CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

PHYSICAL, MECHANICAL, SURFACE, AND STRUCTURAL PROPERTIES OF SODIUM CASEINATE AND SOY PROTEIN ISOLATE SINGLE AND COMPOSITE PROTEIN COMPOSITE EDIBLE FILMS

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https://doi.org/10.34302/crpjfst/2025.17.2.3

Article history:
Received:
April 10th , 2025
Accepted:
July 28th, 2025

Keywords
Edible films
Composite
WVP
AFM
DSC
FTIR

ABSTRACT

Edible coatings are an alternative to decrease the use of one-use synthetic materials, since they can extend food stability by controlling moisture exchange. In this research the physical, mechanical, surface and structural properties of soy protein isolate or sodium caseinate edible films, and their composite combinations (mixture and bilayer), were investigated. Composite films were thicker, but with less soluble matter, and higher WVP values. Bilayer and mixture samples were harder and less stretchable. Soy protein isolate and mixture samples presented higher roughness, and bilayer samples presented a flat surface. The structural analysis by FTIR and DSC presented an inherent interaction between the employed proteins, resulting in a heterogenic and thermally compatible structure that changes physical that modifies the single proteins edible film properties.

1. Introduction

Edible coatings have been proposed as an alternative to reduce the use of one-use plastic materials for food packaging. Coating or edible films can be used to extend the stability of food by reducing the exchange of moisture and gases between the food and the surrounding environment (Mohamed et al., 2020). The idea to use edible films and coating is centered on the reduction of the exposure to environmental conditions of the product to preserve. Among other things, it seeks to establish barriers to moisture and oxygen, although it is difficult to conceive that the edible films and coatings are going to replace non-edible packaging, but it may be the case that they complement each other (Barbosa-Canóvas, 2012).

The term edible implies that structured films must be manufactured with natural biodegradable materials, such as polysaccharides and/or proteins. Food grade proteins represent a natural biopolymer that can be employed to elaborate edible films. Protein-based edible films, under proper conditions of pH, ionic strength and/or temperature, offer a high potential for forming numerous linkages at intermolecular level, creating different structures. In addition, these edible films can supplement the nutritional value of the foods, besides to be completely biodegradable and environmentally compatible (Bourtoom, 2009).

Proteins need a denaturant agent, such as heat, pH shifter, and/or a solvent in order to form the more extended structures that are required for film formation, and these extended structures allow chain-to-chain interaction to produce a cohesive film (Krotcha, 1997).

Composite or multicomponent edible films have the advantage of each component properties, enhancing permeability and mechanical properties. Bilayer films involve four stages: two casting and two drying, where first is required to create a thin layer and over this the secondary layer (Hassan et al., 2018). Mixing proteins is a technological approach to create protein-based materials with a more complete set of specific properties, such as mechanical properties, improving processability and material uniformity as well (Hu et al., 2012).

Edible films have been made from soy protein isolate (Cho et al., 2004; Kokoszka et al., 2010a; Song et al., 2011; Wang et al., 2024) or sodium caseinate (Khwaldia et al., 2004; Schou et al., 20005) or mixtures of both proteins (Monedero et al., 2010; Koshy et al., 2015; Liu et al., 2021). No bilayer edible films made from these two proteins have been reported. The distinct edible film properties of separated layers could not present the synergistically improved bilayer properties without the layer-by-layer interaction (Easdani et al., 2024).

Since soy protein isolate and sodium caseinate are one of the most employed ingredients in edible film formulation, the objective of this research was to determine the physical, mechanical, surface, and structural properties of edible films from each one of the proteins, besides composite formulation (mixture and bilayer). Physical properties were thickness, total soluble matter, transparency, and water vapor permeability. Mechanical properties determined were puncture and tensile tests. Surface properties were studied by phase contrast microscopy and atomic force microscopy. Finally, structural properties were determined by differential scanning calorimetry and Fourier transform infrared spectroscopy.

2. Materials and methods

2.1. Materials

Sodium caseinate was obtained from FABPSA (Mexico City, México). Soy protein isolate (90% protein) was obtained from Food Technologies Trading (Atizapan, México). Glycerol as glycerin USP was obtained from Sani Productos (Mexico City, México).

2.2. Methods

2.2.1. Edible films elaboration

The pouring in plate technique was employed to elaborate edible films. For sodium caseinate edible films, 6% (w/v) of this protein was dissolved in 100 mL of distilled water with 6 % (v/v) of glycerol as plasticizer. The mixture was heated to 80 ± 2 °C, cooled at room temperature and poured into glass plates (20 cm per side) during at least 24 h. For soy protein isolate edible films, the same methodology was employed, this is, 6% of protein with 6% of glycerol, heated, cooled, poured and dried. A mixture of sodium caseinate and soy protein isolate (3% of each one, w/v) was dissolved in addition to 6% of glycerol, heated to 80 ± 2 °C, cooled at room temperature and poured into glass plates to follow the same procedure. Finally, a bilayer edible film was elaborated at the same experimental conditions, the soy protein isolate suspension (6%, w/v) was first pouring and let dry during at least 24 h, and then sodium caseinate suspension (6%, w/v) was poured to dry at least for 24 h.

2.2.2. Physical properties

Edible films thickness was measured with a Walfront® manual micrometer (0-25 mm), taking from four to six random measurements of each sample.

Edible film solubility, as total soluble matter, was determined according to the technique reported by Jangchud and Chinnan (1999). Samples (20 mm × 20 mm) were weighed and immersed in 20 mL of distilled water during 24 h at room temperature, and the remaining material was quantitatively filtered (Whatman #1), dried and weighed again, calculating the total soluble matter as the

percent of the initial weight and final weigh ratio.

Edible films transparency was determined according to Yoo and Krotcha 2011) methodology. A piece of edible film was attached to an acrylic cell without front and back walls to measure the percent of transmittance at 560 nm, multiplying by films' thickness (in cm).

Water vapor permeability was determined according to the ASTM E96 cup method, as reported by Kokoszka et al. (2010b). In a glass jar distilled water was let one cm from the surface, covered with edible films of each treatment. Jars were weighed every 15 min for 6 h, and the water vapor transfer rate (WVTR) was calculated using Eq. 1:

$$WVTR = \frac{\Delta w}{\Delta t} A^{-1} \quad (1)$$

Where Δw/Δt is the slope of the weight change during time (g/s), and A is the jar mouth area (m^2). The water vapor permeability was then calculated as rate of the WVTR between the water vapor pressure difference between both sides of the film, Δp= 3169 Kpa at 25 °C, in agree with Wexler (1976), multiplied by the film thickness (*e*, in m), with Eq. 2:

$$WVP = \frac{WVTR}{\Delta p} e \quad (2)$$

2.2.3. Mechanical properties

Punction test properties were determined according to the methodology proposed by Sobral et al. (2001). An edible film sample was fixed in an acrylic cell with an aperture diameter of 52.4 mm and punctured with a 3 mm aluminum probe at a speed of 1 mm/s in a Brookfield LFRA 4500 texturometer (Brookfield Engineering Laboratories, Middleboro). From the force-distance curves, puncture strength was reported as the maximum force before sample breakdown, and puncture deformation at the breaking point was calculated as (Eq. 3):

$$\text{Puncture deformation (\%)} = \frac{\sqrt{D^2 + l_0^2}}{l_0} \times 100 \quad (3)$$

Where D is the probe distance route at breakdown, and l_0 is the initial length of the film (half of the cell diameter).

Tension properties were determined according to ASTM method D882-91, as reported by Gennadios et al. (1993). Films samples (25×100 mm) were analyzed in a Brookfield CT-3 texturometer (Brookfield Engineering Laboratories, Middleboro) with an initial grip separation of 40 mm and a crosshead speed of 1 mm/s. From the force-tension curves, tensile strength was calculated as the peak load at breaking point divided by the film cross-sectional area, and the percentage of elongation was reported as the elongation change at break point and the original length between the grips.

(1)

2.2.4. Surface properties

Edible film samples were randomly cut into 1.5 cm squares and placed between the microscope slide and the cover slip for surface optical observation with a contrast phase microscope Velab VE-B10 (Velab, Texas) at 20X magnification. The microscopy images were captured with a 64 MP cell phone digital camera fixed to microscope. Brightness and contrast of the images were optimized in Microsoft Power Point software (Bastos et al., 2016).

(2)

Atomic force microscopy was employed to analyze the edible film surface of the different samples, in a Nanosurf NaioAFM microscope (Nanosurf, Liestal). The roughness average (Ra), the roughness mean square (Rq), the maximum profile peak height (Rp), and the maximum profile valley depth (Rv) were calculated with the Naio Control Software. The results are the average of three perpendicular measures.

2.2.5. Structural properties

Thermal properties of the different edible films were determined by differential scanning calorimetry in a Mettler DSC1 equipment (Mettler Toledo, Columbus), calibrated with indium. Edible film sample (5-6 mg) was

placed in aluminum pans, sealed, and heated from 25 to 200 °C at 10 °C/min. Significant peaks were selected, smoothed for peak continuity and integrated function to obtain peak temperature and enthalpy (J/g).

Fourier Transform Infrared spectroscopy was employed to analyze the different edible film samples in a Perkin Elmer FTIR model Frontier (Perkin Elmer, Massachusetts) equipped with a deuterated triglycine sulfate detector. Spectra were scanned over the range of 4000 to 550 cm^{-1}, at a resolution of 4 cm^{-1}, accumulating 64 scans in absorbance units. FTIR spectra were collected using a background spectrum of air and the spectrum of every sample was recorded in triplicate using the Spectrum software version 3.01.00 (PerkinElmer, Massachusetts, USA).

2.2.6. Experimental design and data analysis

Data analysis was performed by an analysis of variance using the R studio® platform (https://www.rstudio.com/), and the significant difference ($P<0.05$) between means was determined by Tukey's honestly significant difference in the same platform.

3. Results and discussions

3.1. Physical properties

For the physical properties, single protein edible films were significantly ($P<0.05$) thinner than mixture or bilayer samples. The total soluble matter was significantly ($P<0.05$) higher for soy protein isolate films, followed by sodium caseinate samples; and the lower total soluble matter values were detected in mixture and bilayer samples. The sodium caseinate samples presented significant ($P<0.05$) higher values for transparency, and the lower transparency values were observed in soy protein isolate films. The water vapor permeability of edible films was significant ($P<0.05$) higher in mixture samples, followed by the bilayer samples. Lower values were observed in the soy protein isolate films (Table 1).

Table 1. Physical properties of the different edible films

Edible film	Thickness (mm)	Total soluble matter (%)	Transparency (%T cm)	Water vapor permeability ($\times 10^{-10}$ g/s m² Pa)
Sodium caseinate	0.125±0.069 c	17.10±2.79 b	1.029±0.11 a	1.14 ± 0.47 c
Soy protein isolate	0.124±0.009 c	22.66±3.36 a	0.008±0.09 d	0.74 ± 0.20 d
Mixture	0.217±0.016 b	8.79± 0.89 c	0.027±0.70 c	3.20 ± 1.03 a
Bilayer	0.318±0.044 a	6.19±1.60 d	0.032±1.46 b	2.55 ± 0.62 b

a, b, c, d… Means with same letter are not significant ($P<0.05$) different.

Composite edible films were thicker than the single protein edible films, although in the mixture formulation the same amount of total protein was employed. Understandably, the bilayer samples were thicker than the rest of the treatments. Since the same concentration of protein in weight was employed in the film-forming solution, differences in thickness were related to the protein network formed during the drying process. The differences in thickness suggest that proteins formed different film matrix patterns (Tsai & Weng, 2019). The protein-based edible films depends on the solvent (water) evaporation after the proteins thermal denaturation during dispersion to form a more extended structure required for film formation, since polypeptide chains interactions as a result of the thermal denaturation produced a consistent film where the nature and sequence of amino acid residues that interact will

promote different degree of interactions with different permeability and structural properties (Wittaya, 2012). It is important to consider that, according to Kokoszka et al. (2010a), final thickness is not a linear function of the dry matter content of the film-forming solution.

The lower total soluble matter values indicate a highly stable protein network due to interaction resulted from the thermal treatment during protein dispersion (Galus & Kadzińska, 2016), indicating that composite edible films have a stronger intra-molecular interaction in the aqueous condition as compared to single protein edible films (Saremnezhad et al., 2011). Films formed with cross-linked proteins are stabilized via electrostatic interactions, hydrogen bonding, van der Waals forces, covalent bonding, and disulfide bridges, are stable but biodegradable and compostable (Dangaran et al., 2009). The capacity of edible films to remain partially insoluble in water is desirable to ease the handling and manipulation of edible films, and less soluble films were the thicker ones.

In the films' transparency, sodium caseinate seems to form a more organized network, reflected in a thinner and more translucid film. Soy proteins resulted in a less structured and opaque film network, and since the composite samples were more transparent, this indicates a certain degree of interaction, since transparency is an indicator of miscibility or compatibility of polymer blend (Tsai & Weng, 2019; Su et al., 2012). The addition of sodium caseinate reduced the opacity of the soy protein isolate film, showing that the two proteins had good compatibility and could form a uniform film (Liu et al., 2021; Song et al., 2011). Edible films' opacity represents protection in the packaging of light sensitive foods (Zhang et al, 2022).

Although it has been reported that the WVP of soy protein isolate and sodium caseinate mixture edible films was lower than soy protein isolate edible films (Monedero et al., 2010; Liu et al., 2021), at the experimental conditions employed in this research, composite films presented higher WVP than single proteins samples. Koshy et al. (2015) reported that caseinate in soy protein-based films presented higher water vapor properties. Proteins interactions during dispersion, cooling and casting during film forming process resulted in different hydrophilic matrixes, with different resistance to water transport profiles, since the WVP was consistently related to film thickness, as previously reported (Kokoszka et al., 2010a; Song et al., 2011). In this view, in the composite formulations the combination of both proteins resulted in a stable interaction during and after the filmogenic process, since no phase separation was observed, improving the WVP in less soluble protein matrix.

3.2. Mechanical properties

In the mechanical properties, the sodium caseinate edible films presented significant ($P<0.05$) higher punction strength values, followed by the soy protein isolate samples. For puncture deformation, sodium caseinate presented significant ($P<0.05$) higher values as well, and the lower deformation values were observed in bilayer samples. In the tensile strength, the same behavior was observed, where the sodium caseinate edible films presented the significant ($P<0.05$) higher values, being both mixture and bilayer samples the less resistant. For the elongation of the edible film, the higher significant ($P<0.05$) percentage was detected in the sodium caseinate samples, with the lower values in mixture and bilayer samples (Table 2). The different film-forming capabilities of protein solutions, and their mixtures, may be explained by their different film-forming mechanisms, reflected in their inherent mechanical properties. In one hand, caseinate film formation is extensive hydrogen bonding, electrostatic interaction, and hydrophobic bonding (Chen, 1995). On the other hand, soy proteins upon drying involved unfolded proteins link through intermolecular interactions, such as disulfide bonds and hydrophobic interactions (Cho & Rhee, 2004). Although both proteins present differences in their respective filmogenic properties, theoretically, the extended side-chain structures would allow more protein interaction, reflected

in lower permeability and higher strength (Bourtoon, 2009; Mei & Zhao, 2003), as in the case of composite samples, i.e., mixture or bilayer. It has been reported that caseinate incorporation into soy protein isolate based films modify the mechanical properties of the blend films (Song et al., 2011; Monedero et al., 2010; Koshy et al., 2015). Bilayer edible films enhanced tensile strength by reducing elongation at break (Dhumal & Sarkar, 2018). Since no interlaminar separation was observed in bilayer films during the tensile testing process, this indicates adhesion between the layers with a strong structural stability and solid linkage between layers, with good practical application potential (Chen et al., 2024; Zhang et al., 2022; Easdani et al., 2024). In this view, the composite formulation resulted in a less ductile structure, thicker, more water vapor permeable, and less soluble edible films.

Table 2. Mechanical properties of the different edible films

Edible film	Punction strength (N)	Punction deformation (%)	Tensile strength (N/mm^2)	Elongation (%)
Sodium caseinate	10.32±0.06 a	68±3 a	19.93±0.91 a	438±28 a
Soy protein isolate	9.65±0.01 b	68±2 a	17.60±0.80 b	412±28 b
Mixture	8.90±0.15 c	56±8 b	14.61±0.61 c	341±21 c
Bilayer	8.03±0.04 d	48±3 c	13.92±1.32 d	315±45 d

a, b, c, d… Means with same letter are not significant ($P<0.05$) different.

3.3. Surface properties

Fig. 1 presents the surface structural morphology of different edible films. For sodium caseinate edible films, in the optical micrograph at 20X (Fig. 1-left) an even and continuous surface was observed, and in the atomic force micrographs (Fig. 1-right) lower roughness and peak profile values were detected. In the soy protein isolate samples, optical microscopy (Fig. 1-left) exhibits a non-uniform surface, and in atomic force micrographs (Fig. 1-right) relatively higher values for roughness (Ra and Rq) and higher peak profile and valley depth were detected in a scattered surface. For the mixture of soy protein isolate and sodium caseinate, a more continuous surface with considerable voids can be appreciated (Fig. 1-left), and in the atomic force surface image (Fig. 1-right) the higher roughness values were obtained, with higher profile peak values and higher valley depth as well. And for the bilayer soy protein isolate/sodium caseinate edible films the optical micrograph (Fig. 1-left) presented a continuous but coarse surface, although in the atomic force micrographs (Fig. 1-right) the lower roughness values and lower profile peak values with lower valley depth were observed. The bilayer samples presented lower roughness because sodium caseinate was the upper layer of the composite film, which is why sodium caseinate edible films presented both average roughness and mean square roughness close to the bilayer sample.

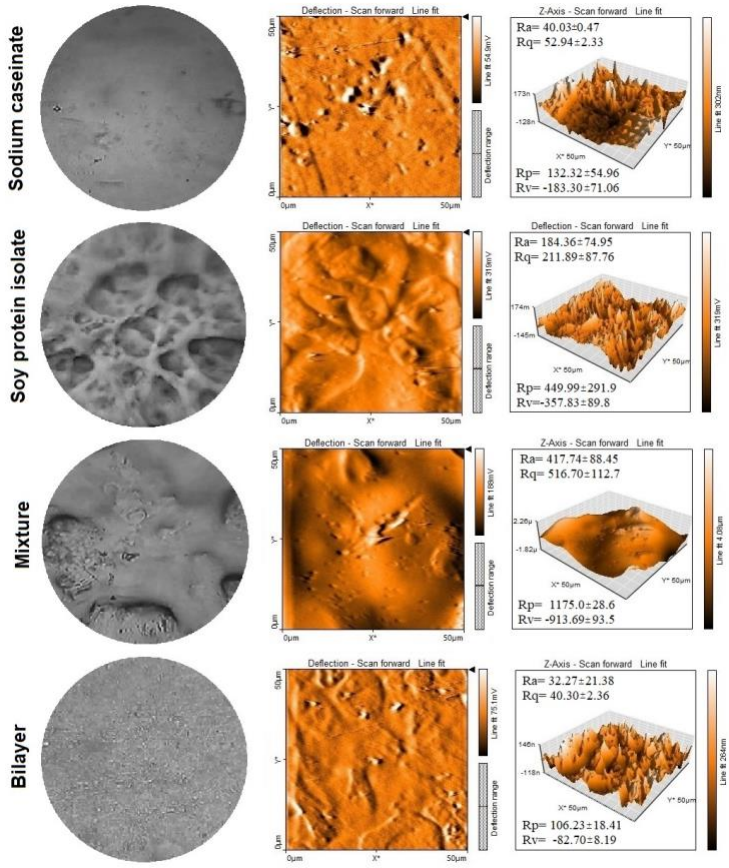

Figure 1. Optical microscopy images (at 20X, left) and bi- and three-dimensional atomic force micrographs (right) of the different single and composite protein edible films (Ra: roughness average, Rq: roughness mean square, Rp: maximum profile peak height, and Rv: maximum profile valley depth).

3.4. Structural properties

The thermal properties of edible films can be appreciated in Fig. 2. Interestingly, the edible films presented exothermic peaks. Four different exothermic peaks were observed in sodium caseinate edible films (peaks at 38.14 °C, 56.25 °C, 66.78 °C, and 79.29 °C, with enthalpies of 7.01, 22.54, 10.24 and 55.55 J/g, respectively). In the soy protein isolate samples two different peaks were detected (59.84 °C and 65.36 °C, with enthalpies of 5.61 and 43.77 J/g, respectively). In the mixture samples, three exothermic peaks were present (40.79 °C, 44.51 °C, and 53.14 °C, and enthalpies of 16.56, 2.83 and 37.76 J/g, respectively). Bilayer samples only presented two exothermic peaks (47.50 °C and 52.25 °C, with 29.45 and 9.71 J/g of enthalpy, respectively). Only soy protein isolate edible film and mixture edible film samples presented endothermic transition above 140 °C. at this respect, Composite films presented the lower enthalpies of crystallization. When the temperature heats above Tg, the proteins entanglements and internal cohesion release energy at the same time, resulting in an exothermic peak in the curve, indicating components miscibility (Yuan et al., 2022). Depending on the material complexity, during temperature scanning, aggregation of proteins exhibits as exotherms, and the transition temperatures reflect the thermal stability of the phase or state going through the transition (Kaletunç, 2009).

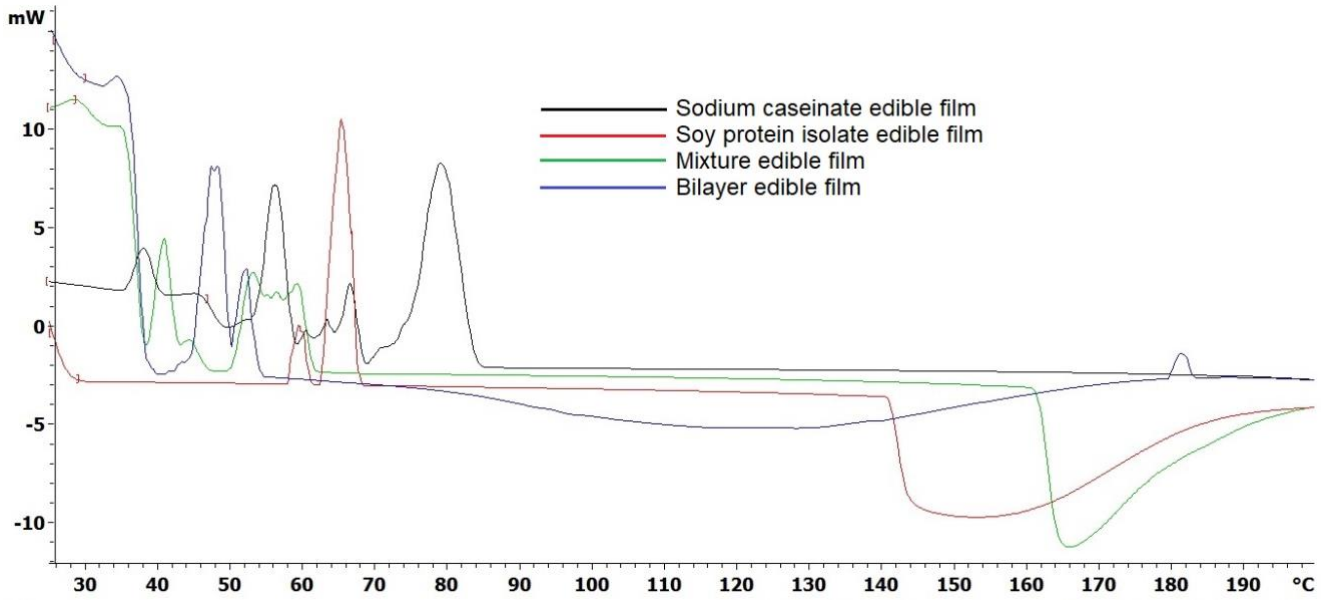

Figure 2. Thermal behavior of the different single and composite protein edible films.

Only both composite edible films presented a glass transition (step in the heat flow trace). The glass transition temperature (Tg) is defined as the physical change from the glassy state to the rubbery state promoted by heat in amorphous materials, and a single Tg indicates a good compatibility of the components (Chen et al., 2024). The glass transition for the bilayer edible film started at 36.06 °C, midpoint at 37.17 °C, and end set at 32.88 °C. For the mixture edible film, the glass transition onset at 36.03 °C, mid-point at 36.95, and ended at 38.50°C. Nonetheless both samples presented practically the same onset and peak temperatures, but mixture sample Tg was longer, indicating more interactions between the proteins. Additionally, the glass transition temperature range depends on the system heterogeneity, where more heterogenic systems present a wider transition range (Roudaut et al., 2004). In this view, the composite edible films resulted in a heterogenic and thermally compatible structure, since there was an inherent interaction between the employed proteins during the dispersion, heating, and casting of the mixture, since the improvement in thermal stability may be due to the interaction and the higher compatibility between the components (Zhang et al., 2022).

In Fig. 3 the FTIR spectra of the different edible films were compared, displaying certain differences in the absorption intensity in certain specific spectral regions. In same manner, the bands observed in the 3600 cm^{-1} to 3000 cm^{-1} wavelength region are attributed to bounded and free O–H and N–H groups, and the range 3000-2800 cm^{-1} observed is related to C–H stretching in CH_2 and CH_3 residues, where the O–H and N–H groups in proteins, O–H in glycerol and O–H in adsorbed water formed inter- and intra-molecular hydrogen bonds with the proteins C=O groups as peptide and carboxyl (Barreto et al., 2003). The samples presented the major bands between 1700-900 cm^{-1}, with clear differences in intensity at 1630, 1539 and 1450 cm^{-1} bands. The band at 1634 cm^{-1} represents amide-I, related to C=O stretching and hydrogen bonding connected to COO, the band at 1539 cm^{-1} represents amide-II related to bending vibrations of C–N groups and angular changing of N–H group (López et al., 2017; Khedri et al., 2021), and the absorption band at 1450 cm^{-1} is associated to amide III CH_2 symmetric bending (Wang et al., 2024) and to C–H deformation (Barreto et al., 2003). FTIR is the main technique employed to identify the covalent bonds formed between the components' functional groups (Zhou et al., 2021). Differences in molecular weight

contributed to differences in the rate of substrate deposition and interactions among the different components, affecting their oriented arrangement, resulting in a lower densification in the structure of the bilayer film, as compared with single protein films, affecting their properties (Chen et al., 2024), suggesting that at the interface between both films there was a lot inter- and intra-molecular interactions between O–H and N–H of both proteins, as seen using FTIR (Zhang et al., 2022). At these specific bands (1630, 1539 and 1450 cm^{-1}), sodium caseinate edible film presented the higher absorbance, and soy protein isolate presented the lower one. The absorbance of both composite edible films, bilayer and mixture, were observed between the absorbance of both single protein samples, indicating an interaction between both proteins, since these bands are associated with amide changes, related to proteins structure.

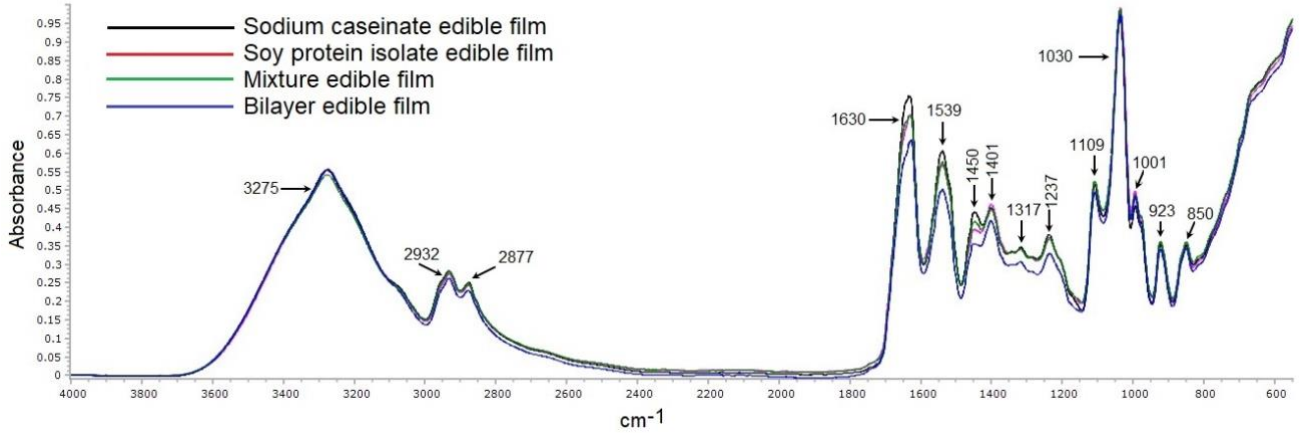

Figure 3. FTIR spectrum for the different single and composite protein edible films.

4. Conclusions

At the experimental conditions employed in this research, the formulation of composite edible films from sodium caseinate and soy protein isolate, either for mixtures or in bilayer, demonstrated an improvement in physical and mechanical properties, demonstrated by FTIR and DSC. In the physical attributes, composite edible films result in thicker and more insoluble material, desirable to allow the handling and manipulation during application, with improved water vapor permeability capacity. This was related to a rigid structure, as compared to the single protein sodium caseinate or soy isolate protein edible films. The interaction between the proteins in the composite mixtures related to the modification of the physical and mechanical properties was established by the thermal behavior, resulting in a heterogenic and thermally compatible structure, due to the inherent interaction between the employed proteins during the dispersion, heating, and/or casting of the mixtures. Changes in the intensity in bands related to amide functional groups, associated with changes in protein structure, supports the interaction in composite formulations as well.

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Acknowledgments

Autor González-Cayetano thanks to the Secretaria de Ciencias, Humanidades, Tecnologia e Innovacion, formerly CONAHCYT, the grant for his graduate studies.