

Physico-chemical Properties of Protein-Based Edible Films

Nidhi Parmar¹, Viraj Roghelia^{2*}

P. G. Department of Home Science, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

*Corresponding Author

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Abstract— The present study focused on the development and characterization of protein-based edible films using soy protein isolate (SPI), mung bean protein (MBP), and their blends with corn starch (CS). A total of six formulations were prepared, namely SPI, MBP, corn starch–soy protein isolate blends (CSSP 40:60 and CSSP 50:50), and corn starch–mung bean protein blends (CSMBP 40:60 and CSMBP 50:50). The films were developed using the casting method with sorbitol as a plasticizer. The prepared films were evaluated for their physical properties including thickness, solubility, transparency, colour parameters (L^* , a^* , b^*), mechanical properties such as tensile strength, and structural characteristics using Fourier-transform infrared spectroscopy (FTIR). The results revealed a significant ($p \leq 0.01$) variation among all film formulations in terms of physical, optical, and mechanical properties. The thickness of the films ranged from 0.196 mm to 0.263 mm, with SPI-based films exhibiting the highest thickness (0.263 mm), while MBP films showed the lowest thickness (0.196 mm). Blended films displayed intermediate thickness values, indicating that incorporation of starch influenced film structure. Solubility values varied from 31.34% to 31.48%, with CSMBP 50:50 (31.48%) and CSSP 50:50 (31.34%) exhibiting significantly higher solubility compared to other formulations, suggesting improved interaction and dispersion of components in blended systems. Transparency also differed significantly among the films, ranging from 0.436 to 1.66. The highest transparency was observed in CSSP 40:60 (1.66), indicating better light transmittance and a more uniform film matrix, whereas MBP films exhibited the lowest transparency (0.436), reflecting higher opacity. Colour analysis demonstrated notable differences, with L^* values ranging from 64.36 to 84.56. CSSP 40:60 films showed the highest lightness, while MBP and CSMBP 40:60 films appeared darker. The a^* values ranged from 3.623 to 7.516, with MBP films exhibiting higher redness, whereas SPI films showed lower values. The b^* values ranged from 4.356 to 14.196, indicating that MBP incorporation increased yellowness in the films. Mechanical analysis indicated significant differences ($p \leq 0.01$) in tensile strength among formulations. The highest tensile strength was recorded for CSMBP 40:60 (4.093 MPa), followed by MBP (3.170 MPa), while SPI films showed the lowest value (1.24 MPa). Blended films generally exhibited improved mechanical performance due to enhanced intermolecular interactions between protein and starch components. FTIR analysis confirmed the presence of characteristic functional groups in all films. Strong O–H and N–H stretching bands around $3273\text{--}3278\text{ cm}^{-1}$ indicated hydrogen bonding, while Amide I and II bands confirmed the protein structure. Additional peaks in starch-blended films verified the contribution of polysaccharides and improved compatibility within the matrix. Overall, the results suggest that blending proteins with corn starch significantly enhances the functional properties of edible films. Among all formulations, CSMBP 40:60 demonstrated superior mechanical strength, while CSSP 40:60 showed excellent optical properties, indicating their potential application in biodegradable and sustainable food packaging systems.

Keywords— Edible film, soy protein isolate, mung bean protein, physical properties.

I. INTRODUCTION

In recent years, the food packaging sector has been under increasing pressure to adopt sustainable and eco-friendly materials. Traditional packaging options, particularly plastics like polyethylene, are non-biodegradable and tend to accumulate in the environment, raising concerns about their impact on human health. As a result, significant progress has been made in the

development of edible films and coatings, which are emerging as effective alternatives to conventional packaging. These biomaterials are especially suitable for perishable foods, as they help protect against chemical changes and microbial spoilage [1,2,3,4].

Edible films and coatings perform multiple functional roles in food preservation systems. They provide protection against ultraviolet (UV) radiation and regulate the mass transfer of solutes such as salts, additives, and pigments between the food matrix and the external environment. These materials also modulate the permeability of water vapor and respiratory gases, including oxygen (O₂), carbon dioxide (CO₂), nitrogen (N₂), and ethylene, thereby contributing to the establishment of a controlled or modified atmosphere around the product. In addition, they serve as protective barriers against mechanical damage during handling and distribution, ultimately extending the shelf life of food products. Furthermore, edible films and coatings can function as delivery systems for bioactive compounds, such as antioxidants and probiotic or bioprotective microorganisms, and may exhibit antimicrobial and antifungal activities that enhance both product safety and potential health benefits [5].

Protein-based edible films offer structural stability and are known for their good mechanical properties and high water vapor permeability [6]. Despite their hygroscopic nature, protein-based coatings (milk, soy, wheat, whey) help maintain the structure of perishable foods [7]. Protein-based coatings significantly improve the appearance, texture, and preservation of food. These films act as protective layers that shield food products from external environmental factors that could compromise their quality, safety, and stability. As effective physical barriers, protein-based films can reduce the permeation of oxygen, moisture, and microbial agents, thereby extending the shelf life of perishable food products [8].

Soybean is a major oilseed and protein crop valued for its high grain quality and substantial vegetative mass. It is widely utilized in food, feed, industrial applications, and medicine [9]. On average, soybean seeds contain 37–42% protein, 19–22% oil, and up to 30% carbohydrates. The vegetative biomass harvested during the pod-filling stage is also rich in proteins (16–18%), carbohydrates, and vitamins [10]. Soybeans, with their higher protein content compared to other legumes, are well positioned to help fulfill this demand [11]. Soy protein isolate (SPI) is an affordable, accessible, and nutritious biopolymer with low oxygen permeability, ideal for protecting against oxidative damage [12,13]. However, like other polar polymers, SPI films are poor moisture barriers [14]. Cross-linking treatments can improve their properties [15].

Mung bean (*Vigna radiata*), widely grown in Asia, contains 25–28% protein and numerous bioactive compounds [16]. Dry grains, sprouts, starch for noodle preparation, and paste are the main products of the mung bean market [17]. In the starch extraction process, the protein-rich byproduct is often discarded. This byproduct can be used for the preparation of edible films, thereby reducing waste disposal costs and enhancing sustainability [18]. It contains approximately 25–28% protein and serves as a rich source of bioactive constituents, including flavonoids, phenolic acids, organic acids, and polysaccharides [19]. These phytochemicals contribute to a range of health-promoting effects, such as antioxidant, antidiabetic, anti-hypercholesterolemic, anticancer, anti-tumor, and detoxifying activities [20,21].

Protein-based edible films exhibit superior mechanical strength, barrier performance, and nutritional benefits compared to those derived from polysaccharides and lipids [22]. Soy protein isolate and mung bean protein have been relatively less explored for the development of edible films. Therefore, the present study aims to develop and analyse edible films prepared from these protein sources at different concentrations. The films were fabricated using the casting method with sorbitol as a plasticizer, and subsequently evaluated for their thickness, solubility, opacity, colour characteristics, and mechanical properties.

II. MATERIAL AND METHODS

Protein extraction from mung beans was done by the method described by El-Adawy [23], with some modifications. The dried mung beans are crushed into powder. 5% w/v of the crushed powder is dispersed in the distilled water to obtain a mixture. 0.1 N NaOH is added to the mixture to adjust its pH value up to 9 at 35–37 °C under magnetic stirring at 300–350 rpm for up to 1 hour, and then centrifuged at 2000 rpm for 15 min to collect supernatant therefrom (solid residue is discarded). In order to obtain increased yields, the distilled water is added on the residue, pH is adjusted under magnetic stirring, and the mixture is centrifuged repeatedly. The supernatants are combined in a container, and the pH is adjusted to 4.5 with 0.1 N HCl under magnetic stirring to precipitate the protein. The proteins are recovered by centrifugation at 2000 rpm for 5 min followed by removal of the supernatant by decantation and the sediment is dried in a hot-air oven at 60°C for 24 hours. The dried material was then ground, sieved (Mesh size-125 MIC), and stored in an airtight container at 4°C until further use.

2.1 Preparation of Edible Film:

All protein samples at their respective concentrations were mixed in 100 mL of distilled water, after which sorbitol was incorporated as a plasticizer, as shown in Table 1. The pH of each was adjusted to pH 9.0 for soy protein-based edible film and pH 12 for mung bean protein-based edible film using 1 N sodium hydroxide. The higher pH for mung bean protein was required to achieve adequate protein solubilization and molecular unfolding, as mung bean protein exhibits different solubility characteristics compared to soy protein isolate. The solutions were then heated on a hot plate with continuous stirring at 85 ± 5 °C for 15 ± 5 minutes. Following heating, the solutions were allowed to stand at ambient temperature for 5 minutes to facilitate the release of entrapped air bubbles.

Subsequently, the film-forming solutions were uniformly poured onto glass plates to regulate film thickness, ensuring that the volume of solution used for each casting was constant. The films were dried in a hot air oven at 60 °C for 24 hours. Once dried, the films were carefully peeled off and stored in a desiccator for subsequent analysis.

TABLE 1
COMPOSITION OF PROTEIN-BASED EDIBLE FILMS

Edible film	Corn starch (gm%)	Mung bean protein (gm%)	Soy protein isolate (gm%)	Sorbitol (gm%)
CS	4	-	-	3
MBP	-	4	-	3
SPI	-	-	4	3
CSMBP 40:60	1.6	2.4	-	3
CSMBP 50:50	2	2	-	3
CSSP 40:60	1.6	-	2.4	3
CSSP 50:50	2	-	2	3

Note: CS - Corn starch based edible film (100%), MBP – Mung bean protein edible film (100%), SPI – Soy protein based edible film (100%), CSMBP 40:60 - Corn starch (40%) and mung bean protein (60%) based edible film, CSMBP 50:50 - Corn starch (50%) and mung bean protein (50%) based edible film, CSSP 40:60 - Corn starch (40%) and soy protein isolate (60%) based edible film, CSSP 50:50 - Corn starch (50%) and soy protein isolate (50%) based edible film

2.2 Physical Parameters of Edible Film

- Film thickness (hand-held micrometer):** The thickness of developed films from different starches was determined by the handheld digital screw gauge (Insize company) with a precision of 0.001 mm. The samples were taken in triplicates, and the average of three measurements at different locations in the film were recorded.
- Transparency (UV-visible recording spectrophotometer):** Film transparency was measured using a UV-visible spectrophotometer at 600 nm (M/s PerkinElmer/Lambda, 25).
- Solubility:** The solubility of the edible films was assessed following the method of Wu et al. [24]. Before the water solubility test, the films were stored in a desiccator at 0% relative humidity (RH). The initial weight of the 20 mm × 20 mm dry film sheet (W0) was recorded. The film was then immersed in 5 mL of distilled water at 25 ± 1 °C for 5 minutes at 50 rpm. The insoluble portion of the film was carefully separated and dried in an oven at 100 °C until a constant weight (W1) was achieved.
- Tensile strength (by Texture analyzer LLOYD, UK):** Tensile strength was measured following the standard test method using a Texture Analyzer (TA Plus, Lloyd Instruments, Largo, FL) at a crosshead speed of 2 mm/s. Films were cut into 10 cm × 2 cm strips and tested. Only samples rupturing at the centre were included in the analysis. The mean and standard deviation were calculated for all valid measurements.
- Colour analysis (Hunter L*, a*, b*):** The colour of the films was determined using a colour flex EZ 45°/0° spectrophotometer (Hunter Lab). After standardization of the instruments using Hunter Lab Colour standards, 'L' (*lightness*), 'a' (redness to greenness) and 'b*' (yellowness to blueness) values were measured by placing the sample in a sample cup.

- 6) **Fourier transform infrared spectroscopy:** The Fourier transform infrared (FTIR) spectroscopy of the developed edible film was conducted using a Spectrum GX (Perkin Elmer, U.S.A). The spectra were obtained as an average of 45 scans with a spectral resolution of 2 cm⁻¹ using FTIR spectrum of 500 to 4500 cm⁻¹ [25].

Statistical analysis: The obtained data were analyzed for mean, standard deviation, and ANOVA using SPSS version 20.0.

III. RESULTS AND DISCUSSION

Total six protein-based films were developed namely soy protein isolate (SPI), mung bean protein (MBP), corn starch and soy protein isolate blend (CSSP 40:60), corn starch and soy protein isolate blend (CSSP 50:50), corn starch and mung bean protein blend (CSMBP 40:60), as well as corn starch and mung bean protein blend (CSMBP 50:50). The results of protein-based edible films are discussed below.

The physical properties of various protein-based edible films are presented in Table 2. A significant ($p \leq 0.01$) difference was observed in thickness, transparency and solubility among these edible films.

TABLE 2
PHYSICAL PROPERTIES OF PROTEIN-BASED EDIBLE FILMS

Edible films	Thickness (mm)	Solubility (%)	Transparency
SPI	0.263e ± 0.015	27.370a ± 0.236	1.583d ± 0.476
CSSP 40:60	0.256d ± 0.011	29.293c ± 0.587	1.660e ± 0.137
CSSP 50:50	0.240c ± 0.010	31.334d ± 0.090	1.466d ± 0.086
MBP	0.196a ± 0.011	28.503b ± 0.179	0.436a ± 0.045
CSMBP 40:60	0.203b ± 0.005	29.356c ± 0.372	0.683c ± 0.035
CSMBP 50:50	0.213b ± 0.015	31.486d ± 0.291	0.503b ± 0.065
F - Value	16.677**	69.719**	22.624**

SPI: soy protein isolate based edible film, CSSP 40:60: corn starch (40%) and soy protein isolate (60%) based edible film, CSSP 50:50: corn starch (50%) and soy protein (50%) based edible film, MBP: mung bean protein based edible film, CSMBP 40:60: corn starch (40%) and mung bean protein (60%) based edible film, CSMBP 50:50: corn starch (50%) and mung bean protein (50%) based edible film

*Values are Mean ± SD of three observations, ** indicates significant difference at $p \leq 0.01$. Different alphabetical superscripts indicate significant difference within a column.*

A significant ($p \leq 0.01$) difference was observed in thickness of various formulations of protein-based edible films which follows the given trend: 0.196 mm (MBP) < 0.203 mm (CSMBP 40:60) < 0.213 mm (CSMBP 50:50) < 0.240 mm (CSSP 50:50) < 0.256 mm (CSSP 40:60) < 0.263 mm (SPI). SPI based edible film showed the highest thickness whereas MBP based edible film showed the lowest thickness. The thickness of soy protein isolate based edible film ranged from 0.136 mm to 0.170 mm in previous studies, which was found to be lower than the value obtained in the present study [26]. Similarly, the thickness of the fenugreek protein concentrate films ranged from 0.23 to 0.30 mm and increased as the pH of the film-forming solution was raised from 9 to 12. This effect is likely associated with enhanced protein solubility and molecular unfolding at higher pH levels. As alkalinity increases, protein molecules become more dispersed and better solubilized, which decreases intermolecular spacing and ultimately results in greater film thickness [27].

With respect to solubility, CSMBP 50:50 exhibited the highest solubility (31.48%) followed by CSSP 50:50 (31.34%) that was significantly ($p \leq 0.01$) higher than solubility of other protein-based edible films. pH strongly affects films made from water soluble proteins. As pH rises and moves away from the isoelectric point, protein solubility increases, leading to denaturation, unfolding, and easier dissolution. This solubilization weakens cohesive forces and promotes better molecular alignment, forming a fine, uniform network [28]. The lower solubility (17.44% - 23.08%) was observed in whey protein isolate based edible films as compared to present study [29]. Solubility value of mung bean protein based edible film at different time and temperature ranged from 12.76% to 49.41%, which was similar to recorded data of the present study [30].

A significant ($p \leq 0.01$) variation in transparency value was observed for different edible films. CSSP 40:60 films demonstrated the highest transparency (1.66), which was significantly ($p \leq 0.01$) higher than other edible films. The least transparency was

observed for MBP films (0.436). Similar transparency values were observed by [29] for the whey protein isolate based edible films which ranged from 0.202 to 1.687. Film transparency is governed by light-matter interactions, which are a function of compositional density. Higher polymer concentrations and greater film thickness increase light scattering and absorption, thereby diminishing transparency [31]. Consequently, thinner films demonstrate superior light transmittance [32]. pH was the main factor affecting film opacity, causing a tenfold increase at pHc (coacervation) over pH 11. The effect of polysaccharide concentration was minor and contradictory, increasing opacity slightly at high pH but decreasing it at low pH. This suggests that pH fundamentally alters the film's morphology [33].

A significant difference ($p \leq 0.01$) was observed among all the protein-based film formulations with respect to their color parameters (Table 3). The L^* values of the films ranged from 64.36 to 84.56, indicating notable variation in their lightness. Films prepared with CSSP 50:50 exhibited the highest L^* value (84.560), suggesting a lighter and more visually transparent appearance. In contrast, films developed using pure MBP and CSMBP 40:60 showed the lowest L^* values (64.356 and 64.610, respectively), indicating that these formulations produced comparatively darker films. L^* values for fenugreek protein based edible film ranged from 34.06 to 64.62, which was in range with MBP based edible films whereas for SPI based edible film it was lower [27]. The L^* value of different concentration of SPI-CMC based edible films (78.37 to 82.7) was in line with soy protein based edible film. The L^* values declined as the pH increased, indicating a darker appearance of the films [33]. Under alkaline conditions, proteins can interact with polyphenolic compounds to form complexes, which likely contributed to the increased discoloration observed in films produced at higher pH levels. The colour becomes more yellow and darker as the heating temperature and pH increases [30].

TABLE 3
COLOUR ANALYSIS (L^* , a^* , b^*) OF PROTEIN-BASED EDIBLE FILMS

Edible films	L^*	a^*	b^*
SPI	79.526d ± 0.810	3.623a ± 0.005	5.716c ± 0.055
CSSP 40:60	75.570c ± 3.015	4.846b ± 0.215	4.443b ± 0.219
CSSP 50:50	84.560e ± 2.000	5.363c ± 0.574	4.356a ± 0.211
MBP	64.356a ± 1.852	7.516e ± 0.032	14.196f ± 0.035
CSMBP 40:60	64.610a ± 3.077	6.113d ± 0.142	10.503d ± 0.295
CSMBP 50:50	73.920b ± 3.070	5.570c ± 0.130	12.066e ± 0.488
F - Value	32.462**	73.094**	770.950**

SPI: soy protein isolate based edible film, CSSP 40:60: corn starch (40%) and soy protein isolate (60%) based edible film, CSSP 50:50: corn starch (50%) and soy protein (50%) based edible film, MBP: mung bean protein based edible film, CSMBP 40:60: corn starch (40%) and mung bean protein (60%) based edible film, CSMBP 50:50: corn starch (50%) and mung bean protein (50%) based edible film

*Values are Mean ± SD of three observations, ** indicates significant difference at $p \leq 0.01$. Different alphabetical superscripts indicate significant difference within a column.*

The a^* values varied widely among samples, ranging from 3.623 to 7.516. Films made from pure MBP showed the highest a^* value (7.516). Conversely, SPI-based films, particularly SPI (3.623), showed lower a^* values, indicating a greener, less reddish appearance. Blended films such as CSSP 50:50 and CSMBP 50:50 showed intermediate a^* values, indicating moderate redness that was neither too intense nor too minimal. The b^* values of protein-based edible film exhibited a significant difference ($p \leq 0.01$), which ranged from 4.356 to 14.196. MBP-based films showed the highest b^* values, with MBP reaching 14.196, indicating a strong yellowish hue. In contrast, CSSP 40:60 and CSSP 50:50 showed much lower b^* values (4.356 and 4.443, respectively), implying a less yellow appearance. CSMBP 50:50 showed relatively higher b^* values than SPI formulations, suggesting that the incorporation of MBP increased yellowness in the blended films.

a^* values (5 to 14.79) and b^* values (2.25 to 18.13) for soy protein-based edible films observed by [27] were similar to the obtained data. SPI-CMC based edible films showed lower a^* values (-0.18 to 4.04) and higher b^* values (28.53 to 43.19) as compared to present study. Films prepared at the coacervation pH also became darker, as shown by a lower L^* value, while exhibiting more intense red and yellow hues (higher a^* and b^* values) with increasing polysaccharide concentration [33].

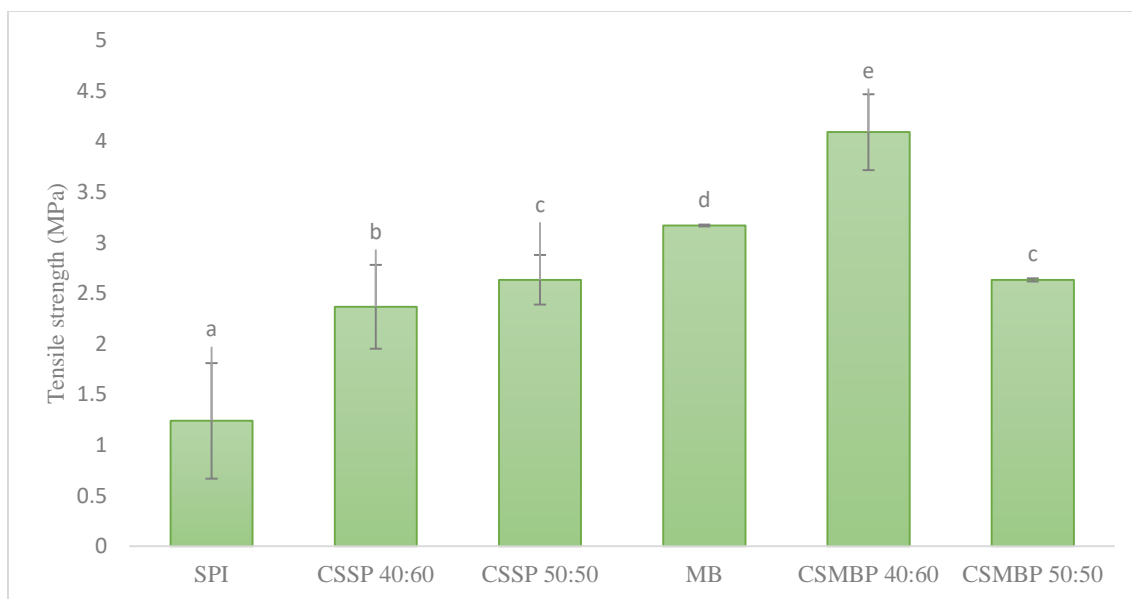
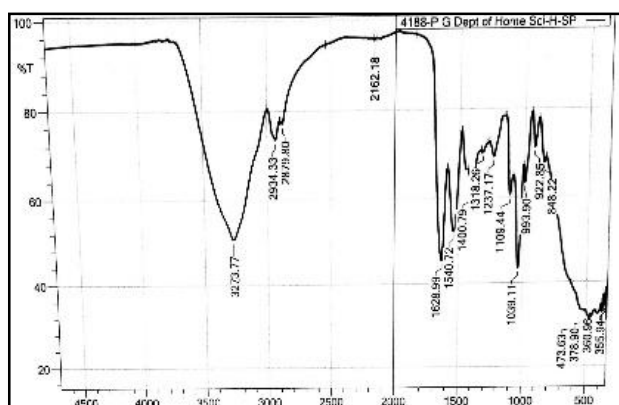


FIGURE 1: Tensile strength (MPa) of protein-based edible films

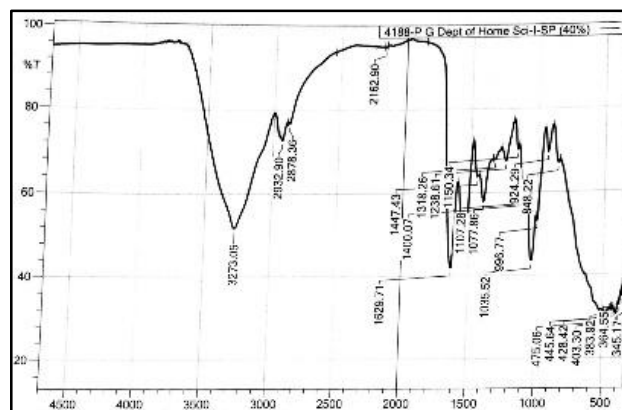
SPI: soy protein isolate based edible film, CSSP 40:60: corn starch (40%) and soy protein isolate (60%) based edible film, CSSP 50:50: corn starch (50%) and soy protein (50%) based edible film, MBP: mung bean protein based edible film, CSMBP 40:60: corn starch (40%) and mung bean protein (60%) based edible film, CSMBP 50:50: corn starch (50%) and mung bean protein (50%) based edible film

Values are Mean ± SD of three observations; different alphabetical superscripts indicate significant difference ($p \leq 0.01$)

High tensile strength reinforces the film, allowing it to maintain continuous protection even under stressful conditions [34]. The tensile strength varied significantly ($p \leq 0.01$) between different combinations (Figure 1). The order of tensile strength was CSMBP 40:60 (4.093 MPa) > MBP (3.170 MPa) > CSMBP 50:50 (2.633 MPa) = CSSP 50:50 (2.633 MPa) > CSSP 40:60 (2.366 MPa) > SPI (1.24 MPa). Similar results for mung bean protein based films were found by [35]. Glycerol softens protein-based packaging films by reducing polymer interactions, decreasing tensile strength, and improving flexibility. Cavities or holes can further weaken the material [36]. Increase in pH showed the increase in tensile strength of mung bean protein based edible films [30]. A similar trend was reported by [37], who observed that the tensile strength of pea protein films increased from 6.9 to 8.4 MPa as the pH of the film forming solution was raised from 7 to 10. The similar values for tensile strength of the soy protein based edible films was observed by [38], which ranged from 3.9 MPa to 6.4 MPa. Similar results of tensile strength for CMC and SPI based edible film at different pH (2.01 MPa to 4.09 MPa) were observed by [33].



(a)



(b)

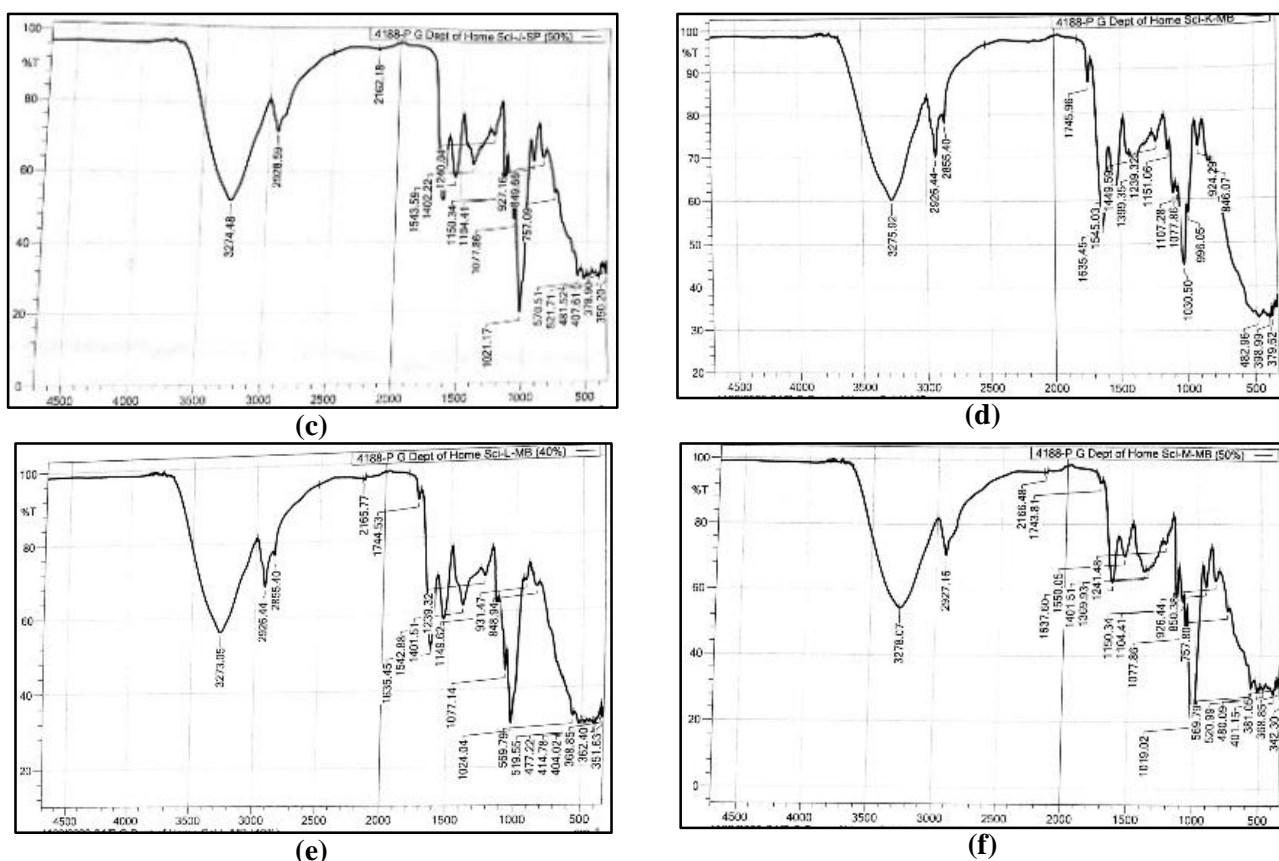


FIGURE 2: FTIR spectra of protein-based edible films

(a) SPI: soy protein isolate based edible film, (b) CSSP 40:60: corn starch (40%) and soy protein isolate (60%) based edible film, (c) CSSP 50:50: corn starch (50%) and soy protein (50%) based edible film, (d) MBP: mung bean protein based edible film, (e) CSMBP 40:60: corn starch (40%) and mung bean protein (60%) based edible film, (f) CSMBP 50:50: corn starch (50%) and mung bean protein (50%) based edible film

Figure 2 shows FTIR spectra of protein-based edible films. FTIR spectra revealed that all protein-based films showed strong O–H and N–H stretching around 3273–3278 cm^{-1} , indicating hydrogen bonding is crucial for film structure. Amide I and II bands (1630–1640 cm^{-1} and 1540 cm^{-1}) confirmed protein presence across soy and mung bean formulations. Films blended with corn starch exhibited additional peaks in the 1000–1150 cm^{-1} region due to C–O–C and α -glycosidic linkages, confirming polysaccharide contribution. Peaks near 1745 cm^{-1} in mung bean blends suggested plasticizer or ester presence. Overall, blending enhanced intermolecular interactions, balancing strength and flexibility. In a protein's FTIR spectrum, the most notable signals are the Amide I (~1650 cm^{-1}) and Amide II (~1540 cm^{-1}) bands. Amide I mainly reflects C=O stretching, while Amide II corresponds to N–H bending and C–N stretching within the peptide backbone [39]. The 1400–1550 cm^{-1} region corresponds to N–H bending, characteristic of the amide II band. The 1600–1700 cm^{-1} range represents C=O and C–N stretching vibrations associated with amide I. Peaks from 2850–2980 cm^{-1} relate to C–H stretching, while the 3000–3600 cm^{-1} region reflects vibrations of free and bound O–H and N–H groups [27]. The results are in line with the study of fenugreek protein based edible film where the Amide I band appears in the 1600–1700 cm^{-1} range, confirming C=O and C–N stretching associated with protein structure, while the Amide II band is consistently observed around 1400–1550 cm^{-1} due to N–H bending. Both results also identify prominent O–H and N–H stretching signals in the 3270–3380 cm^{-1} region (Amide A), indicating hydrogen bonding [27].

IV. CONCLUSION

In conclusion, the study demonstrated that protein-based edible films developed from soy protein isolate and mung bean protein, alone and in combination with corn starch, exhibit promising functional properties. Blending proteins with starch significantly improved the physical, optical, and mechanical characteristics of the films. Among the formulations, CSMBP 40:60 showed superior tensile strength, while CSSP 40:60 exhibited better transparency and overall appearance. The FTIR analysis confirmed strong intermolecular interactions contributing to film stability. Future studies should evaluate water vapor

permeability, antimicrobial properties, and biodegradability of these films to further assess their potential for commercial food packaging applications. Overall, these protein–starch blend films have strong potential as biodegradable and sustainable alternatives for food packaging applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research paper.

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