

Mechanical and Barrier Properties of Edible Starch-Protein-Based Films

J. H. Jagannath, C. Nanjappa, D. K. Das Gupta, A. S. Bawa

Defence Food Research Laboratory, Mysore, India

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ABSTRACT: The present investigation dealt with the mechanical properties, water-vapor transmission behavior at different relative humidity conditions, and DSC thermograms of edible films formulated using various proteins (casein, gelatin, albumin) in combination with starch and nonthermal as well as intense thermal blending. Nonthermal blended film showed in the DSC thermogram a double T_g , indicating poor miscibility of the components and, hence, a poor film-forming property. However, the DSC thermogram of all the films based on intense thermal blending showed a single T_g , indicating the complete molecular miscibility of the components. Casein-based film showed a lower water-vapor transmission rate, water gain at different relative humidity conditions, and higher tensile strength compared to

its counterparts containing gelatin and albumin. Since the casein-starch blend gave better film properties, a blend of hydrophobic carnauba wax and casein was prepared to compare the properties of hydrophilic-hydrophilic and hydrophobic-hydrophilic blends. Both these blends compared well with respect to the water-vapor transmission rate. Wax-based film showed multiphased behavior in the DSC thermograms and the percent elongation was lower as compared to the casein-starch blend. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 88: 64–71, 2003

Key words: biopolymers; blending; miscibility; differential scanning calorimetry (DSC)

INTRODUCTION

The use of edible films as an alternative to a polymeric nonbiodegradable food wrap has been the subject of many review articles.^{1–4} The advantage of an edible coating is that it can be applied to foods by dipping or spraying and may be used to inhibit the migration of ingredients in processed foods, minimally processed vegetables, raw meat, etc., and to improve the mechanical integrity or handling.⁵ Edible films can be prepared from polysaccharides, hydrocolloids, proteins, and their combinations. The protein solutions are known to possess good film-forming properties. Upon casting of film-forming solutions, disulfide bonds are crosslinked together with polypeptide chains to yield a film structure.⁶ Various researchers have noted the important role of a covalent disulfide bond in gluten, gelatin, casein, and albumin film formation.⁷ It also helps in the binding of flavor compounds and water, modification of the surface tension and interfacial surface tension (which influence emulsification and foaming activity), and improvement of the cohesive/adhesive properties (which affect texturization). Crosslinking agents such as lactic acid, tannic acid, calcium chloride, glutaraldehyde, and triethanolamine have been added to protein films to increase the

water resistance but the resulting films have been found to be less flexible and transparent.⁸ Starch-based coatings have advantages in the area of gas exchange, namely, CO₂ and O₂. Bilayer coatings, namely, starch and proteins with wax and lipids, which are hydrophobic in nature, have been extensively studied.^{9–10} However, the bilayer protein and starch blends have not been studied much because both are hydrophilic in nature.

In search for a new polymer material, scientists have polymerized new monomers or made new random, block, or graft copolymers from existing monomers. A third alternative has been to blend existing biopolymers such as protein and starch to produce material with new properties. For example, normal protein and starch films are highly permeable to water vapor, whereas completely miscible or blended or compatible film may be less permeable to water vapor. The particularly important aspect of all biopolymer mixtures is the phase behavior in the solid state as well as the melt. The behavior in the solid state is traditionally assessed by examining the number and location of the glass transitions (T_g) or the melting endotherm (T_m) using the thermal analysis technique. T_g is taken as the leading edge of the melting of amorphous phases, that is, T_g and T_m are one and the same physical phenomenon in the polymer blend.¹¹ For a binary blend of amorphous polymers, if a single T_m intermediate between those of the parent polymers is observed, the mixture is considered to be miscible. If two T_g 's iden-

Correspondence to: A. S. Bawa.

tical to those of the parent are observed, but are shifted from the parent polymer, then the mixture is multiphase with some intermixing of the polymers. This is a kind of semi-interpenetrating network.¹² Thermal analysis techniques, such as differential scanning calorimetry (DSC), are generally employed for the measurement of the T_g/T_m . The mechanical, barrier, and water-soluble properties of the film are affected by the value of the T_g .¹³

The present investigation was undertaken to thermally blend starch with different proteins having disulfide bonds, namely, albumin, casein, and gluten. The mechanical properties, water gain at different relative humidities (RHs), the water-vapor transmission rate, and DSC thermograms were determined to evaluate the suitability of protein-starch blends for making edible coating film. Carnauba wax was also thermally blended with casein to compare its properties with those of a casein-starch blend. A nonthermal homogenized blend was also prepared for the above compositions to ascertain the effect of intense thermal blending on the above-mentioned parameters. Propylene glycol and sodium salt of ethylenediamine tetraacetic acid (EDTA) were used as a plasticizer and a crosslinking agent, respectively

EXPERIMENTAL

All the ingredients for making edible film were food grade, meeting food chemical codex requirements. Corn starch, casein, albumin, gelatin, carnauba wax, and EDTA were of analytical grade obtained from S.D. Fine Chemicals.

Coating liquid preparation

Intensive thermal blending

Protein (15 g) was mixed with water (85 mL), heated, and stirred on a magnetic stirrer hot plate while slowly adding 14 mL of ammonium hydroxide (4.96N) to provide an alkaline condition to disperse the protein. Corn starch slurry (15 g) was prepared in hot water (100 mL) and placed in a three-necked flask. The flask was kept over a heating mantle and the blending was done in an inert atmosphere of nitrogen. Propylene glycol (10 mL) was added to ensure flexibility to the film. The crosslinking agent EDTA (1 g) was added to the reaction vessel. In one of the necks, a thermometer was inserted while the other was connected to a nitrogen gas cylinder. The middle neck was connected to a remotely controlled glass stirrer fitted with a Teflon blade. The completely dispersed protein was added in parts to the starch slurry in the reaction vessel. The temperature was slowly increased to 90°C using the temperature control of the heating mantle and maintained at this temperature for 6 h.

After this, the temperature was further increased slowly to 140°C and maintained at this temperature for another 6 h to ensure complete intense thermal blending of the starch and protein. The experiment was repeated with carnauba wax dissolved in propylene glycol (10 mL) instead of starch. After the experiment, the hot liquid blend was further homogenized in a homogenizer (Virtis, Gardiner, NY) at 10,000 rpm for 5 min. The homogenized dispersion was kept at ambient temperature and diluted to 250 mL with water.

Nonthermal blending

A slurry of corn starch mixed with the protein prepared as above was homogenized in the Virtis homogenizer. Propylene glycol, 10 mL, was added before the homogenizing. Carnauba wax was prepared as above in 10 mL of propylene glycol. In the case of carnauba wax-casein, the mixture was dispersed in a hot condition and the dispersion was diluted to 250 mL with water.

Casting of free films

After degassing, 250 mL of a liquid dispersion of the protein blend was poured onto a glass plate (34 × 34 cm). The film thickness was controlled by pouring a constant amount of the dispersion onto a level glass plate framed with strips of plastic or wood. The cast film was dried overnight at ambient temperature. A total of four glass plates were used to cast films at the same time for each blend. After the water evaporated, films were removed and conditioned at 65% RH and 25°C in a humidity chamber. The thickness was recorded using a micrometer, according to the ASTM 065 method. The dry films' weights were measured daily until no further measurable weight loss was observed.

Measurement of mechanical properties

The tensile strength and elongation at break of the film were determined using the ASTM D-2370-72 method.¹⁴ Specimens in the form of strips of size 14 × 150 mm were cut from the dried films. The specimens were conditioned for 24 h at 60.8% RH and tested for tensile strength using an universal testing equipment (Instron 1123). The gap between the grips was maintained at 25 mm and the specimens were strained at a rate of 20 mm per minute. For each dried film, 10 specimens were examined and the results of six constant readings of stress were used to report the tensile strength. The strain values at the breaking point were used to obtain the percent elongation of the film.

Water-vapor transmission rate (WVTR)

The WVTR was determined using free film as per method ASTM-D-2350.¹⁵ Free films were cut to obtain a circular test specimen of 12 cm. The test specimen was fastened to a Payne cup containing 8 mL of distilled water using a C-type clamp. The area of the film for water vapor to permeate was 10 sq cm. For each film, four different cups were prepared using portions from different areas of the films. Thereafter, the cups were kept in a desiccator over fused CaCl₂. The cups were weighed every 24 h, until a constant water loss was obtained. The WVTR was expressed as mg sq m⁻¹ day⁻¹.

RH storage

Dried free films were stored in a humidity chamber (Modern Equipment Corp., Mumbai) at RHs of 40, 60, 80, and 100%. RH was maintained with the help of a correct setting of the dry bulb and a wet bulb temperature controller using a "psychometric chart." The films were stored in the humidity oven for 48 h. For each dried free film, six specimens were examined and the results of four constant readings were used to report the weight loss or gain. Conditions of the film were also used to report stickiness or a tackfree nature.

Thermal analysis

A TA Instruments DSC 2010 temperature programmer controller fitted with a thermal analyst was used. The

experimental procedure adopted was described elsewhere.¹⁶ Pieces of the dried free film (about 10 mg) placed into preweighed sample pans were weighed on an automatic Mettler balance. The pan was placed on a spacer insert and sealed by a TA quick press. The reference was an empty pan sealed with a lid to give a suitable heat capacity. Triplicate sample pans were prepared and heated at a rate of 5°C/min from 25 to 350°C in the pressure cell with nitrogen gas passed at a flow rate of 60 mL/min. Indium was used to calibrate the instrument. The weight of the film was used to calculate the enthalpy change (ΔH) and expressed as joules per gram of the dry matter. T_g was taken as the leading edge of the melting of phase transition, and T_m as the lowest point of melting of the phase transition on the temperature axis.

Statistical analysis

The standard deviation (SD) and correlation coefficient were determined using Statistica 99 software.

RESULTS AND DISCUSSION

Film characteristics

All the films obtained by the intensive thermal blending of starch, protein, and wax-casein were shown to be opaque, clear, and of uniform thickness. However, the nonthermally homogenized one did not show any

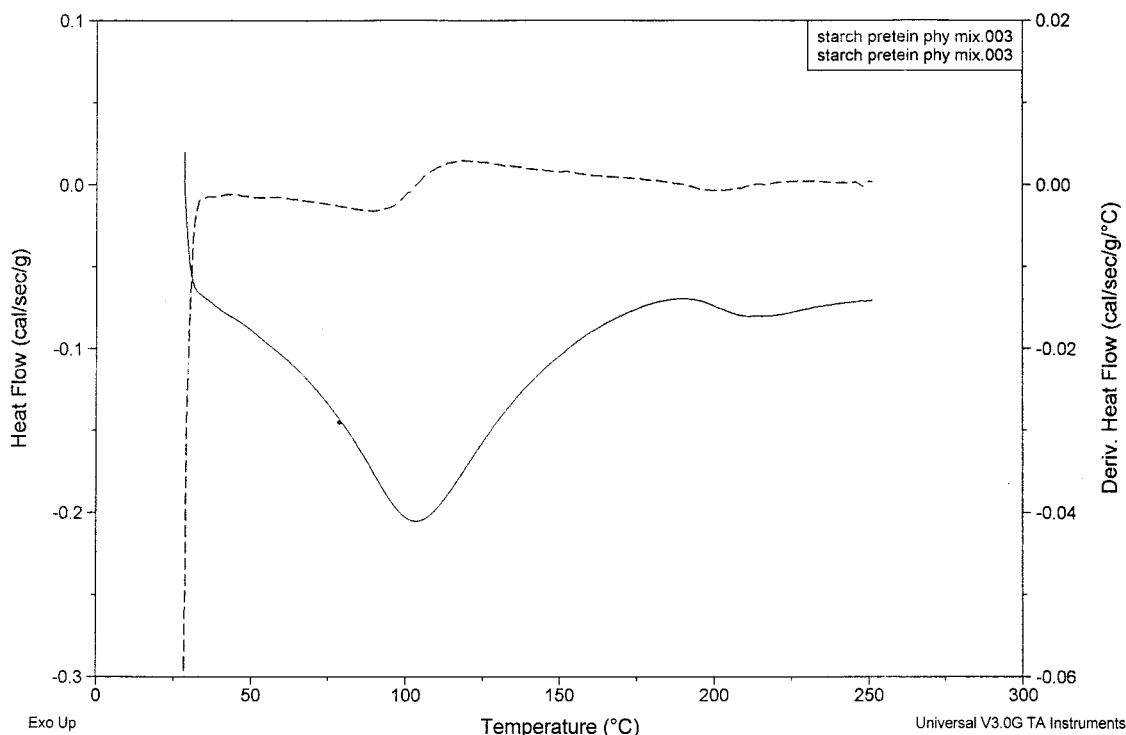


Figure 1 DSC thermogram of nonthermal blend of casein-starch with the (dashed line) corresponding first derivative showing a doublet and a broad melting endotherm.

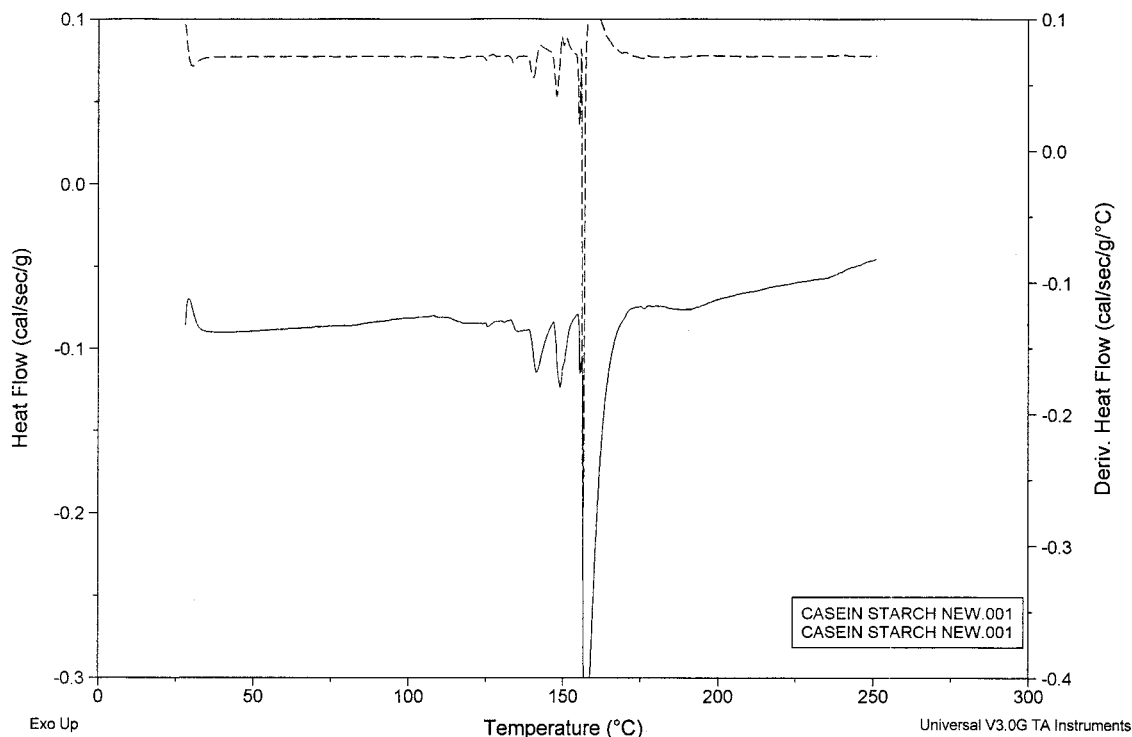


Figure 2 DSC thermogram of thermally blend of casein-starch with the (dashed line) corresponding first derivative showing a singlet and a sharp melting endotherm.

film integrity and was evaluated for all the properties except mechanical ones. Without adding a plasticizer, the film was hard and brittle. A plasticizer, namely,

propylene glycol, was necessary to impart flexibility to the films. All films dried at room temperature were relatively easy to peel off from the glass plate.

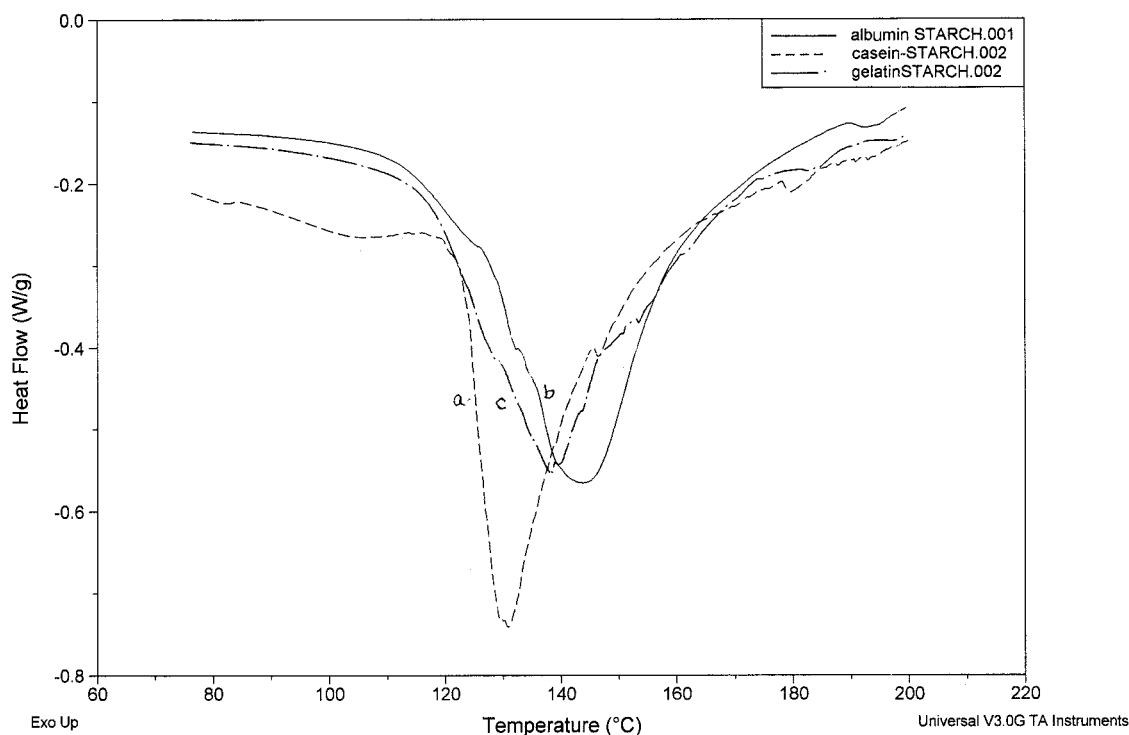


Figure 3 DSC thermogram of thermal blend of (a) casein, (b) albumin, and (c) gelatin.

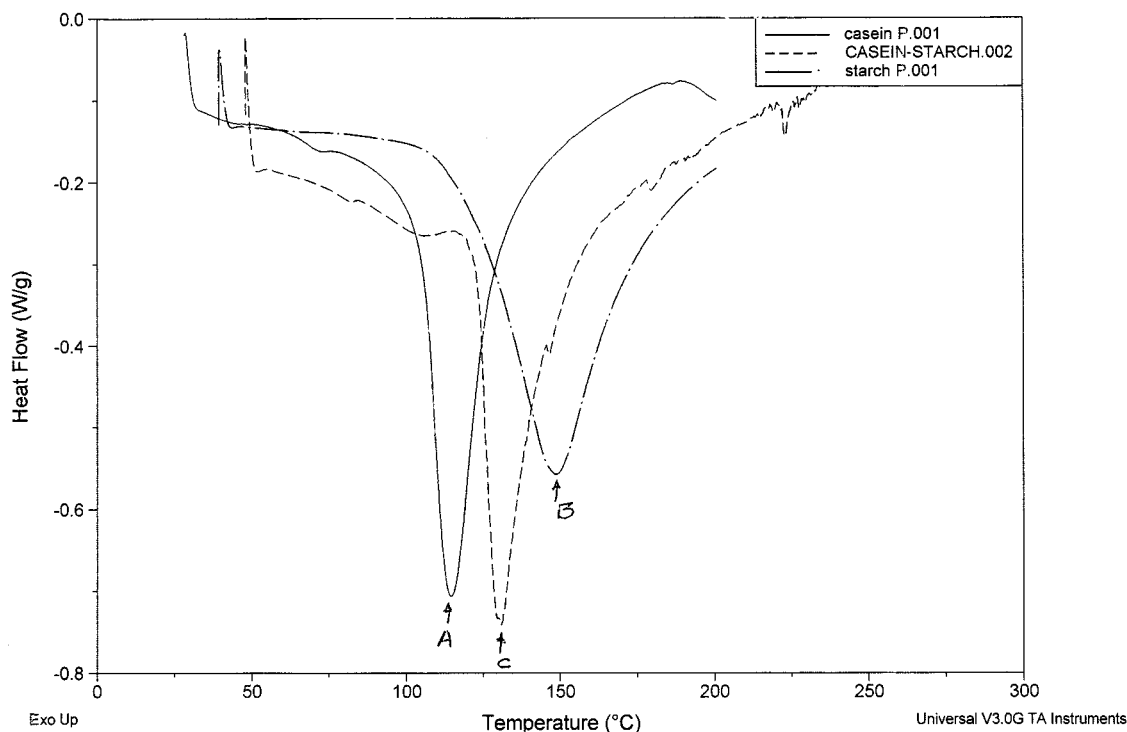


Figure 4 DSC of (A) starch, (B) casein, and (C) thermal blend of starch and casein showing molecular miscibility of the components.

Thermal analysis

DSC thermograms can be used to study the molecular mixing of a blend of starch and protein.¹¹ The non-thermal homogenized blends of protein and starch showed a broad thermogram spanning from 110 to 160°C and another transition at 180°C due to starch. This indicated that the molecular mixing of the components had not taken place (Fig. 1). On the other hand, intensive thermal blending of starch and protein resulted in molecular mixing since a single sharp phase transition was obtained in the DSC thermograms (Figs. 2 and 3). A typical binary blend of a biopolymer, namely, casein and starch, resulted in a sharp T_m intermediate between those of the parent polymers, indicating that they formed a true blend and followed the Fox equation (Fig. 4). Polymer chemists, in synthesizing a blend to specific T_g 's or T_m 's employ the Fox equation.¹⁷ An interesting thermogram was observed for a wax and casein blend. Intensive thermal blending of this resulted in a DSC thermogram showing two peaks. One of the peaks shifted away from the parent polymers, indicating that the blend had a multiphase with some intermixing of the components (Fig. 5). In this type of blend, the Fox equation cannot be applied. The T_m of the starch component shifted from 162.3 to 226.3°C, probably being a reaction product (Table I).

For film flexibility, a lower T_g is better.¹¹ It can be seen in Table I that the casein-based film had the

lowest T_g , indicating more flexibility as compared to the other protein films. On the other hand, the wax-based films showed double T_g 's and one of the T_g 's had shifted from 138.4 to 222.2°C, indicating hardness in the film. Enthalpy changes (ΔH) were determined by integrating the peak areas of the endotherm. Higher ΔH values were obtained with the casein/starch blend films as compared to the other protein films, that is, albumin and gelatin (Table I). The higher value of ΔH indicated that a more extensive network had formed between the starch and protein and this happens in semi-interpenetrating network polymer blends.¹⁸

Mechanical properties

The average tensile strength (TS) and percent elongation at break (% E) is given in Table II. The nonthermal blended and homogenized protein–starch film was fragile and it was difficult to measure its mechanical properties in the Instron as the film was broken while clamping. On the other hand, the intensely blended film showed satisfactory mechanical properties. When molecular mixing due to intense thermal blending takes place, there is an overall improvement in the mechanical properties; both the TS and % E improve, resulting in a toughened edible film. The average % E of the films ranged from 23 to 68, while the casein-based film showed a maximum % E. The effect of

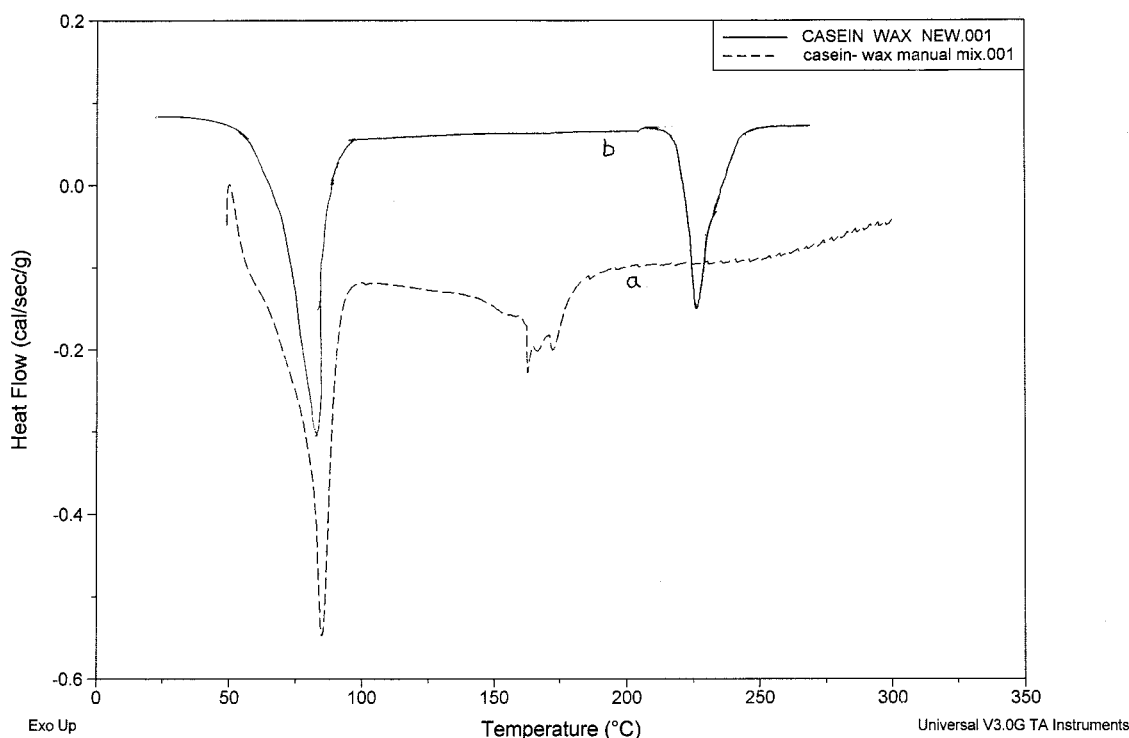


Figure 5 DSC thermogram of (a) nonthermal blend of casein-wax and (b) thermal blend of casein-wax.

intensive blending on the tensile properties of the films varied. Generally, the film having the highest TS showed the lowest % E. On the contrary, it is evident from Table I that intense blending resulted in a significant improvement in the toughness of the protein films, resulting in an increase in the % E and TS values. The casein-based film showed a TS of 40.8 kg/cm². While the wax-based counterpart showed the highest TS (42.3 kg/cm²), the % E, however, was the lowest (23.5%, Table II). This indicated that the wax-based film was harder compared to the other ones.

WVTR

The average thickness of the edible film used for WVTR determination was 200 microns with a stan-

dard deviation (SD) of 6.1 microns. WVTR values for a 200-micron thickness are given in Table II. The WVTR of the casein-starch thermal blend film was almost equal to that of the thermal wax-casein-based blend. Wax is hydrophobic in nature and wax-based film was expected to give a low WVTR. Accordingly, it showed the lowest WVTR, followed by casein-, gelatin-, and albumin-based thermally blended films in increasing order. It was interesting to note that casein-based film exhibited a WVTR almost in the same range as that of the wax film (Table II). This proves an important point, that intensive thermal mixing is more likely a possible crosslinking effect, transforming the hydrophilic-hydrophilic blend into one that acts like a more hydrophobic one. The intensive mixing of protein-starch resulted in a possible crosslinking effect and

TABLE I
Effect of Nonthermal and Thermal Blending on Phase-Transition Temperature and Enthalpy Associated with Melting of Different Blends

Edible composition	Glass transition temperature (°C) ^a		Melting temperature (°C) ^a		ΔH (J/g) ^a	
	Nonthermal	Thermal	Nonthermal	Thermal	Nonthermal	Thermal
Albumin-starch	56.0 ± 1.6	112.9 ± 3.1	87.5 ± 2.9	143.5 ± 3.9	195.6 ± 4.1	131.5 ± 3.5
	153.2 ± 4.1		156.1 ± 4.1		3.7 ± 0.01	
Gelatin-starch	58.3 ± 1.6	109.7 ± 3.0	89.2 ± 2.4	141.5 ± 3.9	194.4 ± 4.1	139.6 ± 3.6
	153.4 ± 4.2		156.3 ± 4.1		4.3 ± 0.01	
Casein-starch	59.7 ± 1.7	102.8 ± 3.0	90.3 ± 2.5	131.6 ± 3.9	195.5 ± 4.2	152.3 ± 4.1
	153.7 ± 4.2		156.4 ± 4.1		5.7 ± 0.01	
Casein-wax	56.8 ± 1.5	82.4 ± 1.9	85.0 ± 2.1	84.0 ± 2.1	143.3 ±	139.1
	138.4 ± 3.9	222.2 ± 4.5	162.3 ± 3.5	226.3 ± 4.5	39.4 ±	151.1

^a Mean + SD values.

TABLE II
Effect of Nonthermal and Thermal Blending on WVTR and Mechanical Properties on Different Blends

Edible film blend	WVTR g cm ⁻² day ^{-1a}		Mechanical properties ^a	
	Nonthermal	Thermal	% E	TS kg/sq cm
Gelatin–starch	1.08 ± 0.01	0.197 ± 0.004	29.3 ± 4.1	34.5 ± 4.4
Albumin–starch	0.938 ± 0.01	0.216 ± 0.005	42.3 ± 5.2	34.6 ± 4.1
Casein–starch	0.892 ± 0.01	0.168 ± 0.003	68.0 ± 6.8	40.8 ± 5.6
Casein–wax	0.416 ± 0.01	0.161 ± 0.003	23.5 ± 3.0	42.3 ± 5.5

^a Mean ± SD values

close packing of the components. There is also a possibility that a small amorphous portion of the starch and protein must have converted into a crystalline phase and this phase in the biopolymer is insoluble in water. The above two factors must account for the low value of the WVTR of the films (Table II). It can be seen from the table that there is almost a five- to sixfold decrease in the WVTR of the intensively blended film as compared to the nonthermal blended one.

RH

Table III shows the water gain due to the adsorption in the nonthermal and thermal blended films from different proteins in combination with starch and wax. Water gain in most of the intensively thermal blended films up to 80% RH was very small (below 1.0 %). However, at 100% RH, the percent gain of water in the film drastically increased (up to 5.0%). It was also noticed that albumin- and gelatin-based films were slightly tacky compared to casein–starch- and casein–wax-based ones. The nonthermal blended films showed two to three times more uptake of water compared to the thermally blended ones. The higher water gain and swelling of the film lowers the barrier efficiency. The films that had a higher water gain showed a higher WVTR (Tables II and III). Water gain was much lower in the wax-based film, which is hydrophobic in nature.

Correlation

The correlation coefficients among the five physical parameters, namely, (a) TS, (b) % E, (c) WVTR, (d) water gain (WG), and (e) ΔH of the thermal blending of starch and protein as well as wax–casein blend are shown in Table IV. While determining the correlation, the % E of the wax-based film was not included since the value was very low and did not fit into the pattern. The correlation coefficient in most of the cases was above 95%. The best correlation exists between DSC and the mechanical properties

CONCLUSIONS

Molecular mixing or intensive thermal blending properties of biopolymers, namely, protein and starch for edible film, can be studied by DSC, WVTR, water uptake, and the mechanical properties. When the blending of a component is complete, that is, leading to a single-phase transition (a single melting endotherm in the DSC), the film exhibits toughness as indicated by the mechanical properties, low WVTR, and water gain.. DSC is best suited for studying the compatibility or molecular mixing or blending between two components, and an endotherm shifted away from individual component as in the case of the wax–casein blend suggests a multiple phase. There was a good correlation between the five parameters studied. Blending phenomena in bilayer edible film can be studied by any one of the physical parameters

TABLE III
Percent Water Gain (WG) of Edible Film Exposed to Different RHs FOR 48 h

Blend	% WG (g) at different RHs ^a							
	40		60		80		100	
	Nonthermal	Thermal	Nonthermal	Thermal	Nonthermal	Thermal	Nonthermal	Intensive
Albumin–starch (AS)	2.21 ± 0.005	0.483 ± 0.01	5.2 ± 0.08	0.585 ± 0.01	9.1 ± 0.09	1.051 ± 0.05	10.1 ± 0.1	5.01 ± 0.08
Gelatin–starch (GS)	1.83 ± 0.050	0.412 ± 0.01	4.9 ± 0.08	0.488 ± 0.01	8.6 ± 0.09	0.786 ± 0.04	9.5 ± 0.09	4.895 ± 0.07
Casein–starch (CS)	1.53 ± 0.040	0.342 ± 0.01	4.3 ± 0.08	0.428 ± 0.01	6.1 ± 0.07	0.512 ± 0.04	6.9 ± 0.07	2.516 ± 0.06
Casein–wax (CW)	0.318 ± 0.010	0.285 ± 0.01	0.406 ± 0.02	0.323 ± 0.01	1.8 ± 0.03	0.42 ± 0.04	2.3 ± 0.04	1.598 ± 0.04

^a Mean ± SD values.

TABLE IV
Correlation Coefficient Between WVTR,
WG, TS, % E, and ΔH

	WG at 85 RH	TS	% E ^a	ΔH
WVTR	0.9854	0.9604	0.9880	0.9898
WG	—	0.9815	0.9631	0.9654
TS		—	0.9851	0.9951
% E			—	0.9748

^a % E of carnauba wax-based film data not included.

mentioned above; however, the authors feel that DSC is more convenient and less time-consuming. By intensive blending, it is possible to change the hydrophilic nature of the protein-starch blend to one that behaves like a more hydrophobic one with respect to the WVTR and water absorption.

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