

Research Article

Mechanical Strength, Solubility, and Functional Studies of Developed Composite Biopolymeric Film

Ankit Bishnoi, Narender Kumar Chandla , Gopika Talwar, Sunil Kumar Khatkar, and Amandeep Sharma

College of Dairy Science & Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Punjab, India

Correspondence should be addressed to Narender Kumar Chandla; chandla84@gmail.com

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The study evaluated the use of corn, quinoa, and tapioca starches with glycerol to develop biopolymer films. The water binding and oil binding capacities of the starches were determined, and tapioca starch was found to have good film-forming properties and produce transparent films. During the study, starches and glycerol with concentrations of 6% *w/v* and 3% *w/v* respectively, were used to develop the biopolymer films. These starches were evaluated for water binding and oil binding, with capacities that ranged from 182.62 to 199.60% and 159.02 to 193.33%, respectively. Quinoa starch presented the highest final viscosity (3584.00 cP), followed by corn starch (3058.00 cP) and tapioca starch (2547.00 cP), which indicate that these starches possess an intermediate range of viscosities required for the development of biopolymeric films. Comparative studies of the properties of starches were done with the intention of developing better quality films among corn, quinoa, and tapioca starch as base raw materials. Tapioca starch exhibits a good film-forming property and produces transparent films. Further, based on the evaluation of physical and mechanical properties, tapioca starch (6% *w/v*) with glycerol (3% *w/v*) was found suitable as a base ingredient to develop the composite biopolymeric films. Results showed that the functional properties of tapioca starch-based films were significantly enhanced after the incorporation of whey protein isolate (WPI). Particularly, the solubility of different films prepared from tapioca with WPI as the base material was measured at three different temperatures, i.e., 5, 25, and 50°C, and significant differences in solubility at different temperature values were observed. An increase in the tensile strength (TS) after the incorporation of WPI in tapioca starch is an indication of the establishment of a strong starch-protein matrix network in developed biopolymeric films. Also, TGA analysis was performed to determine the percentage degradation in weight of the film samples. The SEM micrographs of the optimized composite biopolymeric film showed a smooth surface without surface cracks.

1. Introduction

Packaging plays a critical role in ensuring the safety, quality, and convenience of food products throughout the entire supply chain. The use of appropriate packaging materials and techniques can help to minimize food waste, reduce environmental impact, and ensure the availability of safe and nutritious food for consumers. Packaging protects food from external abuse, namely, dust, water, pollutants, sunlight, and direct touch. Packaging is an essential component of food processing, as it provides a protective barrier between the food product and the external environment,

which helps to maintain the quality, safety, and shelf life of the product during storage, transportation, and distribution [1]. However, materials greatly contribute to the generation of plastic waste, thus creating serious disposal and ecological problems. Their disposal and incineration also cause soil, water, and environmental pollution. Therefore, these concerns must be addressed on a timely and hot topic with thoughtfulness by technologists and engineers to provide suitable substitutes for reinforced biopolymeric novel packaging materials for the future packaging industry. The development of composite biopolymeric films is an important area of research, as it has the potential to lead to the

production of sustainable and environmentally friendly packaging materials that can replace traditional petroleum-based plastics.

The biological macromolecules, such as starch, protein, cellulose, and chitosan, are used to substitute polymeric packaging materials (PE, polyethylene; PS, polystyrene; etc.) because of their ecofriendliness and nontoxicity. Although starch is the most significant polysaccharide polymer used to create biodegradable films, film has several drawbacks, including poor mechanical qualities and a high hydrophilic character when compared to traditional synthetic polymers, which makes it unsuitable for packing. Simultaneously, the biological macromolecule-based biopolymeric films also exhibited some challenges, especially those dominated by hydrophilic attributes [2]. Thus, there is a need to seriously entertain the highlighted challenges so that compatible substitutes of polymeric materials can be proposed with better engineering properties and enhanced material strength. Several researchers have worked to overcome the problem associated with biopolymeric films, employing certain modifications and hydrocolloids to achieve the desired mechanical, thermal, and surface finish properties [3], but with less emphasis on the hydrophilic properties (solubility and water vapor transmission rate) and their impact on surface and mechanical properties. Hence, improving the characteristics of native films by modifying raw materials is necessary to develop biopolymeric films with functional mechanical properties. Already existing studies focus on developing polymeric films and improving their functional properties to represent similar attributes as conventional synthetic biopolymeric materials, such as tensile strength, elongation at break, bursting strength, transparency value, and water/gas permeation resistance.

Starch polymers are one of the most important commercially available bio-based macromolecules, which are experimented with and explored, to develop conventional biopolymers. These polymers have high biodegradability and solubility rates, resulting in a negative impact on the global market for biodegradable polymers; therefore, there is a need to develop copolymers made of thermoplastic starch [4]. Thus, recent progress has been made at the industrial level in manufacturing bio-based composite polymers from native, modified starched polymers and with added additives and hydrocolloids; thus, this will contribute to the development of a sustainable bio-based economy of biopolymers. Amylopectin and linear amylose are the two main molecules that make up starch. Normal starch has about 25% amylose and 75% amylopectin. More than 90% of a recently created genetically modified starch is amylose [5]. Starch granules are extracted from various plant sources, and the size of starch can be as small as a submicron or as large as 100 μm . Wheat A granules have a diameter of 18 to 33 μm , while B granules have a diameter of 2 to 5 μm . Quinoa, corn, rice, amaranth, and tapioca starches have diameters that range from 1 to 3 μm , 5 to 20 μm , 3 to 8 μm , 5 to 35 μm , and 5 to 25 μm , respectively [6]. Various starches from different sources like cereals (corn, rice, wheat, oats, millets, etc.) and tubers (cassava, tapioca, yam, potato, etc.) were studied. It was reported that due to their abundant

resources, starch could be added at a lower cost to manufacture biopolymeric films [3]. The maize starch and cellulose acetate-based film had a lower water sorption capacity and a slower breakdown rate in an aqueous medium when compared to other starch films [7]. Tapioca starch-based edible film was prepared with different concentrations of chitosan; there was an increment in the tensile strength value of the edible films when chitosan was added compared to native starch film [8].

Corn, quinoa, and tapioca starches possess unique properties suitable for different food and nonfood applications. These natural biopolymers form biopolymeric films with good mechanical strength and gas barrier properties. Corn starch contains 25–30% amylose, which affects gelatinization and retrogradation, making it ideal for water-resistant films. Quinoa starch has smaller granules with a high water holding capacity, making it suitable for films having thermal stability. Tapioca starch has large granules, a low amylose content (15–20%), and high clarity and forms a gel quickly when heated, making it ideal for films having flexibility, water resistance, high tensile strength, and low elongation [9–13]. Therefore, starch-based films' properties vary based on the source of starch and processing conditions. While they have good barrier properties against oxygen and carbon dioxide, their solubility in water is high, requiring additives. Wheat starch films have good barrier properties against water vapor and oxygen and lower solubility in water than other starch-based films, despite having lower mechanical properties [12, 14].

The gelation is responsible for biodegradable film formation during the casting process; moderate drying occurs, which results in a three-dimensional network of polysaccharide double helices and a solid film after the solvent is evaporated [15]. Likewise, solvent casting, tape casting, injection moulding, extrusion processing, and compression moulding are used to manufacture polymeric films [16]. Generally, on a laboratory scale, the biopolymeric film production process is standardized using the "solvent casting" method, which consists of pouring the filmogenic solution onto plates (Teflon plates, Petri dishes, etc.), and then, the prepared films are stored in a particular temperature and humidity condition before evaluation. It is observed that the standardized process greatly affects the properties of developed biopolymer films; hence, the type of bio-based raw material (starch, protein, fiber, additives, etc.), formulation, and processing parameters (temperature of heating, time of blending, and estimation of gelation point) are important considerations during the manufacturing of biopolymer films. Protein network accommodates polysaccharide chains (starch), resulting in the formation of a continuous phase and dense matrix in composite films, which leads to improved barrier (WVTR) and mechanical (TS) properties; hence, two biomaterial sources (starch and WPI) are tried for the development of the films from diverse starch sources originated from cereal, pseudocereal, and tuber crops [17–19].

The objective of this study was to standardize the process for the development of composite biopolymeric films and to evaluate the various properties of the developed films. Therefore, in order to achieve this objective, the study

involved the optimisation of various processing parameters, such as the composition of the biopolymeric matrix, the type and concentration of reinforcing agents or additives, and the processing conditions, such as temperature and time. The mechanical and functional properties of the films would then be evaluated using various analytical techniques.

2. Materials and Methods

Corn starch used in the research study was purchased from HiMedia Laboratories Pvt. Ltd. Quinoa seeds were bought from Shri Ram Flour Mills, Ludhiana, and starch was extracted from quinoa seeds as described in Section 1. One tapioca starch is purchased from Istore Direct Trading Pvt. Ltd. Mumbai. WPI-90 (Whey Protein Isolate 90%), which was purchased from "AS-IT-IS Nutrition," Karnataka, India. Glycerol was purchased from HiMedia Laboratories Pvt. Ltd., India.

2.1. Isolation of Quinoa Starch. Starch was extracted from quinoa seeds using the existing methods given by researchers Jan et al. [20] with little modification. Quinoa seeds were thoroughly cleaned to remove all foreign material and dried in an oven at 40°C for 48 hours, then ground into flour using a lab-scale stone mill, and passed through a 100 mesh sieve (British Standard Size). Alkali (NaOH) solution was prepared at a 0.25% (wt./vol.) concentration. On the basis of preliminary experiments, the alkali solution to flour ratio was standardized at 1:5 and steeped at 48°C for 20 hours. Successive filtration was done with screens of 100, 200, and 400 mesh sizes (BSS), respectively. For 24 hours, the mixture was steeped with intermittent manual mixing. The slurry was centrifuged at 5500 rpm for 15 minutes; the supernatant was used for protein recovery, while the residue was collected and subjected to wet milling with added deionised water. The yellowish layer above the residue was carefully scraped off and discarded. The recovered starch cake was resuspended in water and centrifuged; this purification process was repeated four times. Purified starch was then dried in a hot air oven at 40°C for 6 h.

2.2. Physicochemical Properties of Starches

2.2.1. Amylose Content. Starch samples of 70 mg were mixed with 10 ml of urea and DMSO (dimethyl-sulphoxide) solution in 1:9 ratio. The mixed solution was heated for 10 min with continuous stirring for proper mixing and incubated at 100°C for 1 h and then cooled to room temperature. Addition of 0.5 ml mixed incubated sample solution was added to 25 ml of distilled water, along with 1 ml solution of iodine (I) and potassium iodide (KI). This 1 ml solution was made by addition of 2 mg iodine and 20 mg potassium iodide, and the volume was made up to 1 ml by distilled water. Blank sample was also prepared without addition of starch sample, and absorbance was taken at 635 nm [21].

$$\text{Blue value (\%)} = \frac{\text{absorbance} \times 100}{2 \times \text{gm of solution} \times \text{weight of sample}},$$

$$\text{Amylose content (\%)} = 28.414 \times \text{blue value.}$$

(1)

2.2.2. Swelling Power and Solubility. Swelling power (SP) and solubility of starches were performed by studying the methods of various authors [22, 23], at 95°C, and latter, a modified method was developed. The starch (1.0 g) suspension was heated in 25 ml of water with gentle stirring for the first 15 min, and the remaining 10 ml water was added thereafter. Briefly, a homogeneous mixture of starch (1.0 g, dry basis) in distilled water (35 ml) was heated in 80 ml centrifuge tube at 95°C for 30 min. Samples were then cooled in ice bath for 1 h and centrifuged (Model; C-24, BL; Remi Laboratory Ltd., Mumbai, India) at 12,500 rpm for 30 min. The suspended cloudy layer was poured through double-folded cheese cloth by gravitation for 2 min, and the soluble matter which passes on the cheese cloth (filtrate) was considered as supernatants while gel retained on filter cloth was collected back inside the tube as sediments. The weight of sediment was recorded for swelling power, and supernatant collected was poured in previously weighted Petri dish and dried in oven at 100°C for 3.5 h and weighted for the solubility determination.

The swelling power (SP, g/g, dry basis) and solubility (S, %) were calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{mass of dried solids}}{\text{weight of starch taken}} \times 100,$$

$$\text{Swelling power (g/g)} = \frac{\text{sediment weight (wet mass)}}{\text{sample weight of starch taken}}. \quad (2)$$

2.2.3. Water/Oil Binding Capacity (WBC/OBC). Water and oil binding capacities were determined by the method described by Chandla et al. [17]. 5 g starch was taken and dissolved in 75 ml distilled water and oil for water and oil binding capacities, respectively. The sample was agitated for 1 h and centrifuged at 3000 rpm for 10 min. The free water and oil recovered from the sentimental starch sample were removed, and tubes were drained for 10 min to separate out the surface water and oil. The water/oil binding capacity was calculated as follows:

$$\text{OBC (\%)} = \frac{\text{WBC}}{(\text{weight of sediments/weight of sample})} \times 100. \quad (3)$$

2.2.4. Color of Starches. The Hunter colorimeter was used to measure the color of starch samples in terms of "L" value (Hunter Associates Laboratory Inc., Reston, VA., USA). The following value depicts L* (lightness: 0 = black, 100 = white).

2.2.5. Pasting Properties by RVA. The pasting properties of the starch powder (3 g, 12.5% db) were determined by Rapid Visco Analyzer (RVA, Starch Master TM; Model: N17133; Newport Scientific Pvt. Ltd., Warriewood, Australia). The starch samples were programmed within RVA and hold at 50°C for 1 min, then heated to 95°C within 4 min and held at 95°C for 3 min, and then cooled to 50°C within 3 min

and hold at 50°C for 2 min. From the curve, pasting temperature and viscosity profile were obtained.

2.3. Development of Composite Biopolymeric Films. The preliminary trials were conducted to select a suitable source of starch. The starch concentration for the development of composite biopolymeric films is also standardized. As shown in Table 1. Three different commercial starches (corn, tapioca, and quinoa) and pseudocereal starch quinoa were chosen to develop biopolymer films with starch concentrations ranging from 1 to 8% and the addition of the plasticizer glycerol (3.0%). The complete process used to develop the film is the casting method, as described below.

Different types of starch sources (corn, quinoa, and tapioca) and plasticizer (glycerol) were taken by weight. To prepare the filmogenic solutions, different concentrations of three types of starches, namely, corn, quinoa, and tapioca, were taken and added to distilled water (200 ml quantity of water) and then thoroughly blended with the help of a magnetic stirrer. After ensuring complete mixing, the gelatinization of starch was carried out on a hot plate with magnetic stirring. This filmogenic mixture was heated until the gelatinization point was achieved. After gelatinization, glycerol was added to the filmogenic slurry, and these slurries were poured into the Petri dishes (diameter: 150mm). The films are developed using a casting technique and slurry spread over a levelled surface manually. Filmogenic slurry dispersed in Petri plates was aged for 5 minutes to “escape air bubbles” from the film-forming solution. Petri dishes carrying the filmogenic solutions were transferred to a hot air oven for drying purposes. The temperature-time combination is used for the convective drying of filmogenic solution at 50°C for 18–24 hours. Evaporation of filmogenic solutions is carried out in a hot air oven by heating the films at a controlled temperature for a specific time; thus, the mechanical and functional properties of the formed film are not affected by accelerated and inconsistent heating. After drying, films were peeled off of Petri dishes and kept under airtight conditions for the further evaluation of the properties of prepared biopolymeric films.

Based on evaluation of the properties of the biopolymeric films developed, tapioca starch (6%) was selected as a base ingredient along with 3 percent glycerol. The selected base material (tapioca starch) was further formulated by adding the WPI at various concentrations (0.25, 0.50, 1.0, and 2.0%). To check the effect of plasticizer on the properties of films, glycerol was also varied at different concentrations, i.e., 2.0, 2.5, 3.0, 3.5, and 4.0. Based on the mechanical and functional characteristics, glycerol at a concentration of 3.5% was selected. Tapioca starch was screened as the best starch source, and the process was standardized after investigating the mechanical and other functional properties. The tapioca starch (6%), WPI (0.50%), and glycerol (3.5%) formulation was finally standardized to develop the biopolymeric films.

2.4. Properties of Biopolymeric Films. The analysis of properties helps provide a comprehensive understanding of the performance of the developed biopolymeric films and their potential for various applications. The developed films were analyzed for moisture content, thickness, opacity, solubility,

water vapor transmission rate, color, mechanical strength (tensile, elongation at break, and puncture strength), thermal analysis, and scanning electron microscopy.

2.4.1. Thickness. A digital micrometer (Mitutoyo 2046F) with a range of 0-1" was used to measure the thickness of the composite biopolymeric film. All film specimens were conditioned for 48 hours in a humidity test environment at 50% relative humidity. The average of three thickness measurements made at various locations on each film sample was used for the calculations.

2.4.2. Moisture Content. Moisture content (MC) of the biopolymeric film was analyzed by the AOAC [24] standard method. Each piece of film (1.0 g) was dried for approximately 6 hours at 120°C, and drying took place till the weight became constant. The average of three samples in each scenario is taken for statistical analysis. The following formula was used to calculate the moisture content's percentage:

$$\text{Moisture content (\%)} = \frac{(M_i - M_f)}{M_i} \times 100 \quad (4)$$

where M_i is the initial mass of the wet biopolymeric film sample and M_f is the final mass of the dried biopolymeric film sample.

2.4.3. Solubility. The solubility of composite biopolymeric film sample in water was determined using the standard method given by Romero-Bastida et al. [25]. Stripes of composite biopolymeric film measuring 20 mm by 20 mm were cut from the films ($n = 3$). The original dry weight of the film sample was calculated by drying them for 24 hours at temperature of 105°C. After the first weighing, the samples were immersed in a flask containing 80 ml of distilled water at 25°C for 1 hour and stirred slowly. After that, the samples were removed and dried at 60°C until they reached a constant weight. The percentage loss of weight in an hour is used to calculate weight loss. The solubility was determined using the following equation:

$$\text{Solubility (\%)} = \frac{W_i - W_f}{W_i} \times 100, \quad (5)$$

where W_i is the initial weight of dried sample and W_f is the final weight.

2.4.4. Opacity. Opacity analysis was done by using spectrophotometer. Using standard method documented by Ren et al. [26], opacity value was measured. Film sample was cut down into pieces of rectangular shape; after that, the cut piece was put into cuvette, in such a way that it covered the width and length of the cuvette. The blank cuvette was taken as reference. The opacity was calculated using following formula:

$$\text{Opacity} = \frac{\text{Abs}_{600}}{X}, \quad (6)$$

TABLE 1: Properties of biopolymeric film developed from starches (corn, quinoa, and tapioca, 6% w/v) with 3% w/v glycerol concentration.

Starch type	Thickness (mm)	Moisture content (%)	Solubility (%)		Opacity	Color <i>L</i>	Tensile strength (kgf/cm)	Elongation at break (%)
			5°C	25°C				
CS	0.458 ± 0.06 ^b	15.17 ± 0.74 ^a	27.12 ± 0.34 ^b	32.83 ± 0.28 ^b	1.47 ± 0.31 ^a	27.84 ± 0.09 ^a	1.112 ± 0.47 ^c	1.853 ± 0.46 ^c
QS	0.469 ± 0.21 ^a	15.08 ± 0.15 ^b	27.36 ± 0.56 ^a	33.74 ± 0.55 ^a	1.36 ± 0.29 ^b	26.64 ± 0.16 ^b	1.176 ± 0.64 ^b	2.330 ± 0.39 ^b
TS	0.428 ± 0.13 ^c	15.06 ± 0.06 ^b	26.73 ± 0.72 ^c	32.56 ± 0.43 ^b	1.25 ± 0.22 ^c	26.50 ± 0.25 ^c	1.380 ± 0.58 ^a	2.460 ± 0.28 ^a

CS: corn starch; QS: quinoa starch; TS: tapioca starch. Results are expressed as mean value ± standard deviation of three determinations. Means in column with different superscript letters differ significantly ($p < 0.05$).

where, Abs_{600} is the absorbance at 600 nm and X is the thickness of respective sample.

2.4.5. Color. The Hunter colorimeter was used to measure the color of biopolymeric film samples in terms of “ L ” value (Hunter Associates Laboratory Inc., Reston, VA., USA). Before taking sample measurements, the equipment was standardized using white and black tiles.

2.4.6. Tensile Strength and Elongation at Break. Using a texture analyzer TA.XT2i and the ASTM [27] reference technique, the tensile strength and elongation at break of a biodegradable film were evaluated. Biopolymeric film samples were cut into “2 mm × 5 mm” long film strips, which were then inserted between the grips of the texture analyzer. The speed was set at 5.0 mm/s, and the beginning grip separation was 50 mm. Using the Texture Expert tool, the tensile strength and elongation at break were computed directly from the stress-strain curves.

2.4.7. Water Vapor Transmission Rate. An altered technique was used to measure the transmission of water vapor [28]. A beaker filled with 30 ml of distilled water was placed on top of the sealed film. After that, a desiccator containing prehydrated silica gel was used to keep the test cell dry. For these measurements, silica gel was dried for three hours at 180°C. The entire assembly was maintained at 25°C, and after being stored for 24 hours, the test cell’s weight loss was measured. The formula below was used to calculate the water vapor transmission rate (WVTR):

$$WVTR = \frac{\Delta m}{\Delta t \times A}, \quad (7)$$

where $\Delta m/\Delta t$ is the moisture absorbed per unit time and A is the surface area of biopolymeric films.

2.4.8. Puncture Strength. Puncture tests were conducted using the same equipment, the texture analyzer TA-XT2i, to determine the puncture strength (N). Samples with a 30 mm diameter were affixed to the equipment’s plate through a 20 mm diameter hole (3M Scotch, Brazil). A cylindrical probe with a diameter of 5 mm was used, and the sample was pushed perpendicular to the biopolymeric film samples’ surface at a constant rate of 1 mm/s until it penetrates the film. Curves of force deformation were recorded. At the rupture site, force and deformation were measured. For each test, three samples were examined.

2.4.9. Thermal Properties by TGA. Thermal testing was done using a thermogravimetric analyzer (TGA): Make: Perkin Elmer (Model-TGA 4000). The composite biopolymeric film samples were heated from 30 to 500°C at a rate of 10°C/min with a nitrogen gas flow of 30 ml/min using 5 to 7 mg of each biopolymeric film sample. The several phases of deterioration were identified, and the weight losses of the materials were calculated on the dry basis. Analyses were done three times to ensure reproducibility.

2.4.10. Scanning Electron Microscopy (SEM). The external surface of the biodegradable film was examined using scanning electron microscopy. Films were broken and suspended in solvent prior to observation, after which biopolymeric was mounted on an aluminum stub using double-backed cellophane tape and biopolymeric films coated with gold palladium (60: 40 w/w) in an automatic fine coater (Make: JEOL, Smart Coater). The surface morphological characteristics of the films were absorbed at a moisture content of 5-6%. The analysis of the film samples were done at various magnifications. All film samples were stored in a desiccator filled with silica gel before examination (0% RH).

2.5. Biodegradability. The composite biopolymeric sample’s biodegradability was assessed using a standard technique with a little modification described by Shafik et al. [29]. Two-by-two-centimeter film samples were buried eight centimeters deep in a soil-filled container. The pot was kept in the lab, and water was sprayed into the soil at regular intervals to keep it moist. By carefully removing the sample from the soil, the weights of the film samples were periodically taken. A brush was used to gently clear the sample’s surface. To calculate the biodegradation rate, the weight loss % method of the film samples over time was used.

2.6. Statistical Analysis. At least three replicates of the data were used to calculate the average. The data was statistically analyzed using the SPSS 17.0 programme. The values in different tables are expressed as mean value ± standard, and the experimental data was evaluated using a two-way analysis of variance (ANOVA).

3. Results and Discussion

The present study is performed to investigate the process standardization for the development of composite biopolymeric films. Initially, different starches were analyzed for the physicochemical properties. The research work encompasses study of mechanical and functional properties of developed films, and films were analyzed for surface finish/defects by morphological examination; however, thermogravimetric analysis is also done to analyze the rate of degradation of biopolymeric films. The results collected during these investigations are presented and discussed, in this chapter.

3.1. Evaluation of Physicochemical Properties of Starches

3.1.1. Amylose Content. Amylose content of different starches is shown in Table 2. It was found that amylose content was observed lesser for quinoa (12.13%) and is followed by corn starch (16.03%) and tapioca starch (17.00%) [30]. The amylose content of the starch is varied due to climatic conditions, botanical origin, and harvesting periods [31]. Amylose content of quinoa and tapioca starch is consistent with the amylose content values reported by Jan et al. [20].

3.1.2. Swelling Power (SP) and Solubility. Swelling power (SP) and solubility of corn, quinoa, and tapioca starches are shown in Table 2. Quinoa starch presented higher SP than corn and tapioca starches. SP of starches is primarily

TABLE 2: Physicochemical and viscosity profiles of starches.

Starch source	Amylose content (%)	Swelling power (g/g) (90°C)	Solubility (%) (90°C)	Water binding capacity (%)	Oil binding capacity (%)	Color L value	PT (°C)	PV (cP)	HV (cP)	FV (cP)
CS	16.03 ± 0.02 ^a	10.40 ± 0.05 ^c	36.47 ± 0.06 ^b	199.60 ± 0.92 ^a	193.33 ± 0.76 ^a	91.70 ± 0.98 ^c	77.55 ± 0.41 ^a	3616.00 ± 0.52 ^b	1616.00 ± 0.31 ^c	3058.00 ± 0.21 ^b
QS	12.13 ± 0.03 ^c	12.53 ± 0.03 ^a	38.50 ± 0.10 ^a	199.23 ± 0.35 ^b	159.02 ± 0.45 ^c	97.87 ± 0.64 ^a	73.35 ± 0.40 ^b	3389.00 ± 0.26 ^c	3135.00 ± 0.11 ^a	3584.00 ± 0.22 ^a
TIS	17.00 ± 0.01 ^b	11.98 ± 0.07 ^b	32.60 ± 0.20 ^c	182.62 ± 0.78 ^c	163.26 ± 2.03 ^b	92.23 ± 0.32 ^b	71.05 ± 0.61 ^c	4870.00 ± 0.33 ^a	1924.00 ± 0.21 ^b	2547.00 ± 0.55 ^c

CS: corn starch; QS: quinoa starch; TIS: tapioca starch; PT: pasting temperature; PV: peak viscosity; HV: holding viscosity; FV: final viscosity. Results are expressed as mean value ± standard deviation of three determinations. Means in column with different superscript letters differ significantly ($p < 0.05$).

due to their amylopectin content whereas the amylose content of the starch acts either as inhibitor or diluent [32]. Higher SP of quinoa starch may be due to more amount of amylopectin in quinoa starch which assists in enhancing the swelling power. Higher SP results of quinoa starch are in agreement with Jan et al. [20]. Lesser SP of corn starch (10.40 g/g) may be due to stronger binding within the corn starch granules [33]. Overall, the differences in SP between quinoa starch and corn starch may be attributed to differences in their molecular structure and composition. These differences can impact the functional properties of the starches and their suitability for various applications.

3.1.3. Binding Capacity and Color Value. Water binding and oil binding capacities of corn, quinoa, and tapioca starches were ranged from 182.62 to 199.60% and 159.02 to 193.33%, respectively, as shown in Table 2. Water binding capacity of starch granule is the capacity to absorb water. Starch granule of corn, quinoa, and tapioca is composed majorly of amylopectin content which helps in holding the water and oil within the starch molecules. Corn starch has highest water binding capacity which may be due to the high amylose content and chemical composition. Also, high amylose corn starch- (HACS-) based films were produced at low temperatures leading to a decrease in gelatinization temperature [34]. Higher oil binding capacity (OBC) is presented by starches, and it could be due to binding of hydrocarbon side chains of oil with the starches [35]. During sprouting of sorghum grain, it is observed that protein denaturation occurs which makes the availability of lipophilic proteins on the surface. This eventually increased the protein's ability to bind with oil and thus enhanced the oil binding capacity of hydrocarbon side chains of oil. This is in agreement with the findings of a previous study by Elkhalfa and Bernhardt [36]. The hydrophobic amino acids present in the protein structure can interact with the hydrophobic molecules of oil, resulting in an increase in oil binding capacity. In addition, the amylose content and accessibility of oil binding sites in starches have been found to significantly affect oil binding capacity. This is supported by the findings of Singh et al. [37], who reported that the oil binding capacity of starches was influenced by their amylose content and the accessibility of oil binding sites. Smaller starch granules can have a larger surface area per unit weight than larger granules, which can provide more active sites for oil binding. Additionally, the polygonal geometrical shape of starch granules can result in a greater surface area and more exposed surface area for interaction with oil molecules. These factors can increase the number of available binding sites on the starch granules and facilitate stronger binding between the starch and oil. This can ultimately result in higher oil binding capacities of starches with smaller granule sizes and polygonal shapes. Starches showed significant differences (Table 2) in color *L* values ranging from 91.70 to 97.87. More amount of amylose content present on the starch granule is chiefly responsible for opaque appearance as of increase in viscosity and thus reduce the transmittance through edible films; however, clearer viscoelastic and firm gel formation in quinoa starch gel may be due to more amy-

lopectin content. Similar findings states that the lightness of pea flour containing noodles decreased significantly as compared with control samples due to the amount of protein content present in the flour. Quinoa starch may have a lighter color due to factors such as its lower protein content and higher amylopectin content. The amylopectin molecules in starches tend to form more flexible and less compact structures than amylose, which can result in a lighter color due to reduced light scattering. Other factors, such as the size and shape of the starch granules, can also affect the color of starches. Hence, quinoa starch had shown higher lightness values than corn and tapioca starches [38, 39].

3.1.4. Pasting Properties. Pasting properties are important characteristics of starches that provide information about their gelatinization and pasting behavior when subjected to different temperatures. All starches presented increase in the viscosity with the increase in temperature range. Change in viscosity may be due to the given heat moisture treatment to the starches [40]. Peak viscosity is regarded as maximum viscosity gained by starch granule to swell up before it ruptures. The peak viscosity can be influenced by factors such as the amylose content, granule size and shape, and processing conditions. Starches with higher amylose content tend to have higher peak viscosities due to their ability to form more rigid structures during gelatinization. Quinoa starch presented highest final viscosity (3584.00 cP), followed by corn (3058.00 cP) and tapioca (2547.00) which indicates that these viscosity values lie within intermediate range of viscosities which are required for the development of biopolymeric films. Highest peak viscosity (PV) was observed in tapioca (4870.00 cP), followed by corn starch (3616.00 cP) and quinoa starch (3389.00 cP) as shown in Table 2. Similar results showed that cassava starch observed high peak viscosity than the corn starch [41]. This might be because more inflated granules and less free water are occupying the same space at greater starch concentrations, increasing viscosity [42]. The pasting temperature (PT) of all these starches ranged from 71.05 to 77.55°C which indicates that tapioca starch starts the formation of paste at lesser temperature than the corn and quinoa starches. Similar finding of lower pasting temperature was observed in different concentrations of starches [43]. The pasting profiles of different starches varied from those reported in Jan et al. [20] and Kong et al. [22].

3.2. Process Standardization for the Development of Biopolymeric Film

3.2.1. Selection of Suitable Starch Source. Corn starch, quinoa starch, and tapioca starch were experimented to develop the biopolymeric films. Different trials for screening of starch sources were taken, and observations were recorded.

Three different starches were taken, and their concentration varied from 1.0 to 8.0% (*w/v*) to see the possibility of formation of films. Starches (corn, quinoa, and tapioca) and glycerol with 6% *w/v* concentration and 3% *w/v* concentration, respectively, were used to develop the biopolymer films as shown in Figure 1. Three of the starches at 6% presented better formation of biopolymeric film with desired

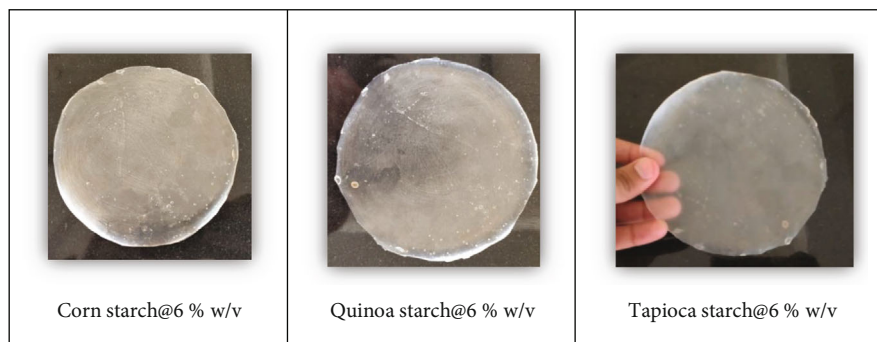


FIGURE 1: Screening of starch source to develop the films with glycerol at 3% *w/v* concentration.

visual appearance, hence chosen to be suitable for the film formation; moreover, the same amount of starch percentage can be better compared to check the strength and functional properties of the developed films. These films found to be were easy to peel off without getting torn off. It was observed that biopolymeric films with lesser concentration of starch, i.e., 1, 2, and 3% *w/v*, were difficult to peel off and mostly stuck to Petri dishes. Also, films with higher concentration, i.e., 7 and 8% *w/v*, presented high thickness which affected the formation, handling, flexibility, and overall appearance of films. Hence, comparative studies of the properties were done to finalize better quality films among corn, quinoa, and tapioca starches. Three different starches at 6% with 3% glycerol were found suitable for the formation of biopolymeric films with desired visual appearance; however, all films were easy to peel off. Table 1 presents different physical and mechanical properties of the earlier selected starch-based films.

3.3. Quality Evaluation of Biopolymeric Films

3.3.1. Thickness and Moisture Content. The thickness of films made from three different starches is shown in Table 1. Thickness values ranged from 0.428 mm to 0.469 mm. Results showed that film made with three different starches have minor differences but presented higher thickness values compared to lesser concentration of starch. This may be due to the increase in starch solid concentration [44]. Similar findings also states that biopolymeric films made with different source of starches have increased in thickness with the increase in solid content, and after drying the film suspension, there is increased residual mass left behind which is responsible for the thickness [45].

The moisture content of films made from three different starches is shown in Table 1 and ranged from 15.06 to 15.17%. Results showed that the film made from tapioca starch has lowest moisture content compared to film made with corn and quinoa starches because tapioca starch has lower amylose content compared to the remaining two starches [46]. The moisture content is the parameter that relates to the amount of water molecules occupying the empty volume of the microstructure of film network [47]. Moisture content of starch films should range from 14.50 to 34.39% [48]. The difference in moisture content of different starch films is affected due to the differences in amylose content, water binding capacity, and film-forming components [49].

3.3.2. Solubility. Solubility is influenced by temperature because as the temperature increases, the kinetic energy of the molecules in the filmogenic solution also increases. This increase in energy causes the starch molecules to become less tightly bound to one another and more readily soluble in water. As a result, the solubility of the film increases with increasing temperature [50]. The solubility of films made from three different starches is shown in Table 1. The solubility of different films was measured at three different temperatures, i.e., 5, 25, and 50°C. Solubility of corn starch, quinoa starch, and tapioca starch films was observed in range from 26.73 to 39.45% at temperature of 5, 25, and 50°C, respectively. Corn starch films presented the solubility values, i.e., 27.12, 32.83, and 37.53% at temperature of 5, 25, and 50°C, respectively. This indicates that with the increase in the temperature, the solubility of the films prepared from corn starch increased and similar trends of increase in solubility were also observed in quinoa and tapioca starch-based films. However, film made with tapioca starch has shown significantly lesser solubility values (26.73, 32.56, and 36.79 at 5, 25, and 50°C, respectively) among other starch-based biopolymer films which may be due to strong intermolecular interaction of tapioca starch water suspensions with plasticizer (Q. [51]).

3.3.3. Opacity and Color (*L*). Opacity and color (*L* value) are important parameters for assessing the quality and suitability of biopolymeric films for various food packaging applications. Opacity refers to the degree to which a material obscures light, while *L* value is a measure of lightness or darkness of a color. The observations are presented in Table 1. Opacity value and color (*L*) value ranged from 1.25 to 1.47 and 26.50 to 27.84, respectively. Results showed that film made with tapioca starch has lesser opacity (1.25) and color *L* values (26.50) than the biopolymer films made with corn and quinoa starches. Lesser opacity value indicates the higher transparency of the biopolymeric films [52]. Also, films of corn and quinoa starch comparatively have higher thickness value than tapioca starch. It can be concluded that higher thickness values result to higher color values; similar finding are also reported by Galdeano et al. [53]. These findings suggest that different starches can be used to produce biopolymeric films with varying opacity and color properties, which can be tailored to specific food packaging applications.

3.3.4. Mechanical Properties (Tensile Strength and Elongation at Break). The mechanical properties of biodegradable films can vary depending on a variety of factors, such as the type and source of biopolymer, processing methods, and testing conditions. As a result, it is important to carefully control these variables to obtain reliable and accurate measurements of film strength and elasticity. Standardized testing procedures, such as those recommended by the American Society for Testing and Materials (ASTM), can help to minimize variability and ensure consistent results [54]. The tensile strength of films made from three different starches is shown in Table 1. The tensile strength measured ranged from 1.112 to 1.380 kgf/cm. Results showed that tensile strength of tapioca starch films has highest value (1.380 kgf/cm) compared to corn starch films (1.112 kgf/cm) which is followed by tensile strength values (1.176 kgf/cm) of quinoa starch films due to the strong adhesion between the fiber and matrix, contributing to its chemical affinity ([55]: [56]).

Elongation at break is a measure of the ability of a film to stretch before breaking, which indicates its flexibility and elasticity. It is an important mechanical property to consider when evaluating the performance of biopolymer films in different applications, such as packaging or coating. A higher elongation at break indicates a more flexible and stretchable film, which may be desirable in certain applications where the film needs to conform to the shape of the product or withstand deformation without breaking. The elongation at break values of three different starch-based films is shown in Table 1. Elongation at break value of all starch-based films ranged from 1.853 to 2.460%. It is also depends upon the amount of plasticizer used with the starch [25]. Tapioca starch and glycerol-based biopolymeric films showed higher elongation at break. This may be due to the amount of amylose content present in starch that can significantly change the elastic properties of biodegradable films. Similar finding states that higher molecular weight of amylose showed higher elongation at break in potato starch-based biodegradable films [57].

Tapioca starch exhibits a good film-forming property and produces transparent films [58]. Based on this evaluation of physical and mechanical properties, tapioca starch (6% *w/v*) with glycerol (3% *w/v*) was found suitable as a base ingredient to develop the composite biopolymeric films.

3.3.5. Whey Protein Isolate (WPI) Biopolymeric Films. Starch and protein have complementary properties that can improve the mechanical and functional properties of biopolymeric films. Starch provides film-forming properties, while proteins offer better tensile strength, flexibility, and water resistance. The interaction between these biopolymers leads to the formation of a more complex network that can enhance the barrier properties of the films [59, 60]. Thus, tapioca starch with WPI was tried for the development of composite biopolymeric films.

3.4. Quality Evaluation Biopolymeric Films

3.4.1. Thickness and Moisture Content. The thickness of films made from varied WPI concentration is shown in Table 3 and ranged from 0.462 mm to 0.629 mm. Results

showed that thickness of biopolymeric films increased with the increase in concentration of WPI. This is because cross-linking of starch strengthens the internal structures of granules, and in particular, the addition of WPI provided higher molar volume of starch granules, which results in increased thickness of biopolymeric films [61].

The moisture content of films made from varied WPI concentration is shown in Table 3, and values ranged from 14.43 to 15.74%. The combination of starch and proteins resulted in moisture content reduction of the composite biopolymeric films [47]. The moisture content of biopolymeric films decreased with increased WPI concentration; this may be due to the difference in moisture content of biopolymeric films rationalized by the differences in the film-forming components, and similar finding was also reported by Sukhija et al. [19].

3.4.2. Solubility. The solubility of films made from varied WPI concentration is shown in Table 3. The solubility of different films was measured at three different temperature ranges, viz., 5, 25, and 50°C. Result showed that solubility of WPI decreased with increased concentration because heat denaturation of WPI occurs during film preparation which leads to the creation of a stronger intermolecular interaction that may lead to decrease in solubility [62]. The results are in agreement with earlier findings that increase in protein concentration leads to decrease in solubility resulting from interaction among free hydroxyl groups of starch and free sulfhydryl (SH) groups of protein as well as formation of hydrogen [63].

3.4.3. Opacity and Color (L). Opacity was also studied of biopolymeric films with varied WPI concentration, and tapioca starch-based WPI film had the lowest opacity (1.59) as shown in Table 3. Results showed that opacity values increased with the increase in WPI concentration which may be due to the change in polyelectrolyte interactions between proteins and starch [59]. Increased concentration of WPI with polymers results in high opacity [64].

Color (*L*) value was also studied of biopolymeric films with varied WPI concentration, and observations are presented in Table 3. Color (*L*) value ranged from 26.50 to 33.38 for the developed tapioca-WPI biopolymer films. Color value (*L*) is decreased with increased concentration of protein content (WPI) which indicates that darkness in film color is increased. This is because pH change and Maillard reaction occurred between starch and protein during heat processing of filmogenic solution to gel transformation which leads to dark yellowish color of films [51, 65].

3.4.4. Mechanical Properties (Tensile Strength and Elongation at Break). To evaluate the mechanical properties of films, tensile strength was measured. The tensile strength of films made from tapioca starch and WPI varied as shown in Table 3 and was ranged from 1.409 to 5.473 kgf/cm. Addition of WPI in tapioca starch resulted in increase in tensile strength which may be due to protein and polysaccharide complexes: formed to have more effective functional properties than proteins and polysaccharides offered individually

TABLE 3: Selection of WPI concentration as a second base ingredient.

WPI variation (% w/v)	Thickness (mm)	Moisture content (%)	5°C	Solubility (%) 25°C	50°C	Opacity	Color L value	Tensile strength (kgf/cm)	Elongation at break (%)
0.25	0.462 ± 0.58 ^d	15.76 ± 0.14 ^a	26.80 ± 0.34 ^a	33.20 ± 0.45 ^a	35.20 ± 0.64 ^a	1.59 ± 0.43 ^d	33.38 ± 0.36 ^a	1.409 ± 0.84 ^d	2.370 ± 0.19 ^a
0.50	0.484 ± 0.64 ^c	15.14 ± 0.25 ^b	26.50 ± 0.47 ^b	32.10 ± 0.71 ^b	34.85 ± 0.57 ^b	1.62 ± 0.68 ^c	31.78 ± 0.45 ^b	1.960 ± 0.97 ^c	2.470 ± 0.28 ^b
1.0	0.564 ± 0.73 ^b	14.43 ± 0.09 ^d	24.75 ± 0.26 ^c	30.42 ± 0.38 ^c	32.97 ± 0.38 ^c	1.72 ± 0.78 ^b	28.48 ± 0.61 ^c	1.981 ± 0.78 ^b	1.220 ± 0.32 ^d
2.0	0.629 ± 0.61 ^a	14.51 ± 0.17 ^c	22.50 ± 0.68 ^d	27.50 ± 0.58 ^d	31.05 ± 0.19 ^d	1.97 ± 0.56 ^a	26.50 ± 0.29 ^d	5.473 ± 0.66 ^a	0.260 ± 0.67 ^c

Results are expressed as mean value ± standard deviation of three determinations. Means in column with different superscript letters differ significantly ($p < 0.05$).

[66]. Similar findings were reported by Jumaidin et al. that protein with starch composite-based biopolymeric films increased the tensile strength. Also, increasing protein concentration/quantity resulted in more protein-protein interactions and thus leads to high tensile strength of the composite biopolymer films [65, 67]. Elongation at break of varied WPI concentration-based biopolymeric films is shown in Table 3, and it ranged from 0.260 to 2.470%. Low level of plasticizer results to increase in tensile strength but reduces the elongation at break of biopolymeric films [63]. Plasticizers are used to increase the flexibility and elongation of biopolymeric films, but at the same time, they may cause a reduction in the degree of cross-linking of the polymer network. This can result in a decrease in the elongation at break of the films [68].

Based on the above evaluation of film formation at varied concentration to the base ingredients, i.e., tapioca starch 6% and WPI 0.50% *w/v* were standardized to develop the composite biopolymeric films.

3.5. Standardization of Plasticizer (Glycerol). Glycerol is a commonly used plasticizer in the development of biopolymeric films due to its low cost, nontoxicity, and compatibility with a wide range of biopolymers. It helps to increase the flexibility and elasticity of films by reducing intermolecular forces between polymer chains, allowing them to move more freely and slide past each other. This results in films that are more flexible and less brittle, with improved mechanical properties such as elongation at break. Hence, glycerol was varied at different concentration, i.e., 2.0, 2.5, 3.0, 3.5, and 4.0% *w/v*.

3.5.1. Thickness and Moisture Content. Thickness and moisture content of biopolymeric films made by varying the concentration of “glycerol” are shown in Table 4. The thickness of different films ranged from 0.418 mm to 0.587 mm. Results showed that thickness of biopolymeric films was increased by increasing the concentration of glycerol, and it can be due to the fact that when plasticizer was added in the concentrated solution, a large amount of dry matter (starch+protein) leads to increase in thickness of films [69, 70]. Similar finding was reported by Lagos et al. that the increased thickness of biopolymeric films was observed when there was an incorporation of glycerol [71].

The moisture content of biopolymeric films made with varied glycerol concentration ranged from 15.14 to 16.00%. Plasticizer addition, in particular glycerol, has a great influence on moisture of starch-based film due to its hygroscopic nature [72]. Results showed that moisture content of films made by varying concentration of plasticizer (glycerol) was increased because hydrophilic nature of it helps in the retention of water in the film matrix and at higher concentration leads to the adsorption of water molecules. Also, increased thickness of films leads to increase in moisture content of biopolymeric films [19, 73].

3.5.2. Solubility. The solubility of biopolymeric films made with varied glycerol concentration is shown in Table 4. The solubility of biopolymeric films made with varied gly-

cerol concentration was measured at 5, 25, and 50°C. Solubility presented incremental trends with increase in concentration of glycerol (4.0%) and elevation in temperature (5, 25, and 50°C). This may be because increased concentration of glycerol is able to increase the solubility of the film due to their hydrophilic properties; therefore, glycerol interacts strongly with water and easily incorporates into a network of hydrogen bonds [74]. Similar trend of the effect of plasticizer concentration on the solubility of biopolymers in water was reported in various studies [75–77].

3.5.3. Opacity and Color (*L*). The opacity values of biopolymeric films made with varied glycerol concentration are presented in Table 4. Lower relative opacity values were observed which indicate that the films are more transparent. In the case of the films obtained from varied glycerol concentration, the opacity values increased from 1.43 to 1.85, and it may be because increased concentration of glycerol with dilution of proteins reduced the opacity of biopolymeric films [78]. Similar finding stated that film based on protein and increased glycerol concentration leads to reduction in opacity [79]. Color attributes are important because they are directly related to consumer acceptance. The color (*L*) values of biopolymeric films made with varied glycerol concentration samples are presented in Table 4. Films with glycerol at 2.0% *w/v* have lower *L* values and at 4.0% *w/v* have the highest; this is because the increase in glycerol level enhanced the light reflection on the biopolymer film surface, thus producing an increment in *L* values [80].

3.5.4. Mechanical Properties (Tensile Strength and Elongation at Break). The tensile strength of biopolymeric films made by varying concentration of glycerol shown in Table 4 ranges from 1.887 to 1.961 kgf/cm. The higher the concentration of the plasticizers, the lesser the tensile strength of developed films was observed. The changes in mechanical properties of biopolymeric film attributed by the plasticizers character of weakening the intermolecular forces between the chains of adjacent macromolecules thus increase the free volume and subsequently cause a reduction of mechanical strength [81]. Therefore, the increase in the glycerol concentration causes a diminution of the tensile strength which is due to the decrease in the intermolecular interactions between the different molecules of composite materials of biopolymeric films.

The elongation at break of films is made by varying concentration of glycerol shown in Table 4. The elongation at break of different films ranged from 0.047 to 2.022%. Results showed that glycerol at 3.5% *w/v* has higher percentage of elongation at break. Increase in elongation value with increasing the thickness of films was reported which may be due to the increase in glycerol concentration [82]. It may also be due to that WPI act like as an additive and might have contributed to some plasticization effect along with glycerol in biopolymeric films, thus resulting in an increase in elongation at break. Similar finding were also reported by Muscat et al. [83] that increase in plasticizer concentration results in an increase in the elongation.

TABLE 4: Selection of glycerol concentration as a plasticizer.

Glycerol variation (% w/v)	Thickness (mm)	Moisture content (%)	Solubility (%)		Opacity	Color L	Tensile strength (kgf/cm)	Elongation at break (%)	
			5°C	25°C					50°C
2.0	0.418 ± 0.78 ^a	15.50 ± 0.12 ^a	24.50 ± 0.12 ^a	28.41 ± 0.36 ^a	34.91 ± 0.49 ^a	1.43 ± 0.37 ^a	28.38 ± 0.45 ^a	1.887 ± 0.10 ^a	0.047 ± 0.09 ^a
2.5	0.477 ± 0.64 ^b	15.59 ± 0.08 ^b	24.89 ± 0.34 ^b	29.67 ± 0.42 ^b	35.04 ± 0.37 ^b	1.56 ± 0.48 ^b	29.96 ± 0.28 ^b	1.890 ± 0.34 ^b	1.201 ± 0.43 ^b
3.0	0.482 ± 0.59 ^c	15.78 ± 0.04 ^c	26.50 ± 0.28 ^c	32.30 ± 0.12 ^c	35.85 ± 0.65 ^c	1.62 ± 0.29 ^c	31.78 ± 0.17 ^c	1.911 ± 0.48 ^c	2.470 ± 0.28 ^c
3.5	0.486 ± 0.97 ^d	15.89 ± 0.17 ^d	26.69 ± 0.43 ^d	32.11 ± 0.65 ^d	36.00 ± 0.54 ^d	1.77 ± 0.53 ^d	31.89 ± 0.67 ^d	1.950 ± 0.75 ^d	2.604 ± 0.10 ^d
4.0	0.587 ± 0.88 ^e	16.00 ± 0.32 ^e	27.10 ± 0.34 ^e	33.40 ± 0.28 ^e	36.97 ± 0.83 ^e	1.85 ± 0.61 ^e	32.03 ± 0.53 ^e	1.961 ± 0.34 ^e	2.731 ± 0.03 ^e

Results are expressed as mean value ± standard deviation of three determinations. Means in column with different superscript letters differ significantly ($p < 0.05$).

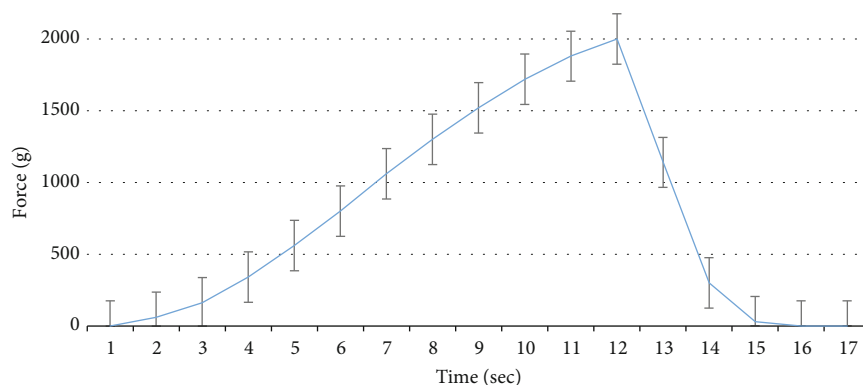


FIGURE 2: Puncture strength analysis of developed composite biopolymeric film.

Incorporation of glycerol at 3.5% *w/v* greatly improved the mechanical property of biopolymeric films. The glycerol not only plasticized the film but also affected the change of crystal structure during heating and blending of filmogenic solutions, which leads to effecting the engineering and other functional properties of the films. Glycerol at 3.5% conc. has shown considerable improvement in the film properties [84].

Finally, tapioca starch was screened as best starch source, and the biopolymeric film production process was standardized at concentrations of tapioca starch (6%), WPI (0.50%), and glycerol (3.5%) The above standardized biopolymeric film was finalized and further checked for puncture strength, thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) as shown below.

3.5.5. Water Vapor Transmission Rate. The barrier properties of composite biopolymeric films, particularly water vapor permeability (WVP), have become extremely important in protecting packaged foods from environmental conditions [85]. WVTR regulates how easily moisture passes through the film [86]. One of the most important characteristics for composite biopolymeric films is that they have as little WVTR as possible, preventing moisture transfer between the local environment and the food. Water vapor transmission rate was observed $0.96 \pm 0.019 \text{ g/m}^2/\text{hr}$. WVP of hydrophilic films increases with film thickness [87]. The reasons for this variability can range from a change in structure because of film thickness, or through swelling of hydrophobic films which can alter the films structure. Because of nonlinear water sorption isotherms of biopolymer films, modeling the water transport through films is extremely complex [88]. Polymeric films typically have water vapor transmission rates (WVTR) in the $0.1\text{--}100 \text{ g m}^{-2}/\text{day}$ range [89] which is usually sufficient for food packaging but not organic electronic applications.

3.6. Puncture Strength. Increasing starch content can increase the density and thickness of the film, leading to higher puncture strength. In addition, the addition of plasticizer can affect the puncture strength, with higher plasticizer content leading to lower puncture force as the film becomes more flexible and easier to penetrate [2]. The presence of other components such as proteins and lipids can also affect the puncture strength, as they can contribute to the overall structure and

mechanical properties of the film. Gontard et al. [90] showed that puncture force of the films was improved as starch concentration increased. Puncture deformation was exclusively affected by the glycerol concentration. Sobral et al. [81] observed that puncture strength values of gelatin film were augmented with increasing the amount of plasticizer content. Puncture strength was $2005.46 \pm 0.35 \text{ gf}$ of standardized composite biopolymeric films as shown in Figure 2. The matrix of the film becomes less dense with glycerol addition, and under stress, movement of polymer chains is facilitated. The similar trend was reported by other authors [91, 92]. This behavior is probably due to the plasticizing effect of glycerol as it combines easily with several polymers increasing the mechanical properties of the composites [93]. Adding glycerol increased the thickness, causing an increase of the burst strength values of biopolymeric films.

3.7. Thermogravimetric Analysis (TGA). In this study, thermogravimetric analysis (TGA) was used to determine the thermal decomposition or degradation and stability of standardized film. The results of thermal degradation for standardized film films was presented in plot curves of weight loss (%) and derivative weight loss of the films as a function of temperature ($^{\circ}\text{C}$) as shown in Figure 3. The weight loss occurred in three main stages. The first stage, from 30 to 250°C , is attributed to the evaporation of water and molecules with molecular weight. The mixture of polymers did not influence this stage. The second stage, around $250\text{--}350^{\circ}\text{C}$, can be attributed to the thermal decomposition of the components present in the films. Protein breakdown starts at around 225°C [94, 95], while the decomposition of starch occurs at $230\text{--}326^{\circ}\text{C}$ [96]. In the third stage, above 350°C , degradation of carbonaceous residues formed during the second stage occurs, with complete oxidation of these materials [94, 97]. When compared to initial weight of film sample before testing, this result showed an increase in weight reduction. It is observed that the biopolymeric film loosed 19.80% weight in 8.288 min. The thermal stability of composite biopolymeric films improved because starch and WPI have an improved binding with film-forming components, thus making them more difficult to evaporate during drying [98].

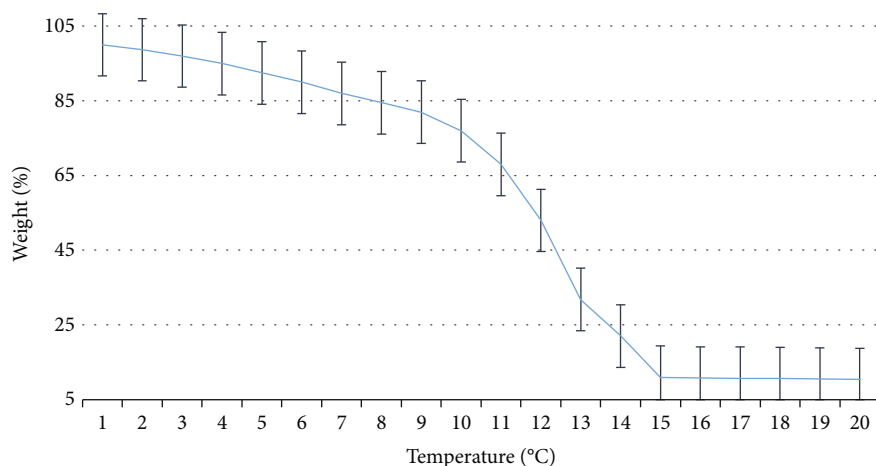


FIGURE 3: Thermogravimetric analysis (TGA) of developed composite biopolymeric film.

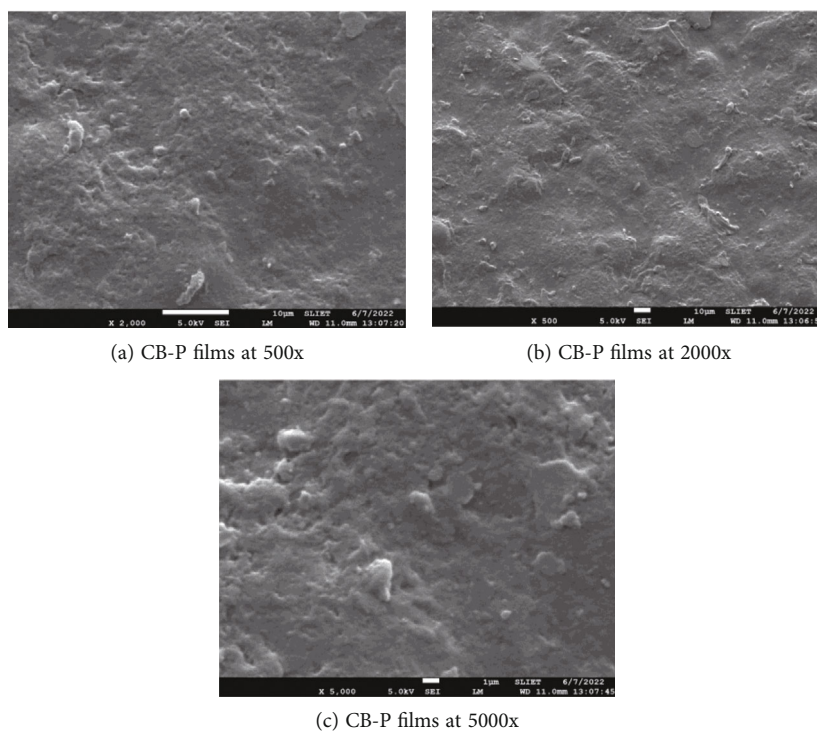


FIGURE 4: Scanning electron microscopy (SEM) images of composite biopolymeric (CB-P) films at different magnifications.

3.8. Scanning Electron Microscopy (SEM). Figure 4 shows the SEM micrographs of standardized composite biopolymeric film presenting a smooth surface without surface cracks and with the presence of small clusters. With the increase in starch concentration, the microstructure of the films became more irregular with greater presence of material accumulation. This behavior can be attributed to the interactions between starch and whey. During interaction, whey protein adheres to the starch granules which results in clumping and aggregation of molecules together [99]. Another hypothesis is that the denaturation of proteins during heating resulted in their aggregation and formation of agglomerates in the films [100]. Similar results have been reported by Huntrakul et al. [101] in starch films with iso-

lated pea protein. The presence of discontinuities and agglomerates in the film matrix can affect its properties [51] as seen in the properties evaluated in this work.

3.9. Biodegradability. Examining the behavior of soil biodegradation is critical for utilizing biopolymeric films in the present environment. Soil biodegradation is the measure of decomposition of biopolymeric materials caused by the action of soil microorganisms (bacteria, fungus, and another organism). The percentage degradation in weight of the sample was observed very less, i.e., 14.28%; biopolymeric film biodegradation took place in 45 days which is the indication of strong network established by starch and protein together, and hence, reinforced films were developed after

standardization of process and ingredients. It was found that the standardized sample took more time to degrade. Previous studies state that the good protein distribution in the biopolymeric film matrix affected migration of extrinsic components (moisture, microorganism, etc.) into the matrix formed of biopolymeric films [102, 103].

4. Conclusion

Biodegradable films' strength and solubility issues can be addressed by incorporating starch and protein and standardizing the film making process. A standardized biopolymeric film was developed using tapioca starch (6.0%), WPI (0.50%), and glycerol (3.5%) with improved engineering properties such as tensile strength (1.950 ± 0.75 kgf/cm), elongation at break ($2.604 \pm 0.10\%$), puncture strength (2005.46 ± 0.35 gf), and thermal gravimetric investigation. Moreover, the studies of films at different temperatures also recommend food product usage at varied storage conditions. Results of the analysis could be used to guide future research and development efforts in this area, as well as to inform the development of new sustainable and environmentally friendly packaging materials. The ban on single-use plastics and their hazardous effects present an important opportunity to explore the commercial development of biopolymeric films. By developing new materials and technologies and improving the properties and performance of biopolymeric films, we can create sustainable and environmentally friendly packaging solutions that can help to reduce the environmental impact of plastic waste.

Data Availability

Data is given in the submitted manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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