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Effects of Carboxymethyl Cellulose and Glycerol on the Physical Properties of Soy Protein -Based Bioplastics

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Abstract

Biodegradable plastic is a food packaging that is widely developed today because of its properties that can be degraded by soil biota. In this study, biodegradable plastics based on soy protein obtained from solid waste of the tofu industry were developed. This study was conducted to see the effect of variations of Carboxymethyl Cellulose (CMC) and Glycerol on the physical characteristics of plastic materials made from soy protein. CMC with concentrations of 1, 1.5, and 2 % (w/w) and glycerol 0, 30, 50, 70% were mixed with soy protein with a concentration of 10% and formed a film by the casting method. The results showed that the addition of CMC and glycerol had an effect on the moisture content and solubility of the resulting plastic. Based on all the physical characteristics tested (moisture content, solubility, thickness, heat resistance and biodegradability tests), the best treatment was obtained at the treatment of CMC concentration of 1.5% and glycerol 30%.

1. Introduction

Plastic has become an integral part of modern food packaging due to its versatility, durability, and relatively low production cost. However, the widespread use of conventional plastic packaging has raised serious environmental concerns because most petroleum-based plastics are resistant to degradation by soil microorganisms and can persist in the environment for long periods. To minimize the environmental impact associated with plastic waste, increasing attention has been directed toward the development of biodegradable plastics that can be naturally decomposed after disposal.

Biodegradable plastics can be produced from renewable natural resources derived from plants, animals, and microorganisms. Nevertheless, the use of plant-based materials presents another important challenge, as many commonly used biopolymer sources, particularly starch, are obtained from staple food crops such as rice, cassava, corn, and sago. The utilization of these food resources for plastic production may create competition between food and non-food applications, potentially affecting food availability and sustainability. Therefore, it is necessary to explore alternative biodegradable plastic raw materials derived from non-food

resources, particularly agricultural by-products, processing residues, and other renewable biomass that do not compete directly with human food supplies [1], [2].

Soybeans are a food ingredient that plays an important role as a source of vegetable protein. Soybeans are generally processed into tempeh, tofu, and soy milk. From the tofu industry, solid waste is produced in the form of tofu pulp that is rich in protein. Tofu pulp contains about 26.6% protein, so this tofu pulp can still be used to make soy protein that can be used as raw materials for making bioplastics [3]. Soy protein has a good capacity to form a plastic film, but this raw material has weaknesses in terms of mechanical properties and sensitivity to moisture [4], [5]. Therefore, in this study, a natural material was used that can improve the characteristics of the plastic film produced from soy protein, where one of the conditions that must be met is that the material must be safe to use and can be applied to food.

Carboxymethyl Cellulose (CMC) is a cellulose-derived compound that is widely used as a food stabilizer, cosmetic and pharmaceutical. This material has degradable properties, is non-toxic, easily binds to other natural materials and is hydrophilic. CMC has also been widely used as an edible film [6], [7]. In addition to CMC, other materials such as glycerol are added to increase the flexibility of the resulting plastic. Glycerol has a small molecular weight and is hydrophilic so it can easily interact with protein-based material components [8], [9]. This study aimed to see the effect of the addition of CMC and Glycerol on the physical characteristics of the plastic from soy protein.

The novelty of this study lies in four aspects. First, the protein matrix is recovered from the solid residue of the tofu industry, an agro-industrial by-product, instead of from starch extracted from staple food crops such as rice, cassava, corn or sago; the film is therefore produced without competing with the human food supply, and a waste stream that is normally discarded or used as low-value feed is valorised. Second, carboxymethyl cellulose and glycerol are varied simultaneously in a complete factorial arrangement of three CMC levels (1, 1.5 and 2% w/w) and four glycerol levels (0, 30, 50 and 70%) at a fixed soy protein concentration of 10%, giving twelve formulations, so the interaction between the reinforcing hydrocolloid and the plasticiser can be observed rather than the effect of each additive examined separately. Third, functional properties and end-of-life behaviour are evaluated on the same set of films, since moisture content, water solubility, thickness and heat resistance are measured together with biodegradability under soil burial; studies on protein-based films more commonly report either the physical performance of the film or its degradation, but not both for identical formulations. Fourth, the combined evaluation identifies an interior optimum at 1.5% CMC and 30% glycerol and shows that the effect of the plasticiser is not monotonic, because glycerol concentrations above this level increase chain mobility to the point where flexibility is gained at the expense of network cohesion and water resistance. The study also states the resulting limitation explicitly, namely that the water sensitivity of the films restricts their use to dry or low-moisture foods, so that the reported optimum is bounded by the application for which it is valid.

2. Research Methodology

2.1 Preparation of raw materials

In this study, soy protein was prepared from soybean pulp using an acid precipitation method. Soybean pulp flour was initially mixed with 2 N HCl solution at a solid-to-liquid ratio of 1:2, and the pH was adjusted to 4.5. The suspension was thoroughly stirred and allowed to stand for 4 h to promote protein precipitation. Subsequently, the mixture was centrifuged at 3,500 rpm for 30 min to separate the solid and liquid fractions.

The recovered fraction was then treated with 0.5 N NaOH, and the pH was adjusted to 6–8 to facilitate further separation of the protein from residual impurities. The mixture was centrifuged again to obtain a protein fraction with improved purity. The resulting soy protein granules were subsequently dried and ground into a fine powder. For biodegradable film preparation, 10 g of the protein powder was dispersed in 100 mL of solution and used as the primary film-forming material.

2.2 Preparation of Biodegradable Plastic Films

Biodegradable films composed of soy protein (SP), carboxymethyl cellulose (CMC), and glycerol were prepared using the casting and solvent evaporation method. The biodegradable plastic films were prepared using SP:CMC ratios of 10:1, 10:1.5, and 10:2 (w/w) with glycerol concentrations of 0, 30, 50, and 70%. First, the SP solution was prepared by dispersing an amount of soy protein in 100 mL of distilled water at room temperature (25°C) for 30 min under continuous magnetic stirring. The pH was adjusted to 10 using 2.0 M NaOH. Separately, CMC solution was prepared by dissolving CMC in 20 mL of distilled water and heating the mixture at 50 °C for 30 min until a homogeneous solution was obtained. The SP and CMC solutions were then combined and continuously stirred at 80 °C. Glycerol was gradually added to the mixture at the predetermined concentration and served as a plasticizer. Stirring was continued until a homogeneous film-forming solution was obtained. The resulting solution was subsequently poured onto Teflon mold to produce films with a uniform thickness. The films were dried at 50 °C for 6 h in an oven. After drying, the films were conditioned at room temperature for 24 h. The dried films were then carefully peeled from the mold surfaces and stored in sealed plastic containers prior to characterization. The resulting biodegradable plastic films were cut into the required dimensions according to the specifications of each subsequent characterization test.

2.3 Sample testing

2.3.1 Determination of Protein Content

Protein content was determined using the Kjeldahl method, which consisted of three main stages: digestion, distillation, and titration. In the digestion stage, 1 g of the sample was placed into a digestion tube, followed by the addition of 7 g of catalyst and 25 mL of concentrated H_2SO_4. The tube was then placed in a digestion apparatus and heated for approximately 1–1.5 h. Digestion was continued until a clear greenish solution was obtained, indicating complete decomposition of the organic matter. The resulting digest was subsequently allowed to cool for approximately 30 min. During the distillation stage, a small amount of distilled water was carefully added along the wall of the digestion flask while the mixture was gently agitated to dissolve any crystals formed during cooling. The digest was then quantitatively transferred into a 100 mL volumetric flask and diluted with distilled water to the calibration mark. An aliquot of 25 mL was transferred into a round-bottom distillation flask, followed by the addition of 50 mL of 30% NaOH solution and three drops of phenolphthalein indicator.

The mixture was then diluted with distilled water as required. A 250 mL Erlenmeyer flask containing 100 mL of H_3BO_3 solution and 2–3 drops of mixed methyl red and methylene blue indicator was positioned at the outlet of the condenser, with the condenser tip immersed below the surface of the boric acid solution. Distillation was continued until approximately half of the initial volume of the sample solution had been collected as distillate. In the titration stage, the collected distillate was titrated with standardized 0.1 N HCl solution until the endpoint was reached, as indicated by a colour change to pink. The nitrogen content obtained

from the titration was subsequently converted to protein content using the appropriate conversion factor (for soy =5.75). Protein content was calculated using the following equation:

$$\%\text{Nitrogen} = \frac{\text{ml titrant x 0.1 N HCl x 14}}{\text{mg sample}}\ x\ 100\%\ x\ \text{dilution factor}$$

$$\%\ \text{Protein} = \%\ \text{Nitrogen x conversion factor}$$

2.3.2 Moisture Content

Moisture content was determined using the oven-drying method. Empty moisture dishes were first dried in an oven at 105 °C for 30 min, cooled in a desiccator, and weighed to obtain their constant empty mass. A known amount of sample was then placed in each dish and weighed. The samples were subsequently dried in an oven at 105 °C for 6 h or until a constant weight was achieved. After drying, the dishes were removed from the oven, cooled in a desiccator to room temperature, and reweighed. The moisture content of the samples was calculated using the following equation:

$$\text{Moisture Content (\%,w/w)} = \frac{\text{weight loss (g)}}{\text{sample dry weight (g)}} \times 100\%$$

2.3.3 Film Thickness Test

The thickness of the film was measured using a micrometre with an accuracy of 0.01 mm. The measurement was carried out at four different places in each sample. The result was the average of the four testing points.

2.3.4 Soluble Power Test

Film solubility in water was determined by cutting the samples into 50 × 50 mm pieces. The film specimens were initially dried at 60 °C for 12 h and subsequently weighed with a precision of ±0.01 g. The measured mass was recorded as the initial dry weight (Wo). Each dried film sample was then immersed in 200 mL of distilled water contained in a 250 mL beaker. The beaker was covered and maintained at 25 °C for the predetermined immersion period. After immersion, the remaining undissolved film was carefully removed from the water and dried in a vacuum oven at 60 °C for 12 h until a constant weight was obtained. The mass of the dried residual film was recorded as the final dry weight (Wi). The percentage of material dissolved in water, expressed as film solubility (S), was calculated based on the difference between the initial and final dry weights using the following equation:

$$\text{S (\%)} = \frac{\text{Wo}-\text{Wi}}{\text{Wo}} \times 100\%$$

Film solubility was evaluated for each formulation at immersion times of 1, 3, and 6 h using the procedure described above.

2.3.5 Biodegradability Test

The biodegradability of the film was evaluated using a soil burial method with soil collected from a landfill site. Each film sample was cut into 3 × 3 cm pieces and buried at a depth of 10 cm. Film degradation was initially monitored over the first four days, and observations were subsequently continued until the samples had completely decomposed.

2.3.6 Heat resistance test

The plastic film samples were cut into 3 × 3 cm pieces and placed in an oven initially maintained at 40 °C. The oven temperature was subsequently increased in increments of 20 °C at 5-minute intervals. Changes in the physical characteristics of the films were systematically observed and recorded throughout the heating process until complete combustion occurred.

3. Results and Discussion

In this study, soy protein (SP) recovered from tofu processing by-products was employed as the principal biopolymeric material for the fabrication of biodegradable plastic films. Protein extraction and concentration were performed using an acidification-based separation technique, followed by quantitative determination of protein content using the Kjeldahl method. The analytical results demonstrated an increase in protein content from 20.4% in the untreated tofu residue to 31.73% in the recovered protein, confirming the effectiveness of the extraction process in enhancing protein concentration.

3.1. Moisture content analysis

The moisture content analysis revealed that increasing glycerol concentration resulted in higher moisture content in soybean protein (SP)-based biodegradable plastic films (Figure 1). This behaviour can be attributed to the hygroscopic nature of glycerol, which contains three hydroxyl (–OH) groups capable of forming hydrogen bonds with water molecules and polar functional groups within the polymer matrix [10]. The incorporation of glycerol increases the availability of hydrophilic sites, thereby enhancing water absorption and retention. Furthermore, glycerol acts as a plasticizer by modifying intermolecular hydrogen bonding between protein chains, reducing intermolecular cohesion, and increasing polymer chain mobility. These molecular interactions promote structural flexibility and facilitate moisture penetration into the film matrix, contributing to the observed increase in moisture content. Additionally, absorbed moisture may function as a secondary plasticizer, further increasing film flexibility and extensibility. Therefore, the relationship between glycerol concentration and moisture content reflects a balance between improved flexibility and reduced structural resistance to moisture absorption [11].

In contrast, the addition of carboxymethyl cellulose (CMC) did not significantly affect the moisture content of the biodegradable films. Although CMC contains hydrophilic hydroxyl and carboxymethyl groups, its interactions with SP may contribute to the formation of a more cohesive polymer network through

intermolecular hydrogen bonding [12]. These interactions may restrict polymer chain mobility, enhance structural integrity, and reduce the accessibility of certain hydrophilic sites to water molecules. Consequently, the hydrophilic contribution of CMC may be counterbalanced by its reinforcing effect on the protein-based matrix. The combined incorporation of glycerol and CMC therefore introduces competing molecular mechanisms, in which glycerol promotes flexibility and moisture retention, whereas CMC may contribute to polymer-network stabilization and mechanical reinforcement.

Moisture content is an important indicator of the water-retention characteristics and potential packaging performance of biodegradable films, although it does not directly represent water vapor permeability. Higher glycerol concentrations may increase water sorption and facilitate moisture diffusion through the polymer matrix, potentially reducing moisture barrier performance[13]. Elevated moisture content may also promote film swelling, dimensional instability, and deterioration of mechanical properties during storage. In food packaging applications, increased moisture transfer may adversely affect product quality by inducing undesirable textural changes, such as softening, and potentially reducing shelf life. Therefore, optimizing the glycerol and CMC concentrations is essential to achieve an appropriate balance between flexibility, structural integrity, moisture resistance, and the functional performance of SP-based biodegradable films.

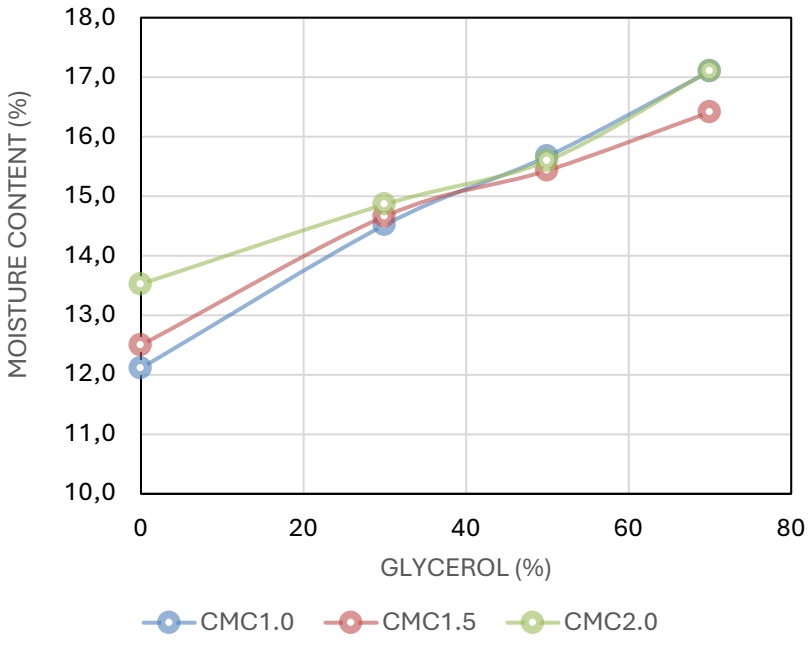


FIGURE 1. Moisture content of biodegradable films formulated with varying concentrations of CMC (1.0, 1.5, and 2.0%) and glycerol (0, 30, 50, and 70%).

3.2. Film thickness analysis

Film thickness is a critical physical characteristic influencing the barrier, mechanical, and functional properties of biodegradable packaging materials. Generally, increasing film thickness can enhance resistance to gas and water vapor transmission by extending the diffusion pathway, although permeability also depends

on the molecular structure and composition of the polymer matrix. Furthermore, film thickness significantly influences mechanical strength, flexibility, and structural integrity. The experimental results demonstrated that films formulated with lower concentrations of carboxymethyl cellulose (CMC) and glycerol exhibited relatively low thickness values (0.2–0.3 mm) and were characterized by greater rigidity and stickiness. In contrast, films containing higher concentrations of CMC and glycerol showed increased thickness (0.4–0.5 mm) and improved elasticity, although they were more susceptible to tearing (Figure 2).

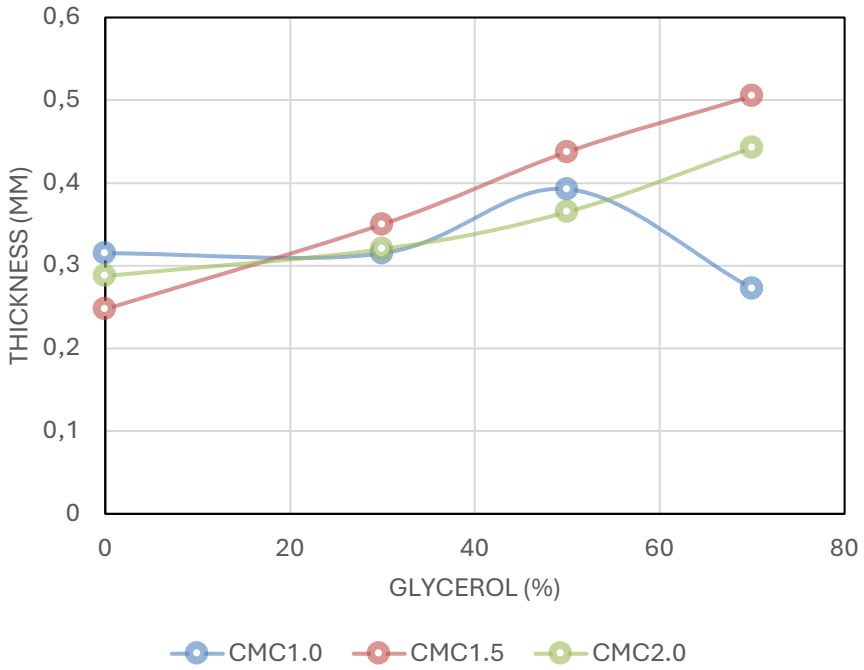


FIGURE 2. Mean thickness of biodegradable films formulated with varying concentrations of CMC (1.0, 1.5, and 2.0%) and glycerol (0, 30, 50, and 70%).

The increase in film thickness with increasing CMC concentration can be attributed to the higher total solids content in the film-forming solution. Increasing CMC concentration generally produces thicker films due to the greater accumulation of polymeric material during film formation. Additionally, CMC may modify the viscosity and intermolecular interactions of the film-forming solution, influencing polymer distribution and structural development during drying. These effects contribute to increased film thickness and may alter the mechanical characteristics of the resulting biodegradable films[14].

Similarly, glycerol incorporation influences film thickness through its plasticizing effect on the soybean protein (SP)-based polymer matrix. Glycerol molecules interact with polar functional groups of protein chains through hydrogen bonding, reducing intermolecular cohesion and increasing polymer chain mobility. These interactions modify the three-dimensional organization of the polymer network, resulting in a more flexible and extensible film structure [11]. Furthermore, glycerol may influence film thickness by increasing the non-volatile content of the film-forming formulation and modifying its drying behaviour. However, previous studies, excessive glycerol incorporation can weaken intermolecular interactions, potentially reduce tensile resistance and increase susceptibility to tearing despite improved flexibility [10], [15].

Visual observations also revealed that thicker films exhibited greater opacity and a more pronounced yellowish coloration compared with thinner films (Figure 3). Increased opacity may be associated with greater

film thickness, which extends the optical pathway and enhances light absorption and scattering within the polymer matrix. The yellowish appearance may be related to the intrinsic coloration of SP and changes in the distribution of polymeric components during film formation. Overall, these findings indicate that CMC and glycerol concentrations influence not only film thickness but also its mechanical and optical characteristics, emphasizing the importance of optimizing their proportions to achieve biodegradable films with desirable properties for packaging applications.

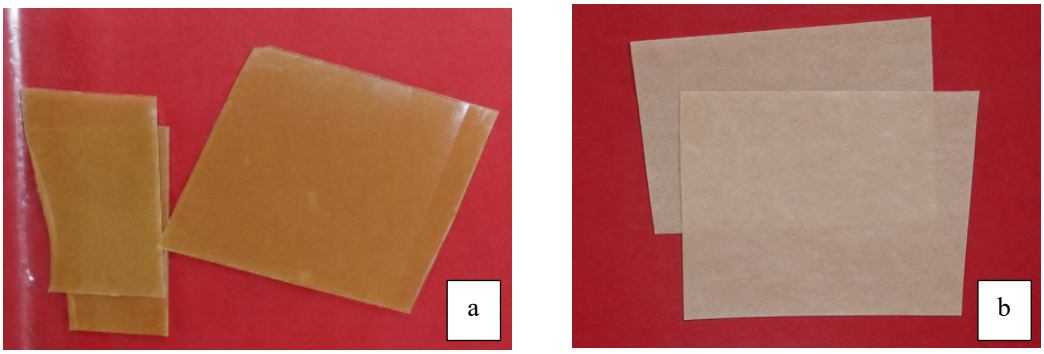


FIGURE 3. Visual appearance of biodegradable film formulations: (a) plastic film containing 2% CMC and 70% glycerol; and (b) film-forming paste containing 1% CMC and 30% glycerol.

3.3.Water solubility analysis

Water solubility is an important physicochemical property influencing the water resistance, structural stability, and potential biodegradation behavior of biodegradable plastic films. The experimental results demonstrated that increasing the concentrations of carboxymethyl cellulose (CMC) and glycerol enhanced the water solubility of soybean protein (SP)-based films. In the absence of glycerol, increasing CMC concentration resulted in an increase in film solubility from 22.9% to 35%. A similar trend was observed with increasing glycerol concentrations (0, 30, 50, and 70%). At a fixed CMC concentration of 2% and an immersion period of 1 hour, film solubility progressively increased from 35% to 43.83%, 46.29%, and 54.18%, respectively (Figure 4). These findings indicate that both CMC and glycerol significantly influence the water sensitivity of the polymeric matrix.

The increase in water solubility can be attributed to the hydrophilic characteristics and molecular interactions of CMC and glycerol within the SP-based polymer network. CMC-based films exhibit considerable affinity for aqueous environments due to the presence of hydroxyl and carboxymethyl functional groups [14]. Similarly, glycerol contains three hydroxyl groups that readily form hydrogen bonds with water molecules, enhancing water penetration and retention within the film matrix. Furthermore, glycerol acts as a plasticizer by modifying intermolecular hydrogen bonding between polymer chains, reducing intermolecular cohesion, and increasing molecular mobility [10]. These effects facilitate water diffusion into the polymer network, promote film swelling, and increase the dissolution or release of water-soluble constituents. Consequently, higher concentrations of CMC and glycerol contribute to greater water solubility through the combined effects of enhanced hydrophilicity and increased polymer chain mobility.

The experimental results demonstrated that the water solubility of biodegradable plastic films increased progressively with prolonged immersion time. At CMC and glycerol concentrations of 1.5% and 50%, respectively, film solubility increased from 29.00% after 1 hour to 30.77% after 3 hours and 42.85% after 6 hours of immersion (Figure 4). This time-dependent increase in solubility can be attributed to the

progressive penetration of water molecules into the polymer matrix, facilitated by hydrogen-bonding interactions with hydrophilic functional groups present in soybean proteins, CMC, and glycerol. As reported by Chai, Xu, and Zhao (2025), these interactions enhance water absorption, promote polymer swelling, and facilitate the dissolution of water-soluble components within the film structure [16]. Prolonged immersion further increases polymer chain mobility and weakens intermolecular associations, resulting in greater material dissolution. Among the investigated formulations, films containing 1.5% CMC and 30% glycerol exhibited the most favorable combination of relatively low water solubility and high flexibility. These characteristics suggest that this formulation provides an appropriate balance between polymer network stability and plasticization, making it a promising composition for biodegradable packaging applications requiring both mechanical flexibility and improved water resistance.

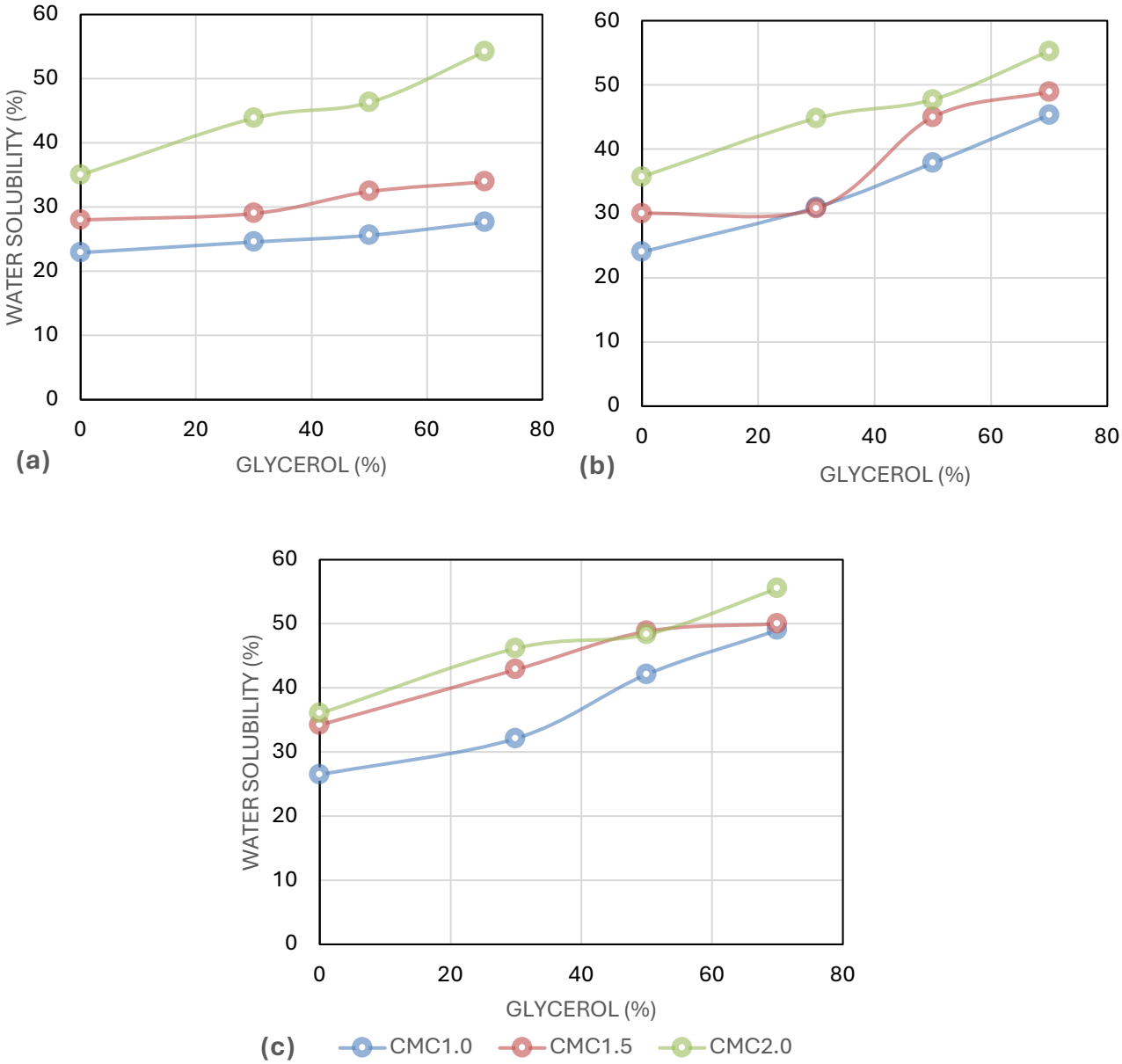


FIGURE 4. Water solubility (%) of biodegradable films after immersion for (a) 1 hour, (b) 3 hours, and (c) 6 hours, at varying concentrations of CMC (1.0, 1.5, and 2.0%) and glycerol (0, 30, 50, and 70%).

3.4.Biodegradability test

Biodegradability testing was performed to assess the susceptibility of biodegradable plastic films to decomposition by soil microorganisms. The experimental results demonstrated that the films underwent progressive degradation during the first 1–4 days of soil burial, followed by complete visual disintegration on the sixth day (Table 1). This rapid degradation behavior can be attributed to the organic nature of the film-forming materials, particularly soybean protein (SP), carboxymethyl cellulose (CMC), and glycerol, which are susceptible to microbial and enzymatic decomposition. Furthermore, the hydrophilic characteristics of these components facilitate moisture absorption, creating favorable conditions for microbial growth and activity while promoting the breakdown of the polymeric matrix. These findings indicate that the developed biodegradable films exhibit promising degradation characteristics and considerable potential as environmentally sustainable alternatives to conventional petroleum-based plastics.

TABLE 1. Results of Biodegradability and Thermal Resistance Tests of Bioplastic Films

No	Concentration of Protein (g)	Concentration of CMC (g)	Concentration of Glycerol (%)	**Film biodegradability**		**Heat Resistance**	
				1-4 Days	4-6 Days	Temperature(°C)	Conditions
1	10	1	0	Partially degraded	Fully degraded	160-200	Late Burned at 200°C
2	10	1	30	Partially degraded	Fully degraded	160-200	Burned
3	10	1	50	Partially degraded	Fully degraded	160-200	Burned
4	10	1	70	Partially degraded	Fully degraded	160-200	Early Burned at 160°C
5	10	1.5	0	Partially degraded	Fully degraded	160-200	Late Burned at 200°C
6	10	1.5	30	Partially degraded	Fully degraded	160-200	Burned
7	10	1.5	50	Partially degraded	Fully degraded	160-200	Burned
8	10	1.5	70	Partially degraded	Fully degraded	160-200	Early Burned at 160°C
9	10	2	0	Partially degraded	Fully degraded	160-200	Late Burned at 200°C
10	10	2	30	Partially degraded	Fully degraded	160-200	Burned
11	10	2	50	Partially degraded	Fully degraded	160-200	Burned
12	10	2	70	Partially degraded	Fully degraded	160-200	Early Burned at 160°C

3.5.Heat resistance test

Thermal resistance is an important physical property of biodegradable plastic films that reflects their ability to withstand elevated temperatures without undergoing significant structural deterioration or thermal degradation. The experimental results (Table 1) demonstrated that the thermal resistance of the films varied depending on glycerol concentration. The films exhibited burning temperatures ranging from 160 to 200 °C, depending on glycerol concentration. Visually, films containing higher glycerol concentrations showed earlier signs of thermal deterioration and began burning at temperatures closer to 160 °C, whereas films with lower glycerol concentrations exhibited greater thermal resistance. In contrast, films without glycerol demonstrated the highest resistance to thermal exposure, with burning occurring at approximately 200 °C. This behavior may be attributed to the plasticizing effect of glycerol, which modifies intermolecular interactions within the polymer matrix, increases polymer chain mobility, and potentially reduces resistance to thermally induced structural changes. These findings suggest that glycerol concentration influences the thermal response of soybean protein (SP)-based biodegradable films. Adequate thermal resistance is particularly important for food packaging applications involving elevated processing temperatures, such as heat sealing and thermal printing, where the packaging material must maintain its structural integrity and functional properties.

4. Conclusion

Biodegradable plastics can be produced from starch-based materials derived from agricultural crops such as corn, sago, and cassava. However, the utilization of these resources for bioplastic production may increase competition with food applications, highlighting the importance of exploring alternative raw materials derived from agro-industrial waste. In this study, solid residues from the tofu processing industry were utilized as a sustainable source of soybean protein (SP) for biodegradable film production. Among the investigated formulations, films containing 10% SP, 1.5% carboxymethyl cellulose (CMC), and 30% glycerol exhibited the most favourable combination of relatively medium to low water solubility, adequate flexibility, and rapid disintegration under soil burial conditions. The superior performance of this formulation may be attributed to balanced intermolecular interactions between SP and CMC, which contribute to polymer network cohesion, while glycerol provides sufficient plasticization to improve film flexibility without excessively compromising structural integrity. In contrast, higher glycerol concentrations resulted in excessive flexibility, potentially due to increased polymer chain mobility and weakened intermolecular interactions. Although the developed films demonstrated promising biodegradation characteristics, their relatively high-water sensitivity limits their suitability for packaging liquid or high-moisture food products. Nevertheless, these films show potential for packaging dry or low-moisture solid foods, provided that their mechanical performance, barrier properties, and food-contact safety are further validated. The utilization of tofu processing by-products also offers a promising strategy for waste valorisation and the development of more sustainable packaging materials.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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