard method E-96 was applied to assess the WVP of the prepared WPC films. Aluminum cups with an outer diameter of 3.5 cm were filled with calcium chloride as a drying agent and the prepared films were placed on the container's mouth. They were then sealed with rubber washers and fastened by screw clamps. The initial weight of the whole container was measured and then transferred to the glass desiccator containing saturated saline water at 25 °C. The presence of saturated NaCl in the desiccator and CaCl inside the containers induced a relative humidity of 75 and 0% in the desiccator and cups, respectively. This humidity difference causes the vapor pressure difference of 1753.55 and, subsequently, water vapor transmission from the desiccator to inside the cups. Within 12 h, the cups were removed from the desiccator once an hour and were weighted with an accuracy of 0.0001 using the digital balance. Diagrams of the weight increase of cups versus time were plotted, and water vapor transmission rate (*WVTR*) and *WVP* were calculated by the Equations (2) and (3):

$$WVRT = \frac{\text{Curve slope}}{\text{Film surface area}} \tag{2}$$

$$WVP = \frac{\text{Thikness} \cdot WVTR}{\text{Pressure difference}} \tag{3}$$

2.4.7. Mechanical properties

Mechanical testing of the prepared films was carried out using the Instron device (Norwood, Massachusetts, United States) according to ASTM-D882 standard. To this end, the films were cut into rectangles of 1×7 cm and preconditioned in a desiccator under 50% humidity at room temperature for 48 h before testing. The distance between the two jaws of the device and the movement speed of the jaws were 30 mm and 5 mm/min, respectively. Parameters including tensile strength, elastic modulus, and strain at the break were obtained from the resulting stress-strain curves. The experiments were repeated three times for each film.

2.4.8. Light transmission of films

To determine the ultraviolet (UV) and visible light barrier properties of the films, three strips in dimension of 4×1 cm were prepared and placed on cuvettes and scanned using a UV-Vis spectrophotometer (Perkin Elmer Lambada 950 spectrophotometer, Waltham, Massachusetts, United States) within the wavelength of 280 to 800 nm. The empty cell was considered a blank or control.

2.5. Statistical analysis

To determine the effects of WPC-based and plasticizers on film properties, a completely randomized experiment was conducted. All experiments were performed at least three times to determine each property. One-way analysis of variance (ANOVA) and Duncan's multiple range tests statistically evaluated the obtained data at a confidence interval of 0.05. Statistical analysis was conducted using SPSS for Windows.

3. Results and discussion

3.1. Fabrication and characterization of GlyLA as a plasticizer

GlyLA was prepared through an esterification process of LA to be applied as a bio-based plasticizer of WPC [32]. The synthesized biodegradable plasticizer of GlyLA was characterized via FTIR and ^{1}H-NMR analyses. To confirm the esterification reaction, the FTIR spectra of LA, Gly, and GlyLA were compared in Figure 1. The characteristic band of C=O stretching vibration of LA (1731 cm^{-1}) could also appear in the GlyLA spectrum with a slight shift in 173 cm^{-1}, indicating the formation of the carbonyl ester group [34]. Moreover, the peaks located at 3383, 2935, and 2881 cm^{-1} could be assigned to the stretching vibrations of O–H and aliphatic C–H, respectively, which are in common with LA and Gly spectra [35]. The bands observed at 1129 and 1454 cm^{-1} could be related to C–O and C–H bending vibrations of the methyl group of lactate [34].

The condensation reaction of the LA and synthesis of GlyLA Were further verified by ^{1}H-NMR analysis. As shown in Figure 2, the peaks labeled **a**, **b**, **c**, and **d** could be assigned to the proton resonances of Gly and LA. In addition, the appearance of the new signals in ~3.8 and 4.3 ppm could be related to the methine and methylene groups of the esterified Gly. Moreover, the degree of esterification (D_e) was calculated using the Equation (4) [36]:

$$D_\text{e} = \frac{4I_{1.3}}{3(I_{4.1} + I_{3.5})} \tag{4}$$

where $I_{1.3}$, $I_{4.1}$, and $I_{3.5}$ are the representative intensities of the methyl groups of the LA moieties and methylene groups of the Gly moieties, respectively.

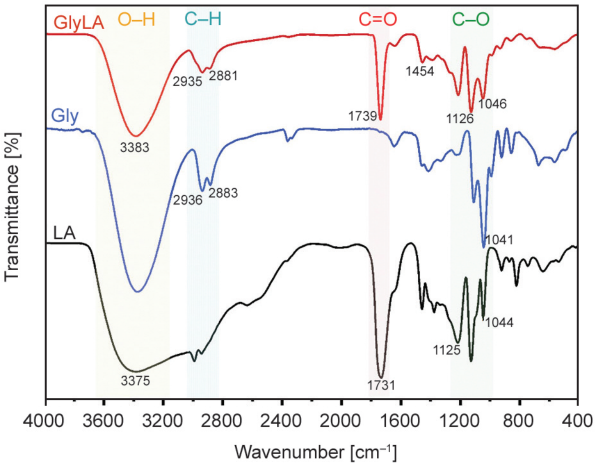

Figure 1. FTIR spectra of Gly, LA, and GlyLA.

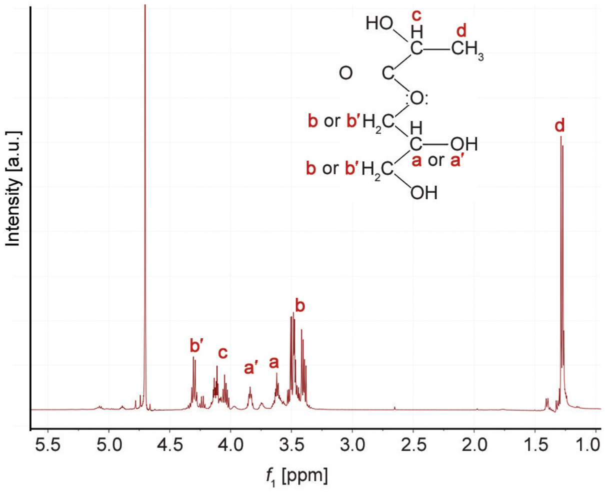

Figure 2. ^{1}H-NMR spectra of GlyLA.

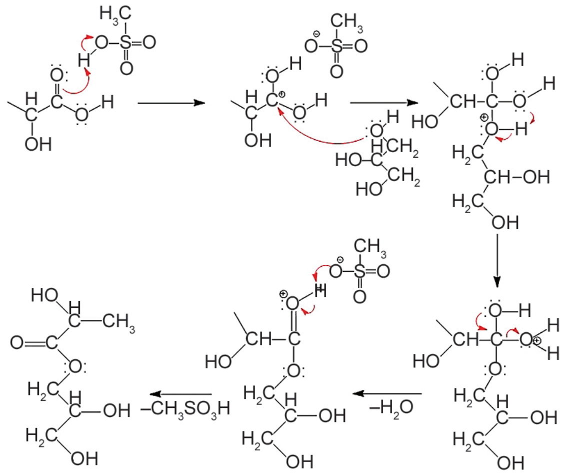

Figure 3. Schematic illustration of GlyLA synthesis mechanism.

The value of 0.27 was calculated as a degree of esterification [32].

A plausible mechanism of esterification of LA in the presence of methanesulfonic acid was proposed (Figure 3). Both LA and Gly, as the reagents of plasticizer synthesis, are from renewable resources. To intensify GlyLA production, the water produced during the dehydration process was collected simultaneously.

3.2. Fabrication and characterization of plasticized WPC-based films

3.2.1. FTIR-ATR

FTIR spectra of WPC-based films containing different amounts of plasticizer are shown in Figure 3. FTIR spectrum of WPC-based film containing Gly was compared with neat WPC and Gly and evaluated in three spectral regions (Figure 4a). Five peaks located between 800 to 1150 cm^{-1} in the spectrum of the prepared film could be attributed to the vibrations of C–C and C–O bonds of Gly. For instance, the bands that appeared at 1040 cm^{-1} could be related to the stretching vibration of the C–O bond in C1 and C3, and the vibration of C–O in C2 could be observed at 1110 cm^{-1} [15]. As shown in Figure 4b the intensity of the peaks in this spectral region increased gradually by increasing the Gly content from 85 to 100%, indicating the increase of free hydroxyl groups of Gly. In the spectral range of 1600 to 1700 cm^{-1}, the amide I absorption band of WPC, which is sensitive to the secondary structure of the protein, could be observed around 1645 cm^{-1}. This peak was mainly governed by stretching vibrations of C=O and C–N groups that appeared in WPC-based film as well [37]. In this study, by adding 85, 90, and 100% w/w of Gly to the film-forming solution, the peak of the neat protein shifted to the low wavenumbers of 1633, 1627, and 1631 cm^{-1}, respectively. This could be evidence of interactions between protein and Gly and the plasticizing effect of Gly as well (Figure 4b) [15]. The last considered spectral region of (3000 to 3600 cm^{-1}) corresponding to the free and bonded O–H and N–H groups of protein was characterized by a broad band at 3420 cm^{-1} for WPC. A significant chemical shift toward the wavenumbers of 3275, 3270, and 3269 cm^{-1} could be observed when the Gly content increased from 85 to 100% w/w, respectively. This band shift could be related to the presence of Gly as a plasticizer with a large amount of hydroxyl groups [38]. It should be noted that by increasing the Gly content in the samples, the intensity of these peaks increased as well, which could be explained by the interaction of protein and Gly through covalent bonds [15].

To better understand the valid reason for the particular functionality of LA- LA-plasticized WPC films, the FTIR spectra of LA-plasticized film with free LA and WPC were initially compared (Figure 4c). The characteristic carbonyl group of LA appeared at 1730 cm^{-1}, which shifted to the lower wavenumber (1721 cm^{-1}), indicating the interactions between the carboxylic group of LA and functional groups of protein [34]. Moreover, Figure 4d showed as the amount of LA increases from 80 to 100% w/w, the peak intensity of the stretching vibrations of C=O decreases, which could be evidence of the consumption of more LA. Although it was expected that the intensity of the peaks would increase with increasing LA content, a reverse trend actually occurred for all

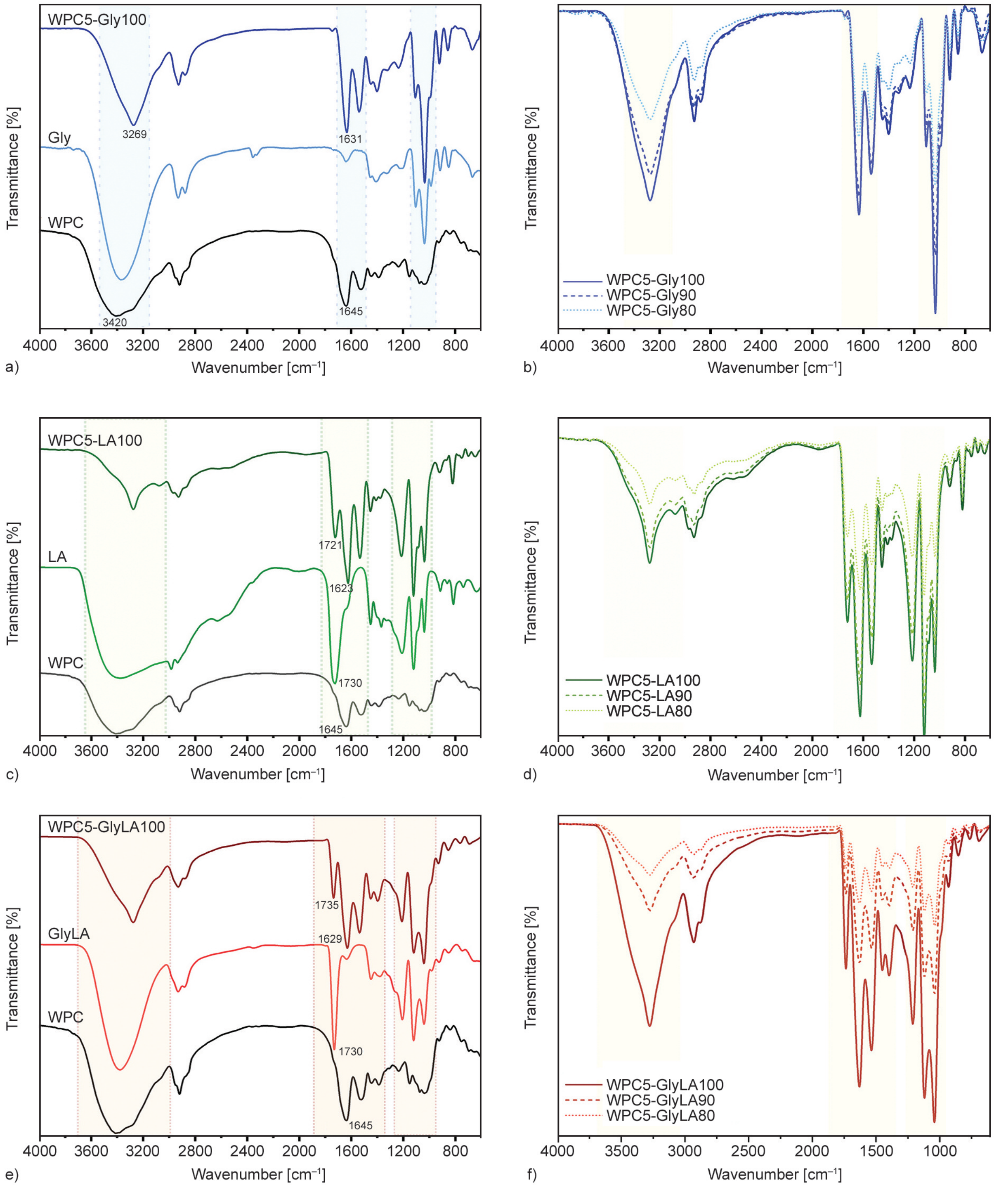

Figure 4. FTIR spectra of WPC-based films with different content of a), b) Gly, c), d) LA, and e), f) GlyLA as plasticizers.

spectral regions. In spectral regions of 1600 to 1700 cm^{-1}, a significant red shift for stretching vibration of WPC from 1645 to 1627, 1622, and 1623 cm^{-1} for WPC-based films containing 80, 90, and 100% w/w LA could be observed, respectively. This remarkable shift could be a sign of very strong hydrogen bond interactions between LA and protein. By increasing the amount of LA from 80 toward 100% w/w, LA functional groups bond to the carbonyl group associated with Amid I as long as with free-NH and –OH groups of protein through hydrogen bonding, so breaks the protein-protein interactions and prevent their reformation. The consumption of most of the free –OH and –NH groups of LA and protein resulted in the reducing intensity in spectral regions of 3000 to 3600 cm^{-1}. Moreover, the solubility parameter of films containing LA increases in this case. On the contrary, with the increase of LA to 100% w/w level and the saturation of the attachment point for electrostatic interactions, the excess amount of LA and film moisture extract from the film structure.

As shown in Figure 4e, the characteristic absorption bands of both WPC and GlyLA confirmed the successful formation of GlyLA plasticized films at different concentrations of plasticizer. As shown in Figure 4f, the intensity of the whole FTIR peaks observed for GlyLA films enhanced with increasing the amount of plasticizer, indicating a suitable penetration rate of the plasticizer through protein chains and the presence of more free –OH and –NH groups. Moreover, the shift of the peak corresponding to the carbonyl groups of Amide I of protein in film spectrum to lower wavenumber (1629 cm^{-1}) compared to neat WPC together with the redshift of carbonyl group peak of GlyLA to 1735 cm^{-1} indicates the effective interactions of protein chains and GlyLA (*e.g.*, hydrogen bonds, not as strong as LA plasticized films). The addition of the excess amount of GlyLA (100% w/w) caused a significant increase in the intensity of the mentioned peaks. The saturation of protein chains with GlyLA and lack of diffusion and the reaction of plasticizer with protein chains led to the presence of more free GlyLA in the structure. Although the rate of plasticizer penetration between protein chains would decrease in high molecular weight molecules [39], GlyLA showed a favorable penetration rate in WPC. It seems that the synthetic plasticizer, due to its great polarity and capability of forming hydrogen bonds, could be inserted into the protein chains and facilitate their mobility. The use of sodium sulfite salt in order to modify the surface of whey protein can prevent the formation of extensively cross-linked bonds during thermal denaturation of the protein and may reduce the formation of disulfide bonds between the protein chains [40]. Therefore, more space is available between chains, allowing GlyLA to penetrate more effectively, and a film with better softening properties is formed.

3.2.2. Thermal analysis

The properties of WPC films with different content of Gly, LA and GlyLA as plasticizers were investigated with DSC. Regardless of the plasticizer concentration, the DSC thermograms shown in Figure 5a–5c demonstrated only one peak for each glass transition of the amorphous part (T_g) and the softening point (T_{sp}) temperatures, which confirmed that all three plasticizers are compatible with whey protein. As depicted in Figure 5a and Table 2, the DSC thermogram of WPC films containing Gly showed a reduction in T_g values with increasing Gly content. This declining trend could be related to the softening effect of Gly molecules, which generally increase the free volume of the polymer network and the mobility of polymer chains. Moreover, higher Gly levels require lower enthalpy to break the interactions between protein chains [41]. It should be noted that with increasing Gly content from 90 to 100% w/w, the plasticizing efficiency did not change notably, resulting in adding more Gly to the system without giving more softening properties to the protein. In the case of protein concentrate films containing LA, increasing the plasticizer content from 80 to 90% w/w led to a considerable decrease in T_g from –6.09 to –13.19 °C, indicating a significant softening of whey protein (Figure 5b and Table 2). However, with the addition of more amount of LA, a significant increase in the T_g value of WPC film could be observed. It seems that in the high amounts of LA, WPC films displayed an anti-plasticization effect owing to the stronger interaction between LA and protein molecules that relatively prevents macromolecular mobility [42]. Moreover, the results showed that in the same content of plasticizers, LA-plasticized WPC films showed higher T_g values than Gly-plasticized protein films. According to a previous study conducted by Sobral *et al*. [43], the glass transition temperature of whey protein films increases as a result of chain stiffness improvement and increasing intermolecular and intramolecular forces. LA reduces the mobility of these chains by establishing stronger hydrogen and electrostatic interactions with protein chains. This result is in line with the results obtained from FTIR spectra and the following tensile test, indicating the manufacture of more flexible films.

Low molecular weight plasticizers have been reported to be more effective in plasticizing efficiency than high molecular weight types. These plasticizers easily penetrate into the protein chains and form hydrogen bonds between the plasticizer's hydroxyl groups and the protein chains [39]. Gly and LA have lower molecular weights than GlyLA, causing more cleavage of protein bonds and hydrophobic interactions between protein molecules. To reduce the number of disulfide bonds between protein chains and consequently increase the spaces between chains for better penetration of GlyLA, the whey protein surface was

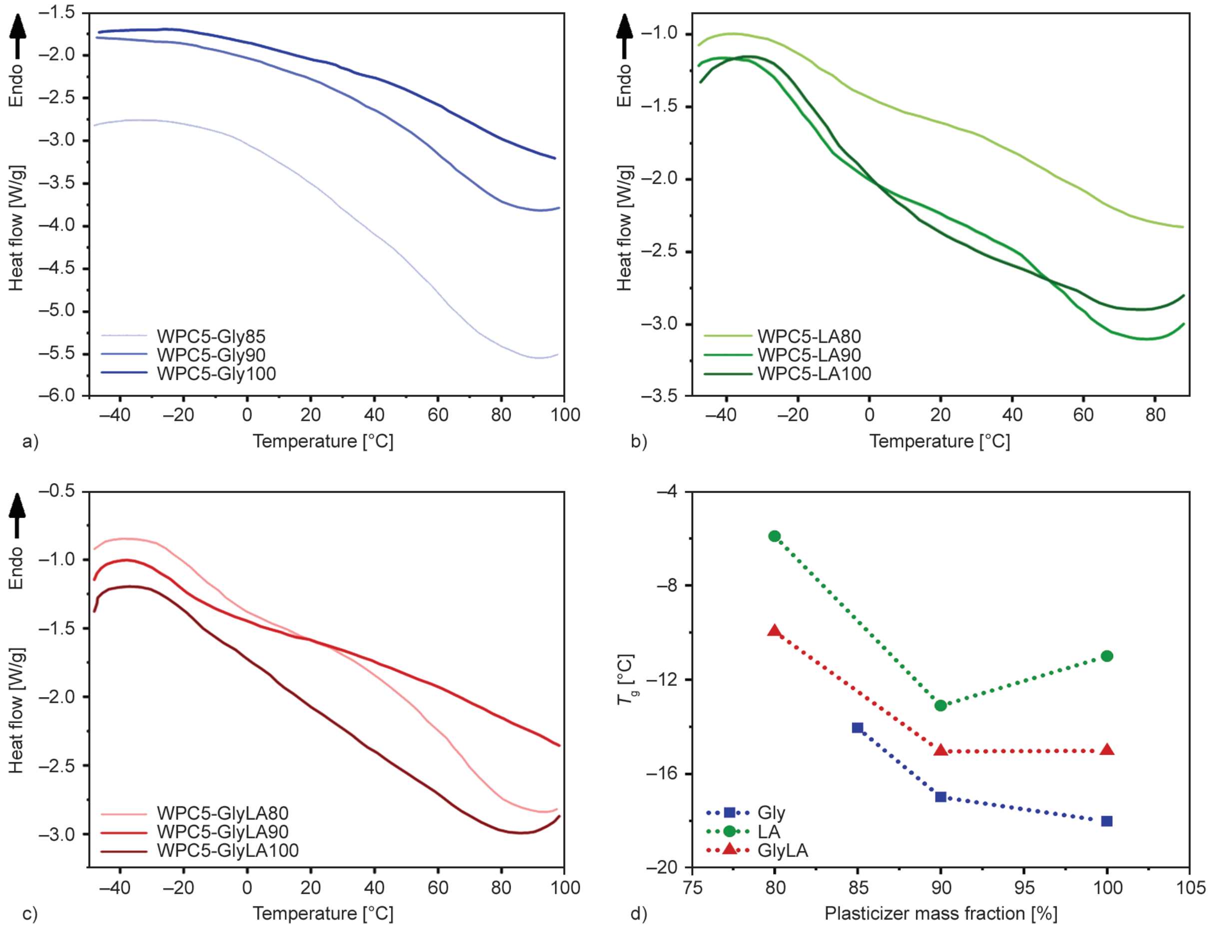

Figure 5. DSC Thermograms of WPC-based films with different content of a) Gly, b) LA, and c) GlyLA as plasticizers. d) T_g values of WPC films as a function of mass fraction of different plasticizers.

modified through sodium sulfite as a reducing salt to form the films with better-plasticized properties. As shown in Figure 5c and Table 2, a similar trend could be observed for GlyLA plasticized films, and the highest reduction in T_g was obtained at 90% w/w of all plasticizers. Compared to the prepared films containing LA, the films prepared with Gly and GlyLA showed more reduction of T_g, which resulted in better-softening efficiency as well (Figure 5d). Due to the amorphous nature of the polymer-plasticizer composite, there is no melting temperature. The peaks observed at higher temperatures represent transition points linked to the softening point temperature (T_{sp}), indicating that at this temperature, the composition becomes completely soft and flexible.

Table 2. T_g and T_{sp} values of WPC films with different content of plasticizers.

Film:* WPC a-plasticizer b	T_g [°C]	T_{sp} [°C]
WPC5-Gly85	–13.84	91.00
WPC5-Gly90	–17.51	90.50
WPC5-Gly100	–18.85	96.33
WPC5-LA80	–6.09	92.17
WPC5-LA90	–13.19	84.16
WPC5-LA100	–11.03	83.73
WPC5-GlyLA80	–10.68	91.50
WPC5-GlyLA90	–15.34	102.00
WPC5-GlyLA100	–15.19	85.33

*a: WPC weight [g] in 100 g of total weight
b: The weight percentage % w/w of Gly, LA, and GlyLA compared to the weight of WPC dry powder.

3.2.3. Light transmission

Figure 6 displayed the Light transmission of WPC-based films at different content of the three plasticizers. Negligible transmission could be observed below 280 nm for all WPC-based films. This excellent barrier property in the UV region could probably be related to the radiation-absorbing amino acids (*e.g.*, alanine, tryptophan, and tyrosine) found in protein-based structures [44]. In contrast, synthetic polymer films cannot generally block UV light [15], suggesting WPC-based films in food systems may retard UV-induced lipid oxidation. However, at

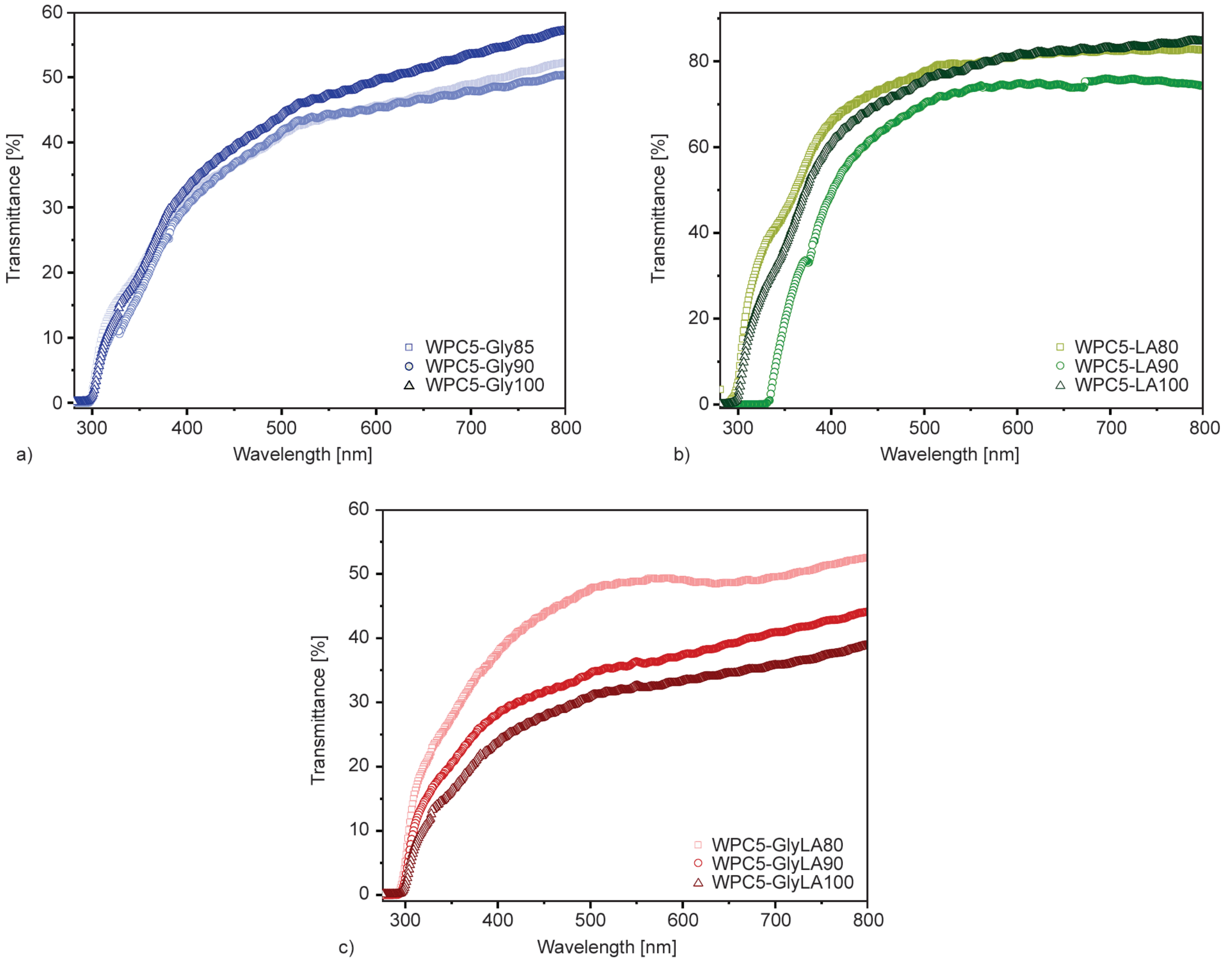

Figure 6. Light transmission of WPC-based films, with various content of a) Gly, b) LA, and c) GlyLA.

300 nm, transmission values ranged from 1.92–0.96, 5.13–2.01, and 5.14–1.54 for the films plasticized with different content of Gly, LA, and GlyLA, respectively. In the visible range (350–800 nm), the maximum light transmission was measured at 800 nm, 52.32–57.34 for Gly, 82.55–84.6 for LA, and 52.56–38.83 for GlyLA as plasticizer with different concentrations. The highest transmittance could be observed for LA plasticized films (≈80%), which could be attributed to the low absorption of $n \rightarrow \pi^*$ transition of the carboxylic group of LA [45]. It should be noted that the films containing GlyLA showed the lowest light transmission due to the presence of sodium sulfite salt. Therefore, increasing the amount of plasticizers in WPC-based films has a notable impact on the transmission of light in films that contain GlyLA. Figure 7 shows the visual appearance of WPC-based films plasticized with GlyLA and LA.

3.2.4. WVP measurements

WVP of the plasticized WPC-based films was evaluated at different contents of three types of plasticizers. As shown in Figure 8a, increasing the plasticizer content caused the higher WVP of all samples, which is probably due to more interruption of the covalent bonds between the protein chains. Moreover, it seems that the WVP of the LA-plasticized films was less affected by plasticizer concentration, which could be related to the evaporation of some amount of solvent and LA in high content [46]. The highest WVP was related to Gly-plasticized films and the lowest one was for LA-plasticized films. Gly, as a low molecular weight plasticizer (92 g/mol), rich in hydroxyl group, renders the films very hydrophilic and consequently showed high WVP. In contrast, in the presence of LA, the formation of stronger hydrogen bonds of plasticizer and protein chains led to less water-permeable sites and acted as a better water vapor barrier [47]. The results showed that GlyLA, with less hydrophilicity compared to Gly, due to the presence of the ester group of LA, reduced the WVP of WPC-based films. It should be noted that the addition of sodium sulfite to whey protein films (up to 10% w/w) did not significantly

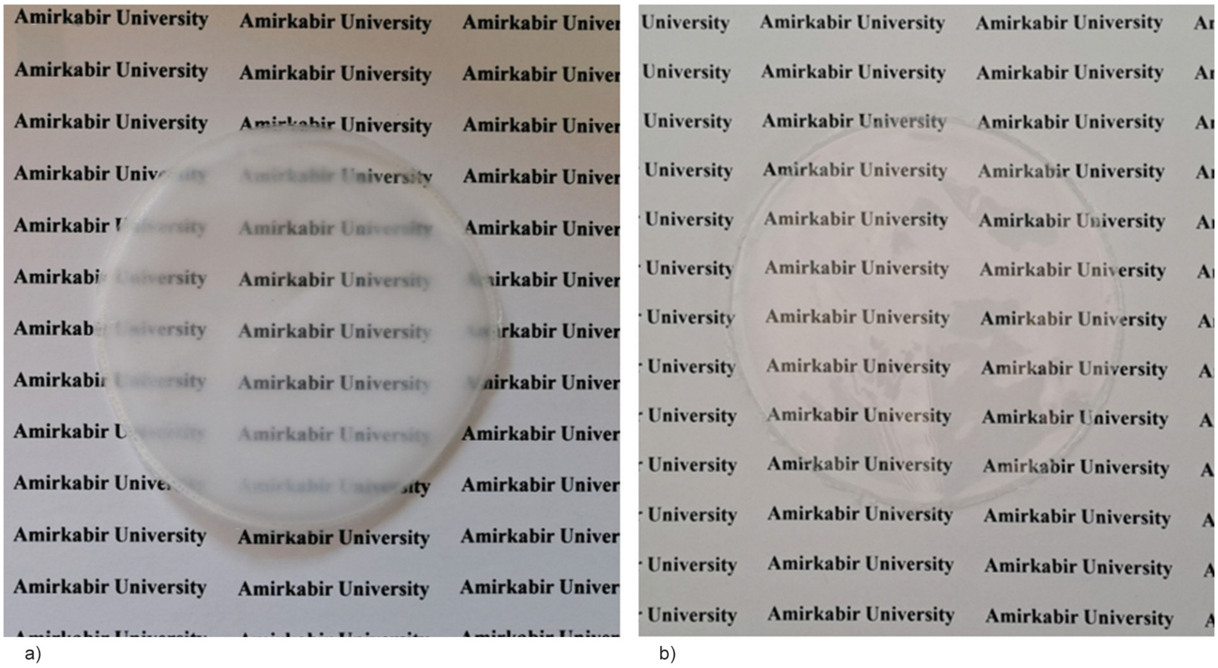
a) b)

Figure 7. Visual appearance of WPC-based films plasticized with a) GlyLA and b) LA.

affect the WVP of the films prepared at a relative humidity of 50% [40].

3.2.5. Solubility

As reported in the literature, the intermolecular disulfide bonds of WPC-based films, make them partially insoluble in water [48]. The solubility of WPC-based films with three types of plasticizers was evaluated. As shown in Figure 8b, an increase in plasticizer content leads to an increase in solubility. The hydrophilicity of plasticizers can affect the solubility properties of the prepared films. According to Cuq *et al*. [49] Gly as a hydrophilic plasticizer can increase the solubility by constitution of the more soluble dry matter from low molecular mass protein chains. The results showed low solubility of the LA-plasticized films compared to those plasticized with Gly. The formation of stronger interactions of LA and whey protein chains could be one of the possible reasons for this lower solubility. These interactions could be supported via the increase in the peak intensity corresponding to the hydroxyl group's vibrations by increasing the LA content in FTIR spectra of LA-plasticized WPC films [17].

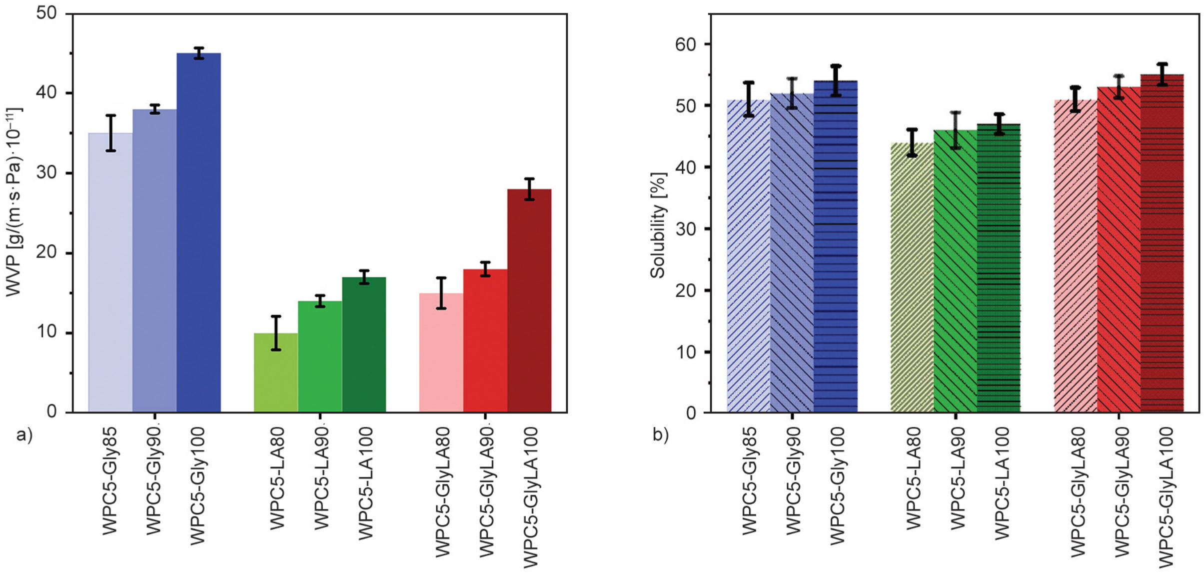

Figure 8. a) WVP and b) solubility of WPC films with different content of plasticizers.

In similar content of plasticizers, the moisture content in films containing high molecular weight plasticizers is lower. However, in the case of films plasticized with GlyLA, due to the preparation of the corresponding films at 40% humidity, we see more soluble films compared to the two other plasticized films.

3.2.6. Mechanical properties

As reported in previous studies, the plasticizing of WPC-based films induces large modifications to their mechanical properties [50]. Figure 9a–9c show the typical tensile curves of WPC films with different plasticizers. An increase in Gly content led to a decrease in tensile strength and an increase in the elongation at break (Figures 9d and 9e). Gly, as a hydrophilic low molecular weight plasticizer, could easily penetrate the protein chains and reduce the number of cross-linked intermolecular bonds of the polymerized protein molecules of the films through the formation of hydrogen bonds with reactive groups of proteins. When plasticizers and proteins are mixed, protein-plasticizer interactions are formed to the detriment of protein-protein interactions [48]. A similar trend could be observed in plasticized films with LA and GlyLA. However, with increasing the amount of these plasticizers from 90 to 100% w/w, tensile strength and elongation of film reduce considerably. This behavior, especially in the case of films including LA, is consistent with the FTIR and DSC results and likely could be attributed to saturation of the protein structure with other compounds. Compatibility between polymer and plasticizer is effective on mechanical properties, and with decreasing compatibility, the possibility of phase separation under stress and loss of tensile properties increases [15, 48, 51]. Increasing GlyLA plasticizer in WPC films, especially WPC5-GlyLA100, will cause phase separation due to the reduction or dominance of intermolecular and intramolecular interactions and lead to incompatibility and, therefore, will result in the loss of tensile properties.

Moreover, according to studies conducted by Schmid *et al*. [40], the mechanical properties of the produced films are affected by the formation of disulfide bonds during thermal denaturation of the protein and the addition of plasticizer to compete for the formation of intermolecular hydrogen bonds after denaturation completion. By the addition of sodium sulfite to protein, there was no change in tensile strength and elastic modulus, but a small decrease in elongation occurred. Therefore, it seems that the reduction in the

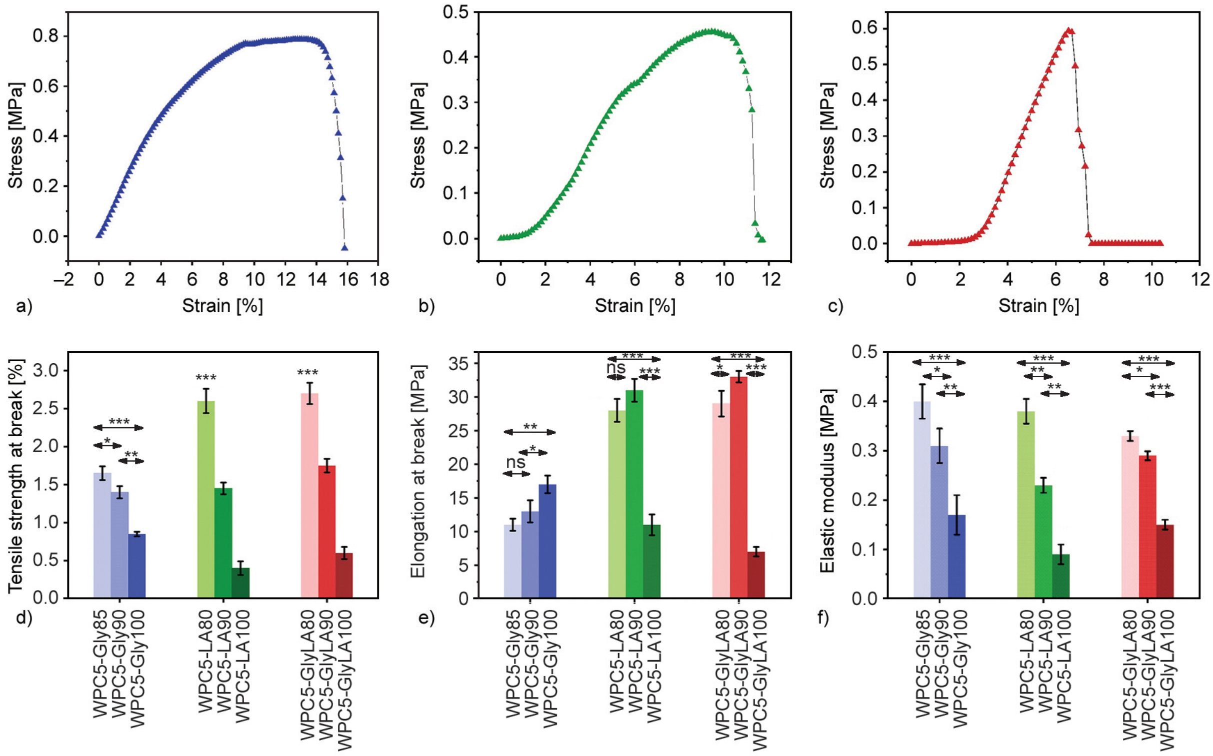

Figure 9. Typical tensile curves of a) WPC5-Gly100, b) WPC-LA100, c) WPC-GlyLA100, and d) Tensile strength, e) elongation at break, f) elastic modulus of WPC films with different content of plasticizers, Data are expressed as the mean ±SD ($n = 3$). ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

strain at the break of Gly LA films compared to LA plasticized films is due to the addition of sodium sulfite to the solution.

The film's elastic modulus, which is an inherent criterion for film stiffness, decreased with the increase of all three plasticizers (Figure 9f). This observation testifies to the decrease in the covalent bonds level of the protein structure as a result of the softening of the prepared protein films. Overall, the above-mentioned results showed that the presence of a lactate group on one side and the Gly group on the other can make GlyLA a suitable plasticizer candidate for improving the mechanical properties of the prepared whey films.

4. Conclusions

This work aimed at introducing two bio-based plasticizers, LA and GlyLA, for WPC, accompanied by investigating the physical, mechanical, and thermal properties of films produced therefrom compared to Gly-plasticized films as control groups. Water-soluble GlyLA with an esterification degree of 0.27, which is compatible with film-forming WPC, was initially synthesized, and its chemical structure was confirmed by ^{1}H-NMR and FTIR analyses. All three plasticizers showed FTIR shifts in carbonyl and hydroxyl spectral ranges, representing the interaction of protein and plasticizers. However, due to the more robust interactions, LA showed the highest FTIR shifts and, consequently, the lowest softening effect. As compared to Gly plasticized films, which showed the most reduction in T_g value, the WPC films containing both LA and GlyLA underwent an obvious drop in T_g values while an increase in T_g of LA films was observed by increasing the content of plasticizer up to 100% w/w. The highest light transmission was observed for LA plasticized films. Furthermore, the water vapor barrier and solubility of WPC films plasticized with LA were better than those of other groups due to the stronger interaction of protein and LA. However, GlyLA showed better barrier properties than control films. Moreover, LA and GlyLA plasticized WPC films exhibited better mechanical compared to Gly plasticized counterparts in similar plasticizer content. The amount of plasticizer used in whey protein structure plays a substantial role in obtaining desired films for application in food packaging since molecular structure, thermal, mechanical, and barrier properties are dependent on utilized plasticizer content in film forming solution. WPC films plasticized with 80 or 90% w/w of LA and GlyLA exhibited not only higher tensile strength and flexibility but also remarkably lower WVP than Gly-plasticized films, which is of great importance in protecting foods from external destructive forces and preventing water vapor uptake through the environment, respectively. So they could be considered good plasticizers for whey protein. However, further studies should be conducted to improve the properties of these bio-based films in competition with non-biodegradable polymer films.

Acknowledgements

The authors are grateful to the Chemistry Department of the Amirkabir University of Technology, Tehran, Iran, for their support and to Somayyeh Mohammadi, Ph.D. student, for her helpful advice in the laboratory to complete this research. The author received no financial support for the research, authorship, and/or publication of this article. Moreover, the authors declare no conflict of interest.

References

[1] Zink J., Wyrobnik T., Prinz T., Schmid M.: Physical, chemical and biochemical modifications of protein-based films and coatings: An extensive review. International Journal of Molecular Sciences, **17**, 1376 (2016). https://doi.org/10.3390/ijms17091376

[2] Kumar N., Pratibha , Prasad J., Yadav A., Upadhyay A., Neeraj, Shukla S., Petkoska A. T., Heena, Suri S.: Recent trends in edible packaging for food applications – Perspective for the future. Food Engineering Reviews, **15**, 718–747 (2023). https://doi.org/10.1007/s12393-023-09358-y

[3] Ali A., Bairagi S., Ganie S. A., Ahmed S.: Polysaccharides and proteins based bionanocomposites as smart packaging materials: From fabrication to food packaging applications a review. International Journal of Biological Macromolecules, **252**, 126534 (2023). https://doi.org/10.1016/j.ijbiomac.2023.126534

[4] Chen H., Wang J., Cheng Y., Wang C., Liu H., Bian H., Pan Y., Sun J., Han W.: Application of protein-based films and coatings for food packaging: A review. Polymers, **11**, 2039 (2019). https://doi.org/10.3390/polym11122039

[5] Schmid M., Müller K.: Whey protein-based packaging films and coatings. in 'Whey proteins' (eds.: Deeth H. C., Bansal N.) Elsevier, 407–437 (2019). https://doi.org/10.1016/B978-0-12-812124-5.00012-6

[6] Milani J. M., Tirgarian B.: An overview of edible protein-based packaging: Main sources, advantages, drawbacks, recent progressions and food applications. Journal of Packaging Technology and Research, **4**, 103–115 (2020). https://doi.org/10.1007/s41783-020-00086-w

[7] Kandasamy S., Yoo J., Yun J., Kang H-B., Seol K-H., Kim H-W., Ham J-S.: Application of whey protein-based edible films and coatings in food industries: An updated overview. Coatings, **11**, 1056 (2021). https://doi.org/10.3390/coatings11091056

[8] Minj S., Anand S.: Whey proteins and its derivatives: Bioactivity, functionality, and current applications. Dairy, **1**, 233–258 (2020). https://doi.org/10.3390/dairy1030016

[9] Rosseto M., Rigueto C. V. T., Alessandretti I., de Oliveira R., Wohlmuth D. A. R., Loss R. A., Dettmer A., dos Santos Richards N. S. P.: Whey-based polymeric films for food packaging applications: A review of recent trends. Journal of the Science of Food and Agriculture, **103**, 3217–3229 (2023). https://doi.org/10.1002/jsfa.12310

[10] McHugh T. H., Krochta J. M.: Sorbitol-vs glycerol-plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation. Journal of Agricultural and Food Chemistry, **42**, 841–845 (1994). https://doi.org/10.1021/jf00040a001

[11] Huntrakul K., Harnkarnsujarit N.: Effects of plasticizers on water sorption and aging stability of whey protein/carboxy methyl cellulose films. Journal of Food Engineering, **272**, 109809 (2020). https://doi.org/10.1016/j.jfoodeng.2019.109809

[12] Pérez L. M., Piccirilli G. N., Delorenzi N. J., Verdini R. A.: Effect of different combinations of glycerol and/or trehalose on physical and structural properties of whey protein concentrate-based edible films. Food Hydrocolloids, **56**, 352–359 (2016). https://doi.org/10.1016/j.foodhyd.2015.12.037

[13] Lin S-Y., Krochta J.: Plasticizer effect on grease barrier and color properties of whey-protein coatings on paperboard. Journal of Food Science, **68**, 229–233 (2003). https://doi.org/10.1111/j.1365-2621.2003.tb14144.x

[14] Abdalrazeq M., Giosafatto C. V. L., Esposito M., Fenderico M., di Pierro P., Porta R.: Glycerol-plasticized films obtained from whey proteins denatured at alkaline pH. Coatings, **9**, 322 (2019). https://doi.org/10.3390/coatings9050322

[15] Ramos Ó. L., Reinas I., Silva S. I., Fernandes J. C., Cerqueira M. A., Pereira R. N., Vicente A. A., Poças M. F., Pintado M. E., Malcata F. X.: Effect of whey protein purity and glycerol content upon physical properties of edible films manufactured therefrom. Food Hydrocolloids, **30**, 110–122 (2013). https://doi.org/10.1016/j.foodhyd.2012.05.001

[16] Kokoszka S., Debeaufort F., Lenart A., Voilley A.: Water vapour permeability, thermal and wetting properties of whey protein isolate based edible films. International Dairy Journal, **20**, 53–60 (2010). https://doi.org/10.1016/j.idairyj.2009.07.008

[17] Perez-Gago M., Krochta J.: Denaturation time and temperature effects on solubility, tensile properties, and oxygen permeability of whey protein edible films. Journal of Food Science, **66**, 705–710 (2001). https://doi.org/10.1111/j.1365-2621.2001.tb04625.x

[18] Osés J., Fernández-Pan I., Mendoza M., Maté J. I.: Stability of the mechanical properties of edible films based on whey protein isolate during storage at different relative humidity. Food Hydrocolloids, **23**, 125–131 (2009). https://doi.org/10.1016/j.foodhyd.2007.12.003

[19] Talens P., Krochta J. M.: Plasticizing effects of beeswax and carnauba wax on tensile and water vapor permeability properties of whey protein films. Journal of Food Science, **70**, E239–E243 (2005). https://doi.org/10.1111/j.1365-2621.2005.tb07141.x

[20] Fernandez L., de Apodaca E. D., Cebrián M., Villarán M. C., Maté J. I.: Effect of the unsaturation degree and concentration of fatty acids on the properties of WPI-based edible films. European Food Research and Technology, **224**, 415–420 (2007). https://doi.org/10.1007/s00217-006-0305-1

[21] Bahram S., Rezaei M., Soltani M., Kamali A., Ojagh S. M., Abdollahi M.: Whey protein concentrate edible film activated with cinnamon essential oil. Journal of Food Processing and Preservation, **38**, 1251–1258 (2014). https://doi.org/10.1111/jfpp.12086

[22] Galus S., Kadzińska J.: Whey protein edible films modified with almond and walnut oils. Food Hydrocolloids, **52**, 78–86 (2016). https://doi.org/10.1016/j.foodhyd.2015.06.013

[23] Dharini V., Selvam S. P., Jayaramudu J., Emmanuel R. S.: Functional properties of clay nanofillers used in the biopolymer-based composite films for active food packaging applications – Review. Applied Clay Science, **226**, 106555 (2022). https://doi.org/10.1016/j.clay.2022.106555

[24] Hassannia-Kolaee M., Khodaiyan F., Pourahmad R., Shahabi-Ghahfarrokhi I.: Development of ecofriendly bionanocomposite: Whey protein isolate/pullulan films with nano-SiO_2. International Journal of Biological Macromolecules, **86**, 139–144 (2016). https://doi.org/10.1016/j.ijbiomac.2016.01.032

[25] Hassannia-Kolaee M., Shahabi-Ghahfarrokhi I., Hassannia-Kolaee M.: Development and characterization of a novel ecofriendly biodegradable whey protein concentrate film with nano-SiO_2. International Journal of Food Engineering, **14**, 20170098 (2018). https://doi.org/10.1515/ijfe-2017-0098

[26] Alizadeh-Sani M., Khezerlou A., Ehsani A.: Fabrication and characterization of the bionanocomposite film based on whey protein biopolymer loaded with TiO_2 nanoparticles, cellulose nanofibers and rosemary essential oil. Industrial Crops and Products, **124**, 300–315 (2018). https://doi.org/10.1016/j.indcrop.2018.08.001

[27] Karimi N., Alizadeh A., Almasi H., Hanifian S.: Preparation and characterization of whey protein isolate/polydextrose-based nanocomposite film incorporated with cellulose nanofiber and *L. plantarum:* A new probiotic active packaging system. LWT, **121**, 108978 (2020).
https://doi.org/10.1016/j.lwt.2019.108978

[28] Ghosh T., Priyadarshi R., de Souza C. K., Angioletti B. L., Rhim J-W.: Advances in pullulan utilization for sustainable applications in food packaging and preservation: A mini-review. Trends in Food Science and Technology, **125**, 43–53 (2022).
https://doi.org/10.1016/j.tifs.2022.05.001

[29] Khanzadi M., Jafari S. M., Mirzaei H., Chegini F. K., Maghsoudlou Y., Dehnad D.: Physical and mechanical properties in biodegradable films of whey protein concentrate–pullulan by application of beeswax. Carbohydrate Polymers, **118**, 24–29 (2015).
https://doi.org/10.1016/j.carbpol.2014.11.015

[30] Lara B. R. B., Araújo A. C. M. A., Dias M. V., Junior M. G., Santos T. A., Ferreira L. F., Borges S. V.: Morphological, mechanical and physical properties of new whey protein isolate/ polyvinyl alcohol blends for food flexible packaging. Food Packaging and Shelf Life, **19**, 16–23 (2019).
https://doi.org/10.1016/j.fpsl.2018.11.010

[31] Zhang X., Zhao Y., Li Y., Zhu L., Fang Z., Shi Q.: Physicochemical, mechanical and structural properties of composite edible films based on whey protein isolate/psyllium seed gum. International Journal of Biological Macromolecules, **153**, 892–901 (2020).
https://doi.org/10.1016/j.ijbiomac.2020.03.018

[32] Yuan Y., Hu Z., Fu X., Jiang L., Xiao Y., Hu K., Yan P., Lei J.: Poly(lactic acid) plasticized by biodegradable glyceryl lactate. Journal of Applied Polymer Science, **133**, 43460 (2016).
https://doi.org/10.1002/app.43460

[33] Gontard N., Guilbert S., Cuq J-L.: Edible wheat gluten films: Influence of the main process variables on film properties using response surface methodology. Journal of Food Science, **57**, 190–195 (1992).
https://doi.org/10.1111/j.1365-2621.1992.tb05453.x

[34] Choubisa B., Dholakiya B., Patel M.: Synthesis and characterization of polylactic acid (PLA) by using SSA, CSA and TPA type solid acid catalyst system in polycondensation method. Journal of Macromolecular Science Part A, **50**, 828–835 (2013).
https://doi.org/10.1080/10601325.2013.802157

[35] Yee T. W., Sin L. T., Rahman W. A. W. A., Samad A. A.: Properties and interactions of poly(vinyl alcohol)-sago pith waste biocomposites. Journal of Composite Materials, **45**, 2199–2209 (2011).
https://doi.org/10.1177/0021998311401073

[36] Li H-Z., Chen S-C., Wang Y-Z.: Thermoplastic PVA/PLA blends with improved processability and hydrophobicity. Industrial and Engineering Chemistry Research, **53**, 17355–17361 (2014).
https://doi.org/10.1021/ie502531w

[37] Orsuwan A., Sothornvit R.: Effect of banana and plasticizer types on mechanical, water barrier, and heat sealability of plasticized banana-based films. Journal of Food Processing and Preservation, **42**, e13380 (2018).
https://doi.org/10.1111/jfpp.13380

[38] Tarique J., Sapuan S., Khalina A.: Effect of glycerol plasticizer loading on the physical, mechanical, thermal, and barrier properties of arrowroot (*Maranta arundinacea*) starch biopolymers. Scientific Reports, **11**, 13900 (2021).
https://doi.org/10.1038/s41598-021-93094-y

[39] Stark T. D., Choi H., Diebel P.: The influence of molecular weight on plasticizer retention. Geotechnical Fabrics Report, **23**, 28–33 (2005).

[40] Schmid M., Prinz T. K., Stäbler A., Sängerlaub S.: Effect of sodium sulfite, sodium dodecyl sulfate, and urea on the molecular interactions and properties of whey protein isolate-based films. Frontiers in chemistry, **4**, 49 (2017).
https://doi.org/10.3389/fchem.2016.00049

[41] Ghanbarzadeh B., Oromiehi A.: Studies on glass transition temperature of mono and bilayer protein films plasticized by glycerol and olive oil. Journal of Applied Polymer Science, **109**, 2848–2854 (2008).
https://doi.org/10.1002/app.28289

[42] Stukalin E. B., Douglas J. F., Freed K. F.: Plasticization and antiplasticization of polymer melts diluted by low molar mass species. The Journal of chemical physics, **132**, 084504 (2010).
https://doi.org/10.1063/1.3304738

[43] Sobral P. J. A., Menegalli F. C., Hubinger M. D., Roques M. A.: Mechanical, water vapor barrier and thermal properties of gelatin based edible films. Food hydrocolloids, **15**, 423–432 (2001).
https://doi.org/10.1016/S0268-005X(01)00061-3

[44] Ribeiro-Santos R., de Melo N. R., Andrade M., Azevedo G., Machado A. V., Carvalho-Costa D., Sanches-Silva A.: Whey protein active films incorporated with a blend of essential oils: Characterization and effectiveness. Packaging Technology and Science, **31**, 27–40 (2018).
https://doi.org/10.1002/pts.2352

[45] Skoog D. A., Holler F. J., Nieman T. A.: Principles of instrumental analysis. Saunders, Philadelphia (1998).

[46] Zhou J. J., Wang S. Y., Gunasekaran S.: Preparation and characterization of whey protein film incorporated with TiO_2 nanoparticles. Journal of Food Science, **74**, N50–N56 (2009).
https://doi.org/10.1111/j.1750-3841.2009.01270.x

[47] Calva-Estrada S. J., Jiménez-Fernández M., Lugo-Cervantes E.: Protein-based films: Advances in the development of biomaterials applicable to food packaging. Food Engineering Reviews, **11**, 78–92 (2019).
https://doi.org/10.1007/s12393-019-09189-w

[48] Galietta G., di Gioia L., Guilbert S., Cuq B.: Mechanical and thermomechanical properties of films based on whey proteins as affected by plasticizer and crosslinking agents. Journal of Dairy Science, **81**, 3123–3130 (1998).
https://doi.org/10.3168/jds.S0022-0302(98)75877-1

[49] Cuq B., Gontard N., Cuq J-L., Guilbert S.: Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. Journal of Agricultural and Food Chemistry, **45**, 622–626 (1997).
https://doi.org/10.1021/jf960352i

[50] Shaw N., Monahan F., O'riordan E., O'Sullivan M.: Physical properties of WPI films plasticized with glycerol, xylitol, or sorbitol. Journal of Food Science, **67**, 164–167 (2002).
https://doi.org/10.1111/j.1365-2621.2002.tb11377.x

[51] Sajimon A., Edakkadan A. S., Subhash A. J., Ramya M.: Incorporating oregano (*Origanum vulgare L.*) Essential oil onto whey protein concentrate based edible film towards sustainable active packaging. Journal of Food Science and Technology, **60**, 2408–2422 (2023).
https://doi.org/10.1007/s13197-023-05763-7