

Research Article

Development and Characterization of Banana Starch– and Guava Peel Extract–Based Sustainable Packaging Films

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In order to compensate the use of plastics, biodegradable starch-based polymer films have been introduced. Banana starch is considered suitable for use in food packaging applications, implying that films made from banana starch can be used directly for the packaging of various food products without causing any health hazards. This study focuses on development of a biodegradable film using banana starch incorporated with guava leaf extract. Guava leaf extract serves as an antimicrobial agent to the formulation. Thereby, banana starch-based film can display antimicrobial properties, which could potentially prolong the shelf life of perishable food items by preventing microbial spoilage. The film was analyzed for solubility, water absorption capacity, water vapor transfer rate, total polyphenol, moisture content, and antimicrobial activity for varied starch (2%–6%), guava leaf extract (1%–3%), and glycerol (10%–25%) concentrations. The analysis affirmed the optimum combinations as 2.28% starch, 1.37% guava leaf extract, and 15.26% glycerol. Corresponding response variables such as thickness, solubility, water absorption capacity, total polyphenol, zone of inhibition against *Escherichia coli*, and moisture content achieved were 0.081 mm, 42.35%, 50.66%, 31.92 mg gallic acid equivalent (GAE)/g, 7.12 mm, and 7.94%, respectively. In summary, antimicrobial banana starch-based packaging film presents a promising alternative for food packaging on commercial basis. Further, this would cause minimum environmental impact and help in food preservation.

Keywords: antimicrobial activity; banana starch; guava leaf extract; optimization; polymer film

1. Introduction

Food packaging is a crucial stage in food production, where synthetic polymers have dominated packaging for many years. However, the environmental and health risks associated with the solid waste produced by synthetic polymers are a major concern in modern era [1]. Most plastics used in food packaging are single-use and used on the go, and the waste produced by these nonrecyclable or nonbiodegradable food packaging wastes possesses a serious threat to the environment on land and in water. As a result, there is an increasing understanding of the necessity for food packaging

materials which adhere to environmental standards [2]. Consequently, in recent years, biodegradable packaging materials have gained popularity because of their safety, affordability, and environmental friendliness [3]. For the creation of biodegradable plastic films, plants containing supplies of starch, including potatoes, rice, and maize, have been investigated [4]. The films must also adhere to the requirements for reducing food loss while maintaining the quality of food products. In addition to greater ecological importance, it can be made from biopolymeric materials that have been incorporated with natural active chemicals that operate as oxygen scavengers, antioxidants, antimicrobials,

ethylene scavengers, and moisture absorbers [5]. The advantages of edible films and coatings made from natural materials over synthetic materials have recently attracted a lot of interest. These films, such as edible films made of proteins, lipids, or polysaccharides (mainly starch), or a combination of these [6], protein-based films modified using various additives and fillers [7], bacterial cellulose-based bioplastics fortified with fermented soymilk extracts [8], and polymer blended films containing food preservatives [9], have demonstrated effectiveness in maintaining food safety and quality. It has been reported that these films stop lipid oxidation and microbiological development, improving the shelf life of food products [10]. These films decompose more quickly than polymeric materials because they are made solely from edible, renewable elements. Because of its widespread availability, ease of extraction, high yield, low cost, and variety of functional characteristics that improve its biocompatibility in the food system, starch is mainly preferred [11]. From a variety of starch sources, including corn, potato, banana, and plantain, edible films have been created and their qualities are being investigated. Active and smart biodegradable films were produced from cassava starch and glycerol with 5 wt.% of different natural extracts, such as green tea and basil leaves, degraded in soil in less than 2 weeks and were thermally stable up to 240°C [12]. Incorporation of plant extracts increases the antioxidant and antimicrobial activities of the starch film. Various plant extracts were incorporated into the starch film previously and the difference between the starch film and the film made up of both starch and leaf extract was reported. Moringa leaf extract (MLE) was added to wheat starch, and it was reported that the addition of MLE possessed good antioxidative activities and ultraviolet light blocking ability, concluding that films containing 1.0% MLE were biodegradable within 30 days [13]. The addition of basil leaf extract to the moth bean starch film proved effective when used against eggplant during the storage period. The results showed that active coating significantly retained moisture loss and firmness, retarded the increase in total soluble solids, and delayed colour changes during the storage period [14]. The antibacterial activity of the biodegradable starch film with the addition of chicory root extract (1%–5%) was tested against Gram-negative bacteria (*Pseudomonas fluorescens* and *Escherichia coli*) and Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) using the microculture method. The most sensitive bacterium in terms of film bacteriostatic activity was *P. fluorescens*. The films obtained were evaluated for their properties such as food packaging material (colour, thickness, permeability, and mechanical strength). Phytic acid improved the tensile strength and barrier properties of the film. The inhibitory effect on growth was noticed in every film acquired across all assessed microorganisms, with the most advantageous impact evident in films incorporating extracts at a 5% concentration obtained through water extraction [15]. The influence of avocado seed starch and orange peel essential oil was investigated and optimized using response surface methodology (RSM) and an artificial neural network on the tensile strength, water vapor permeability, and antimicrobial properties of active

films. The optimum conditions found for orange peel oil were 0.57 g and 30% w/w of avocado seed starch with the values of the corresponding responses of 3.94 MPa, 3.098×10^{-10} g/m²•s•Pa, and 17.273 mm for tensile strength, water vapor permeability, and inhibition zone, respectively [16]. The researchers studied a thin layer made of polyvinyl alcohol (PVA), corn starch (ST), glycerol, and the active elements from watermelon rind extract (WMRE) to understand how it performed in terms of strength, ability to fight harmful substances in food, and its practical uses against specific disease-causing microorganisms found in food. The results showed that the addition of 10% v/v WMRE to the film formulation significantly increased the tensile strength from 19.44 to 33.67 MPa and slightly increased the percent elongation at break (% EAB) from 35.04% to 35.16%. The antioxidant property of the film was significantly increased from 29.21% to 63.42%. Consumer testing of the packaging film indicated that user acceptance of the product was favourable [17].

Similarly, incorporation of several plant extracts not only enhances the antimicrobial properties but also improves the mechanical properties of the film [18]. The EAB, ultraviolet/visible light barrier, and antioxidant properties of sword bean starch (SBS) film improved when goji berry extract (GBE) was added. Moreover, radical scavenging activities of the SBS film with 1.0% GBE were reported to be the highest among the films [19]. Methanolic extract of *Artemisia sieberi* when added to sago starch film enhanced the physical properties of the films, when the concentration of extract was increased from 0% to 10%. The water solubility (WS), water absorption capacity, and water vapor permeability of the films changed from 21.31% to 26.47%, from 1.98 to 3.93 g water/g dry matter, and from 1.63 to 2.93 g/m²•s•Pa, respectively [20]. Guava leaf extract, used in the study, exhibits antimicrobial properties against various bacteria, including both Gram-positive and Gram-negative strains. The extract's antibacterial activity is enhanced by the presence of bioactive substances such as phenol, flavonoids, tannins, and terpenoids [21, 22]. Various techniques, such as disk diffusion and agar well diffusion assays, have been employed to assess the extract's antimicrobial activity. These tests evaluate the extract's inhibitory activity against bacteria [23]. *Salmonella enterica*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *S. aureus*, and *E. coli* are few of the microorganisms that the extract has been demonstrated to have inhibitory action against [24].

The previous findings demonstrated numerous investigations on starch-based packaging films and their optimization. However, very few investigations targeted antimicrobial starch-based polymer film and its characterization. The final qualities of the films were significantly influenced by the morphological and physiochemical parameters of the source starch. In addition, statistical design-based optimization and characterization of packaging are limited in prior art. On the other hand, raw banana starch can be used as a biopolymer for the formulation of packaging film. Further, guava leaf extract, which provides antimicrobial properties to the films, would facilitate enhanced shelf-life of the food material being packaged. Therefore, this investigation emphasizes

TABLE 1: Experimental design for extraction of guava leaves at different combination of independent variables.

S.no.	SP	GLE	GLY	WA	MC	AA	SL	TPC	Thickness	WVTR
1	4	2	17.5	61	10.2	9	33.3	36.7	0.12	5.38
2	6	1	25	64	12.02	6.8	23.4	35.9	0.1	4.15
3	2	1	10	42	6	6	52.09	29.6	0.05	1.46
4	2	1	25	50	6.7	5.2	41.8	31	0.1	3.42
5	0.64	2	17.5	39	5	5.8	46.93	29.1	0.09	2.58
6	2	3	10	44.6	6.3	6.5	48.48	37.4	0.07	2.2
7	6	1	10	60.1	7.1	7.7	49.16	32.1	0.07	3.4
8	4	2	17.5	63	9.5	8.7	32	36	0.11	6.06
9	4	2	17.5	61	10	8.5	33	36.1	0.12	6.6
10	4	3.68	17.5	60	8.6	8.8	20.8	40.2	0.15	6.5
11	4	0.32	17.5	52	9.1	6.2	36.4	31	0.11	3.23
12	4	2	17.5	62.2	9.7	8.5	34.1	36.2	0.11	6.25
13	2	3	25	46	4.6	7.8	38.2	35.2	0.13	4.6
14	4	2	4.89	51.9	6.7	7.2	64	37.8	0.04	1.86
15	4	2	30.1	60	7.57	7.1	34.16	39	0.16	3.9
16	4	2	17.5	59.7	10.1	8.5	32.5	35.7	0.14	5.32
17	6	3	25	68	12.3	10.5	12.13	38.5	0.19	6.1
18	6	3	10	67	11.7	8.6	34.3	38.3	0.13	5.8
19	7.37	2	17.5	73	12.9	9.2	20.6	36.2	0.13	5.64
20	4	2	17.5	62	10.6	9	32	35.3	0.12	6.23

TABLE 2: Analysis of variance showing fitness of model for the dependent variables.

Source	WAC		MC		AA		Solubility		TPC		Thickness		WVTR	
	F value	p value Prob > F	F value	p value Prob > F	F value	p value Prob > F	F value	p value Prob > F	F value	p value Prob > F	F value	p value Prob > F	F value	p value Prob > F
Linear vs. mean	37.75	<0.0001	12.40	0.0002	11.63	0.0003	16.44	<0.0001	14.25	<0.0001	20.45	<0.0001	5.88	0.0066
2FI vs. linear	0.85	0.49	3.84	0.04	1.78	0.20	1.11	0.38	1.11	0.38	2.34	0.12	0.39	0.76
Quadratic vs. 2FI	31.15	<0.0001	21.06	0.0001	37.40	<0.0001	296.57	<0.0001	27.02	<0.0001	2.70	0.10	45.96	<0.0001
Cubic vs. quadratic	1.77	0.25	3.25	0.10	1.57	0.30	1.21	0.40	0.75	0.59	2.10	0.20	0.28	0.88

upon formulation and characterization of raw banana starch-based antimicrobial film incorporated with guava leaf extract. For such investigation, a statistical design-based approach has been adopted with varied starch, glycerol, and guava leaf extract to characterize solubility, total phenolic content (TPC), water absorption capacity, water vapor transmission rate (WVTR), and moisture content of the film. This would facilitate various food packaging industries to adopt such packaging material for better shelf-life and integrity of the food products being packaged.

2. Materials and Methods

2.1. Raw Material. Raw banana was procured from a local market in Phagwara, Punjab, India, in an airtight polyethylene bags. On the other hand, fresh guava leaves were taken from

an agriculture farm, Lovely Professional University, Phagwara, Punjab, India. Thereafter, the samples were thoroughly washed in tap water to remove extraneous materials adhering to them. Raw banana was peeled to take out the starchy material for film preparation. On the other hand, guava leaves were macerated to extract its content to be incorporated into the film in desired quantity according to central composite design (CCD)-based RSM design.

2.2. Film Preparation. Film-forming solution was prepared through gelatinization of banana starch (2%–6% *w/v* of distilled water) at 75°C for 20 min, with continuous stirring at 500 rpm [25]. The solutions were then plasticized with glycerol (10%–25%). On the other hand, guava leaf extract (1%–3%) was dissolved in 100 mL of distilled water at room temperature. Thereafter, the two solutions were combined and slowly stirred at 250 rpm for 20 min at 40°C to eliminate

TABLE 3: Analysis of variance (ANOVA) for various responses of packaging film.

Source	WAC			MC			AA			Solubility			TPC			Thickness			WVTR		
	F value	p value Prob > F	F value	F value	p value Prob > F	F value	F value	p value Prob > F	F value	F value	p value Prob > F	F value	F value	p value Prob > F	F value	F value	p value Prob > F	F value	F value	p value Prob > F	F value
Model	110.09	<0.0001	49.92	<0.0001	<0.0001	59.82	<0.0001	<0.0001	511.73	<0.0001	<0.0001	45.60	<0.0001	<0.0001	20.45	<0.0001	<0.0001	36.56	<0.0001	<0.0001	<0.0001
A—SP	817.16	<0.0001	309.42	<0.0001	<0.0001	212.09	<0.0001	<0.0001	1354.09	<0.0001	<0.0001	89.36	<0.0001	<0.0001	9.55	0.0070	<0.0001	77.35	<0.0001	<0.0001	<0.0001
B—GLE	24.09	0.0006	1.44	0.26	0.0094	1.98	<0.0001	<0.0001	428.86	<0.0001	<0.0001	212.17	<0.0001	<0.0001	15.61	0.0011	<0.0001	64.23	<0.0001	<0.0001	<0.0001
C—GLY	35.65	0.0001	10.30	0.0094	0.0094	4.27	0.07	0.07	1702.002	<0.0001	<0.0001	4.39	0.062	0.062	36.20	<0.0001	<0.0001	36.24	0.0001	0.0001	0.0001
AB	11.81	0.0064	21.90	0.0009	0.0010	0.48	0.51	0.51	73.84	<0.0001	<0.0001	2.82	0.12	0.12	—	—	—	4.67	0.056	0.056	0.056
AC	1.58	0.24	20.87	0.0010	0.0008	0.48	0.51	0.51	154.41	<0.0001	<0.0001	6.34	0.03	0.03	—	—	—	8.67	0.015	0.015	0.015
BC	7.04	0.02	22.16	0.0008	0.0025	45.53	<0.0001	<0.0001	2.67	0.13	0.13	14.27	0.0036	0.0036	—	—	—	7.92×10^{-5}	0.99	0.99	0.99
A ²	37.12	0.0001	6.90	0.025	0.016	36.13	0.0001	0.0001	2.79	0.13	0.13	57.62	<0.0001	<0.0001	—	—	—	38.04	0.0001	0.0001	0.0001
B ²	37.12	0.0001	8.37	0.016	0.016	36.13	0.0001	0.0001	52.35	<0.0001	<0.0001	2.94	0.12	0.12	—	—	—	13.09	0.0047	0.0047	0.0047
C ²	37.77	0.0001	55.57	<0.0001	<0.0001	61.47	<0.0001	<0.0001	788.19	<0.0001	<0.0001	14.91	0.0032	0.0032	—	—	—	106.55	<0.0001	<0.0001	<0.0001
Residual																					
Lack of fit	1.38	0.37	2.40	0.18	0.18	1.12	0.42	0.42	0.81	0.59	0.59	3.05	0.12	0.12	3.31	0.098	0.098	0.19	0.95	0.95	0.95

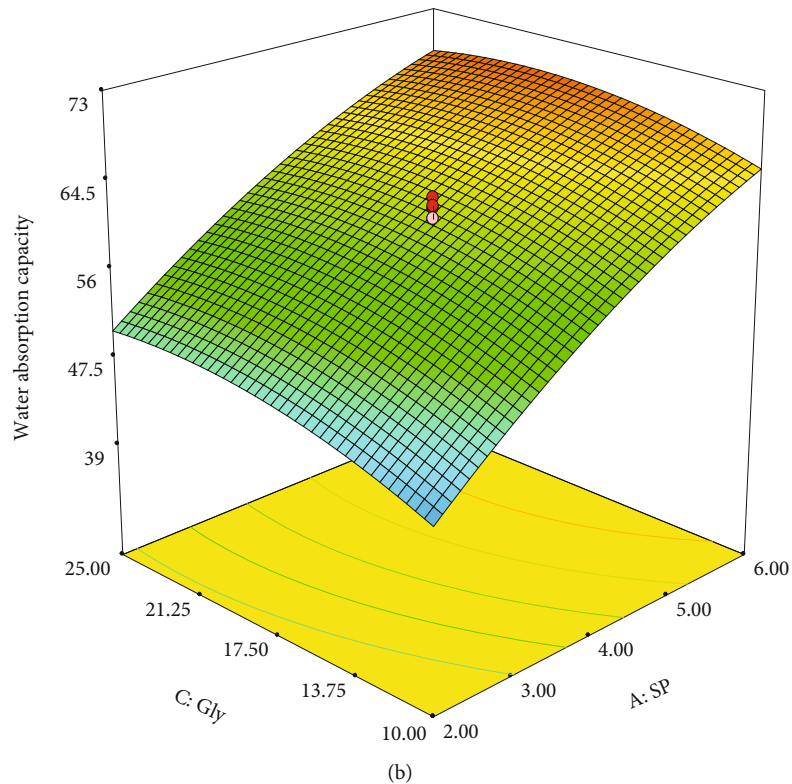
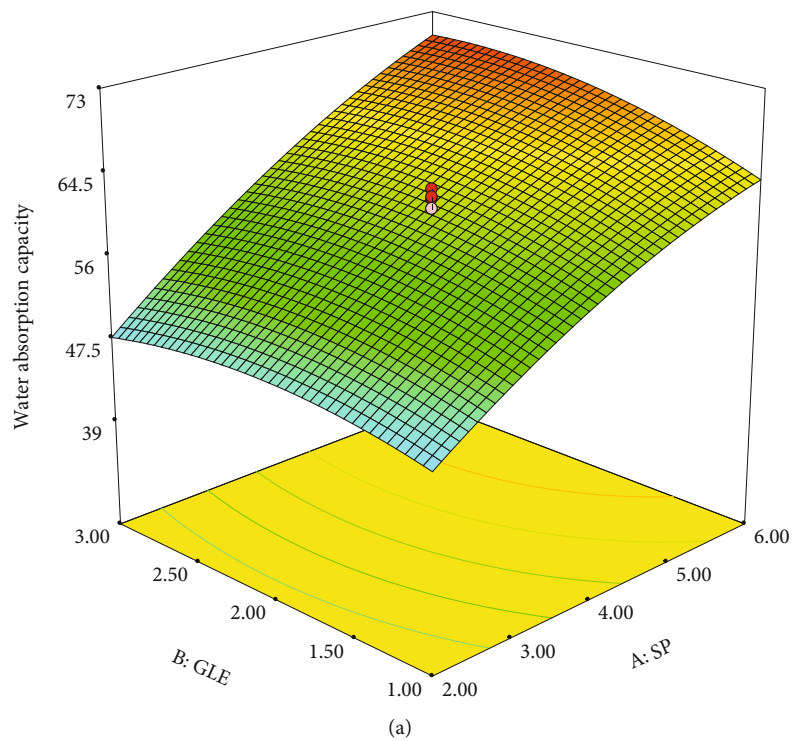


FIGURE 1: Continued.

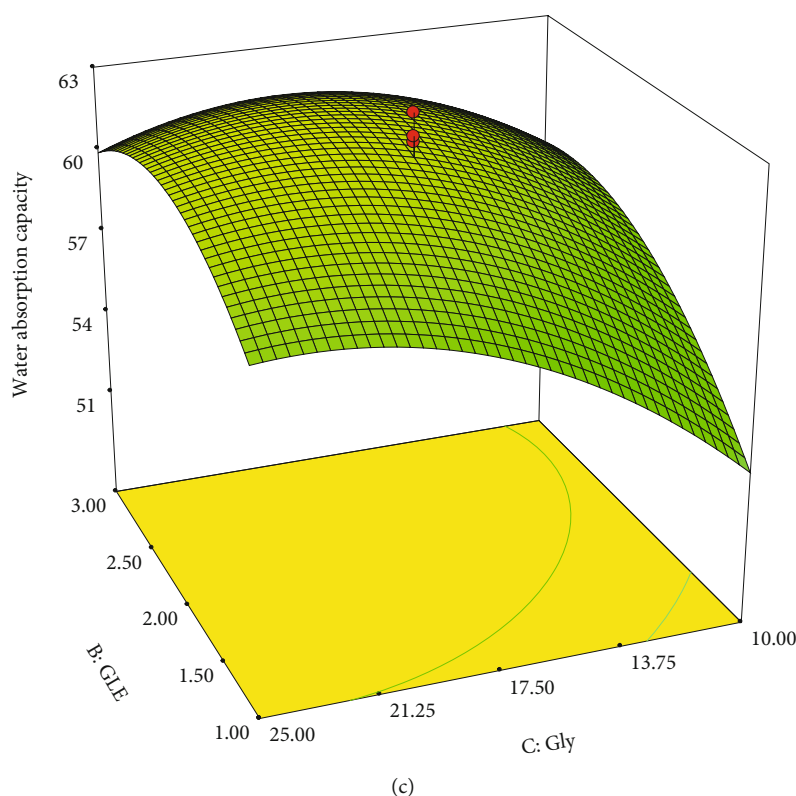


FIGURE 1: Variation of water absorption capacity with (a) guava leaf powder (GLP) and banana starch powder (SP), (b) glycerol (Gly) and banana starch powder (SP), and (c) guava leaf powder (GLP) and glycerol (Gly).

air bubbles and ensure that the mixture was thoroughly mixed. After the homogenization of the mixture, it was spread on top of the glass plate and allowed to dry for 24 h at 70°C. The film was peeled off from the glass plate after being completely dried and examined further.

2.3. Experimental Design and Statistical Analysis. Rotatable CCD-based RSM was used to generate experimental combinations wherein independent parameters such as percentage of starch, glycerol, and guava leaf extract were varied. The percentage variations were 2%–6% starch, 1%–3% guava leaf extract, and 10%–25% glycerol. The mixture was made up to 100% with water. On the other hand, statistical analysis of the data was carried out using Design-Expert software version 7.0. Model fitting and analysis of variance (ANOVA) were performed for all the response parameters with their associated statistical parameters such as *p* value, *F* value, *R*², adjusted *R*², predicted *R*², coefficient of variation (CV) value, adequate precision, and predicted residual sum of squares (PRESS) values.

2.4. Response Analysis

2.4.1. Moisture Content. Moisture content of the film was determined according to the methodology described in the available literature [20]. A rectangular sample (1 × 2 cm) was weighted on an analytical balance to obtain the initial mass (*M*₁). Thereafter, dry mass (*M*₂) was obtained after drying at 105°C for 24 h and the results were expressed as

the percentage of moisture content of the film using the following expression:

$$MC = \frac{M_1 - M_2}{M_1} \times 100 \quad (1)$$

2.4.2. WS. To determine the WS of the film, the film was cut into pieces with dimensions 2 × 2 cm at room temperature as mentioned previously [26]. The initial weight of the film was measured. Thereafter, film was dipped in distilled water for 24 h. Subsequently, the film was removed and dried at 90°C to a constant weight. Further, WS was calculated using the following equation:

$$WS = \frac{\text{initial dried mass} - \text{final dried mass}}{\text{initial dried mass}} \times 100 \quad (2)$$

2.4.3. TPC. About 100-mg film samples were dissolved in 5-mL distilled water, vortexed for 3 min, and placed in a shaking incubator at 37 rpm for 30 min. The solution was centrifuged for 2 min, and the supernatant was collected and used for further experiment. The total polyphenolic content was evaluated using the Folin–Ciocalteu (FC) method [13]. Small rectangular film samples of known masses were placed in glass vials, and water was added to reach a final film concentration of 5 mg/mL, followed by successive addition of FC phenol reagent and 10% (w/v) aqueous solution of Na₂CO₃. Thereafter, it was incubated for 1 h. Further, absorbance of the solution was measured at 765 nm using a

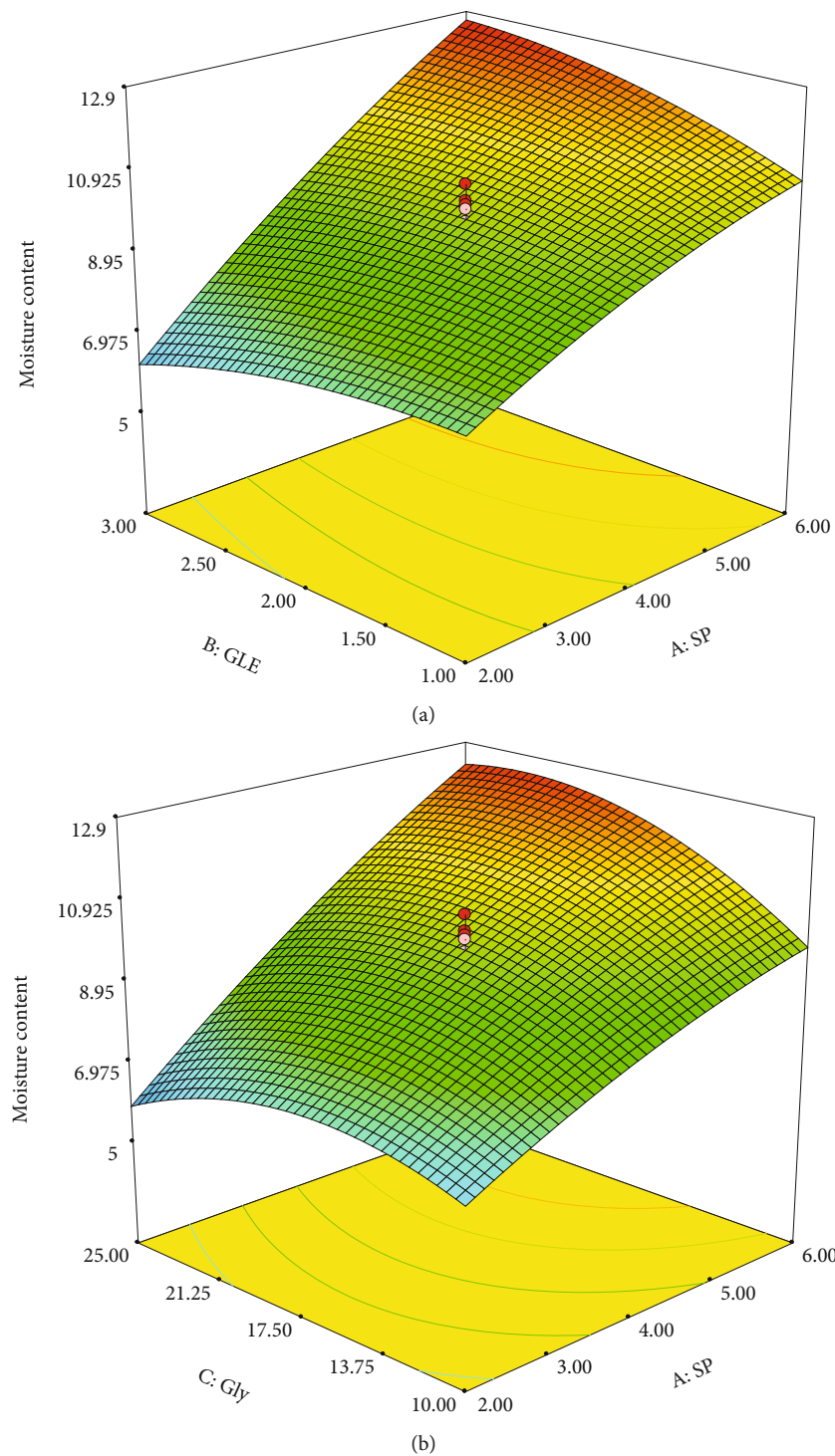


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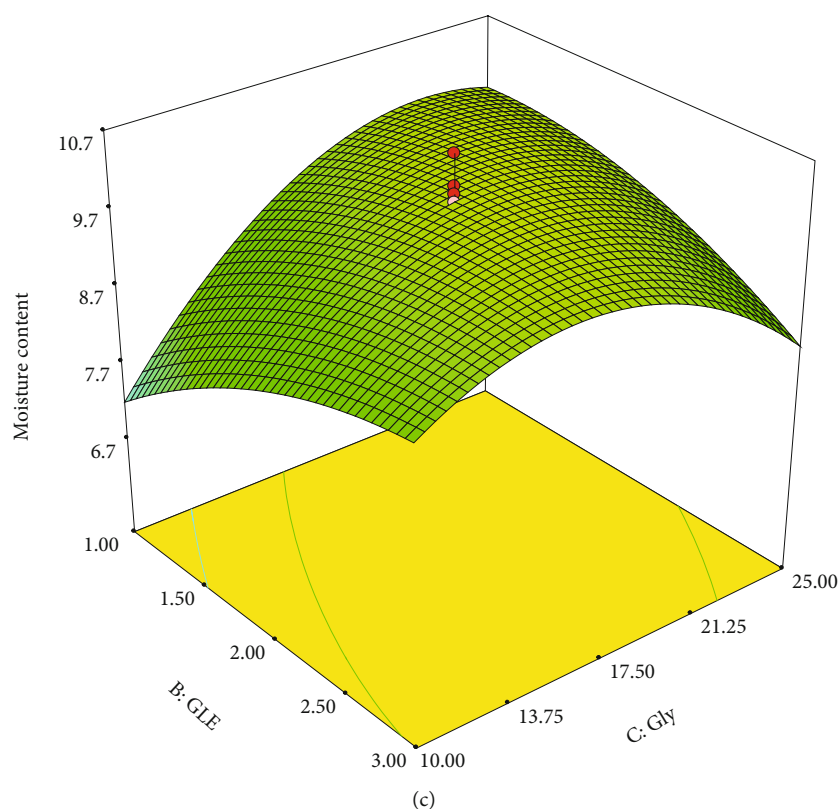


FIGURE 2: Variation of moisture content with (a) guava leaf powder (GLP) and banana starch powder (SP), (b) glycerol (Gly) and banana starch powder (SP), and (c) guava leaf powder (GLP) and glycerol (Gly).

spectrophotometer. The results were expressed as the mass of gallic acid equivalent (GAE) per mass of the film.

2.4.4. Water Absorption Capacity. The absorption capacity of the film was measured using the method summarized in literature [27]. The sample was cut into 1×2 cm pieces. The initial weight of the sample was recorded. The sample was then placed in a beaker containing 60 mL of water at room temperature for 24 h. The sample was then removed from the water and wiped off. The final weight was recorded. The amount of water uptake was calculated using the following formula:

$$\text{Water absorption capacity} = \frac{\text{mass of rehydrated film} - \text{mass of dried film}}{\text{mass of dried film}} \times 100 \quad (3)$$

2.4.5. Antimicrobial Activity. The antibacterial activity of the produced films was determined using the agar disk diffusion method [28]. The films were cut into 6-mm disks and then placed on the prepared culture of *E. coli* containing agar in petri dishes. After incubation of cultures at 37°C for 24 h, the diameters of inhibition zones were examined.

2.4.6. Thickness. The thickness of the film was measured with the help of a vernier calliper having a minimum count of 0.01 mm [29].

2.4.7. WVTR. For WVTR, samples were cut into circular shapes (6-cm diameter) and placed in a test cup filled with 10 mL of distilled water carefully without touching the water. The system was kept in a desiccator. To maintain a 100% relative humidity (RH) gradient across the film, silica gel was placed inside the desiccator. Subsequently, mass of the system (test cup + water + film) was measured at a time interval of 5 h. The WVTR was calculated using the equation expressed as [30]

$$\text{WVTR} = \Delta W / \Delta t \cdot A \quad (4)$$

where ΔW is the amount of water absorbed by silica as a function of time and A is the area of the film (square meter), with the slope ($\Delta W / \Delta t$) of each line determined by linear regression ($R^2 > 0.99$).

3. Results and Discussion

3.1. Effect of the Starch on Response Variables. The variation of various response variables with process independent parameters such as starch, guava leaf extract, and glycerol is summarized in Table 1. For constant guava leaf extract and glycerol (2% and 17.5%, respectively), variations of response variables were thickness 0.09–0.12, solubility 46.93%–33.3%, water absorption capacity 39%–61%, moisture content 5%–10.2%, antimicrobial activity 5.8–9 mm, and WVTR (2.58–5.38) with the increase in percentage of starch from 0.64 to 4. The change in data was further

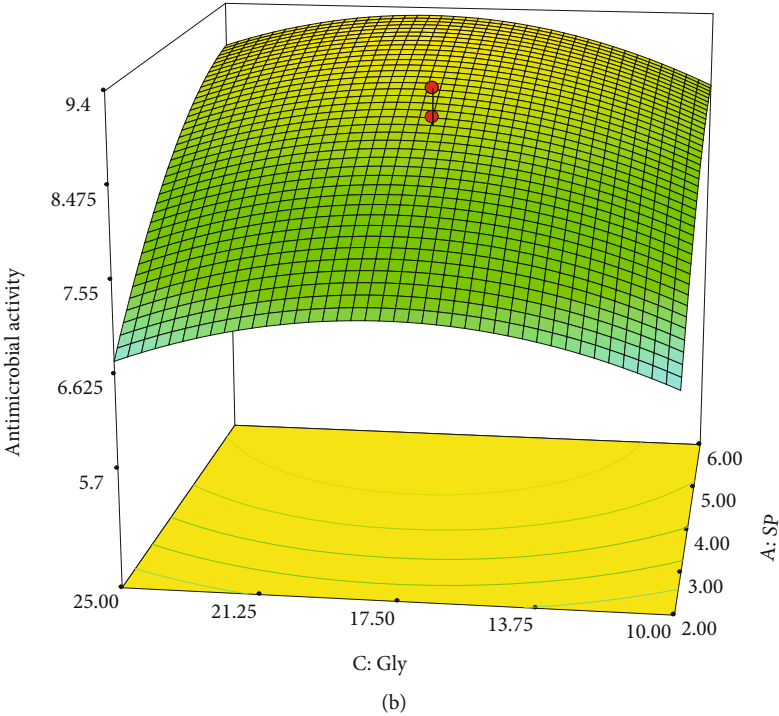
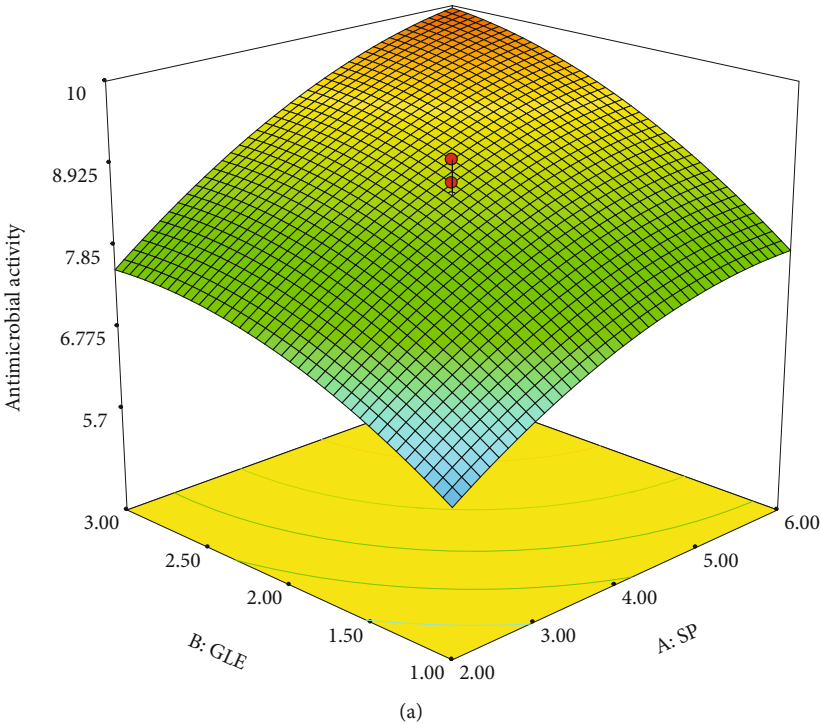


FIGURE 3: Continued.

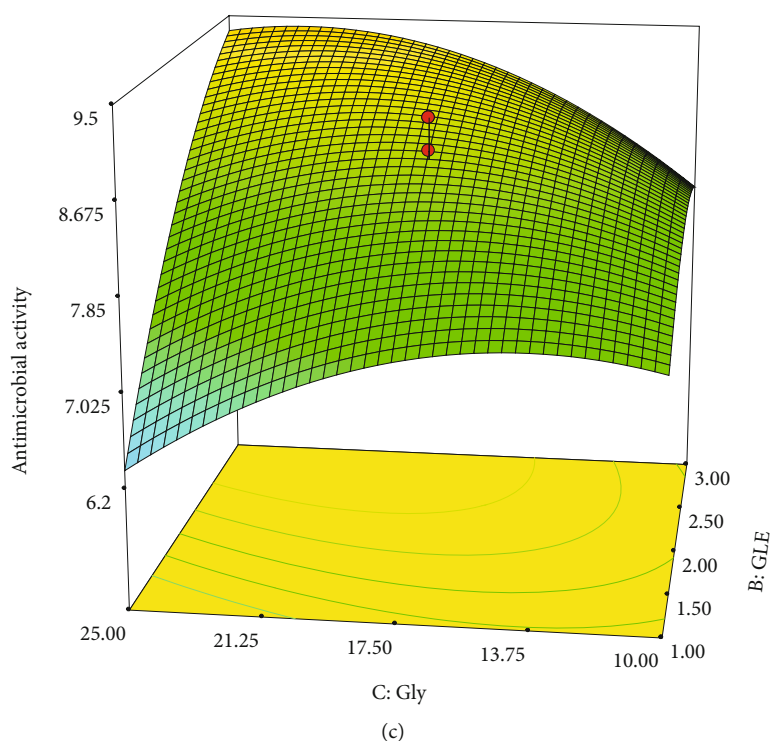


FIGURE 3: Variation of antimicrobial activity with (a) guava leaf powder (GLP) and banana starch powder (SP), (b) glycerol (Gly) and banana starch powder (SP), and (c) guava leaf powder (GLP) and glycerol (Gly).

observed in terms of thickness (0.12–0.13), solubility (33.3%–20.6%), water absorption capacity (61%–73%), moisture content (10.2%–12.6%), antimicrobial activity (9–9.2), and WVTR (5.38–5.64 mg/m²s) for the increase in starch from 4% to 7%. The decrease in solubility with an increase in starch is due to the formation of compact networks between glycerol and starch. A similar trend was reported for a composite edible film consisting of pearl millet starch, carrageenan, and glycerol [31]. For 3% leaf extract and 25% of glycerol, the enhancements of film thickness, water absorption capacity, moisture content, antimicrobial activity, WVTR, and TPC were 0.13–0.19, 46%–68%, 4.6%–12.3%, 7.8–10.5, 4.6–6.1, and 35.2–38.5 mg GAE/g, respectively, for starch enhanced from 2% to 6%. However, solubility declined from 38.2% to 12.13%. Conversely, for guava extract 1% and glycerol 25%, variations of film thickness, WVTR, TPC, moisture content, water absorption capacity, and zone of inhibition were from 0.1 to 0.1, 3.42 to 4.15 mg/m²s, 31 to 35.9 mg GAE/g, 6.7% to 12.02%, 50% to 64%, and 5.2–6.8 mm, respectively, for the enhancement of starch from 2% to 6%. However, the film solubility declined from 41.8% to 23.4%. For the reduced percentage of glycerol (10%) and guava leaf extract 3%, the variations in thickness, water absorption capacity, moisture content, solubility, antimicrobial activity, WVTR, and TPC were 0.07–0.13, 44.6%–67%, 6.3%–11.7%, 48.48%–34.3%, 6.5–8.6, 2.2–5.8, and 37.4–38.3 mg GAE/g, respectively, for starch increased from 2% to 6%. The increase in antimicrobial activity is due to the increase in phenolic compounds, which in turn influences the antioxidant activity and inhibition capacity of the film. Similarly, for the decreased concen-

tration of guava leaf extract (1%) and glycerol (10%), the data obtained showed enhancement of thickness (0.058–0.072 mm), WVTR (1.46–3.4 mg/m²s), TPC (29.6–32.1 mg GAE/g), moisture content (6%–7.1%), water absorption capacity (42%–60.1%), and zone of inhibition (6–7.7 mm) for the variation of starch from 2% to 6%. On the other hand, decrease in solubility of the film was from 52.09% to 49.16%. Enhancement of WVTR is due to higher intermolecular connection between starch and glycerol at high concentrations that creates greater free spaces for water transmission [32].

3.2. Effect of Leaf Extract. The study demonstrated notable improvements across various parameters including water absorption capacity, moisture content, antimicrobial activity, TPC, and WVTR. Specifically, enhancements were observed in these attributes, ranging from 5 to 62.2 mg GAE/g TPC, 3.23–9.7 moisture content, 6.2–8.5 antimicrobial activity, and 31–36.2 mg GAE/g water absorption capacity for extract concentration increased from 0.32% to 2%. Notably, the thickness remained unchanged, while the solubility of the film decreased marginally (36.4–34.1) as the extract concentration increased. Additionally, a decrease in water absorption capacity (60%), moisture content (8.6%), and solubility (20.8%) was observed when the extract concentration increased from 2% to 3%. Conversely, the thickness (0.15), TPC (40.2 mg GAE/g), WVTR (6.5), and antimicrobial activity (8.8) of the film exhibited an increase with the rise in extract concentration. These findings underscore the influence of extract concentration on the functional properties of the film, with implications for its

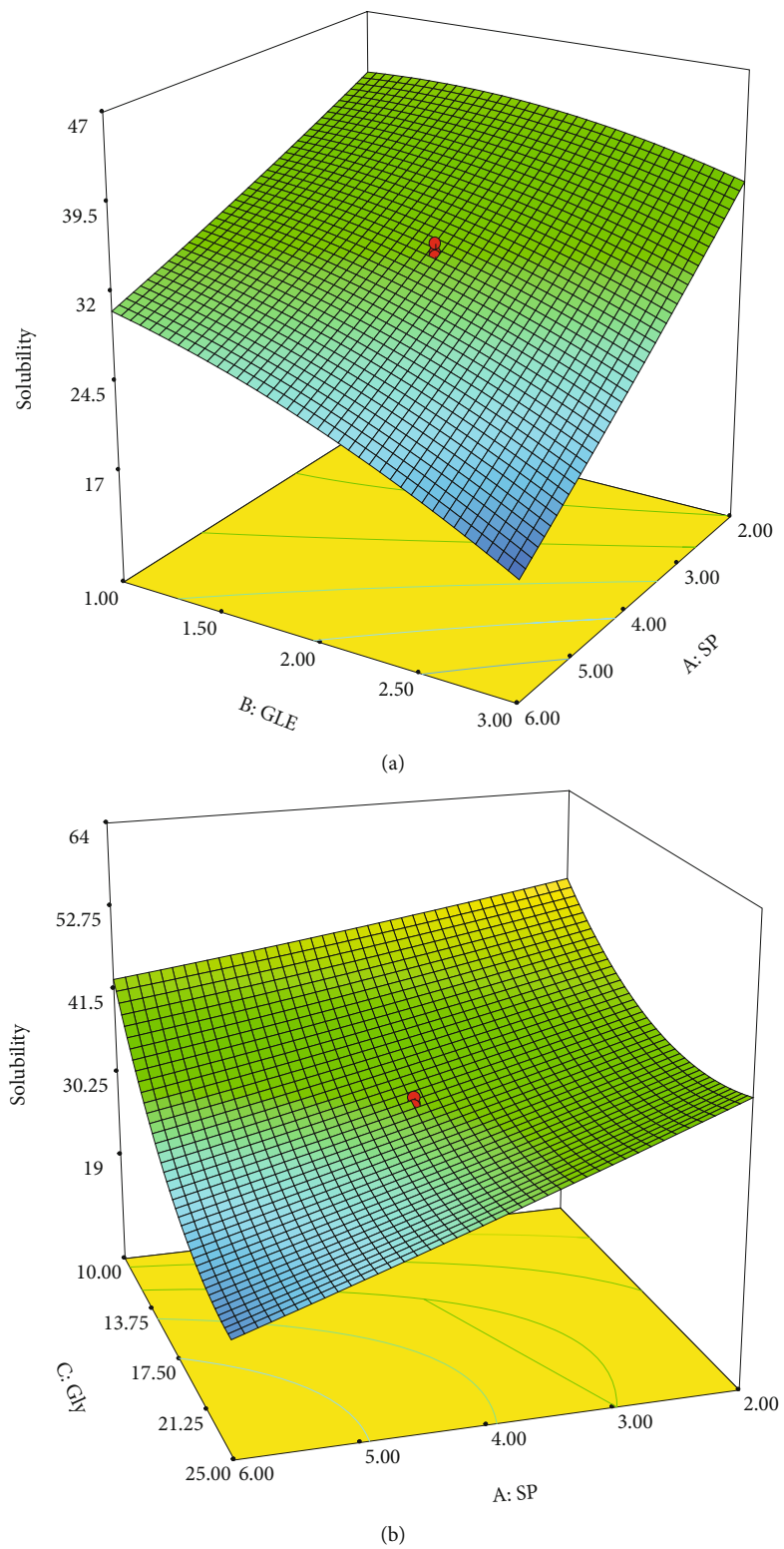


FIGURE 4: Continued.

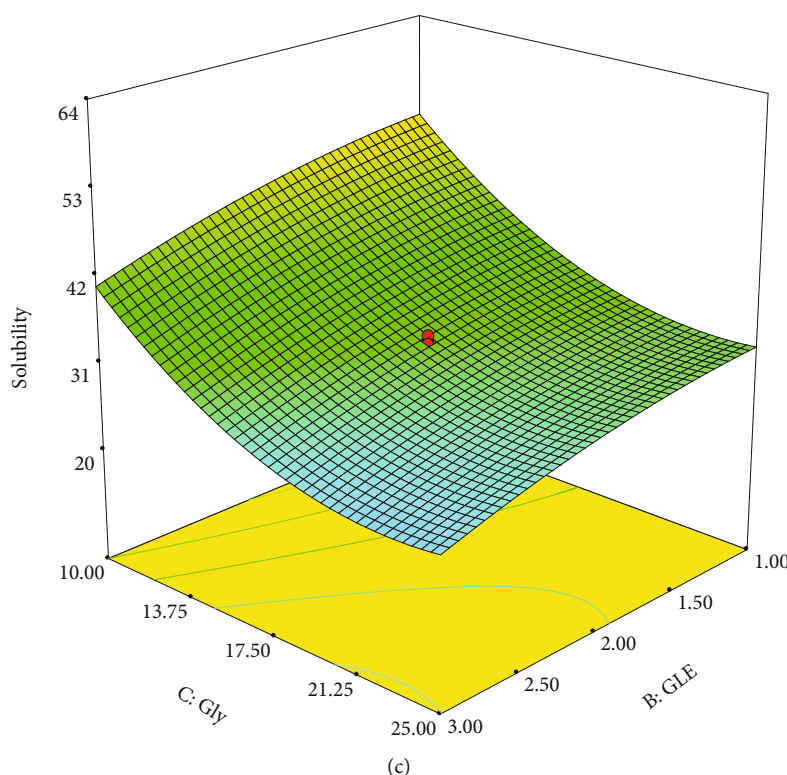


FIGURE 4: Variation of solubility with (a) guava leaf powder (GLP) and banana starch powder (SP), (b) glycerol (Gly) and banana starch powder (SP), and (c) guava leaf powder (GLP) and glycerol (Gly).

potential applications. The variations in responses recorded were thickness (0.1–0.19), solubility (23.4%–12.13%), water absorption capacity (64%–68%), moisture content (12.02%–12.3%), antimicrobial activity (6.8–10.5), TPC (35.9–38.5 mg GAE/g), and WVTR (4.15–6.1). The highest percentage of starch (6%) and glycerol (25%) varied with range 1%–3% of extract. An increase in the inhibition capacity of the film was observed with an increase in the percentage of extract, as previously explained when starch film with antimicrobial chicory root extract was studied [15]. For the reduced glycerol (10) and same amount of starch, the effect of extract was reported to be thickness (0.072–0.13), solubility (49.16%–34.3%), water absorption capacity (60.1%–67%), moisture content (7.1%–11.7%), antimicrobial activity (7.7–8.6), TPC (32.1–38.3 mg GAE/g), and WVTR (3.4–5.8) when the extract concentration increased from 1% to 3%. On the other hand, the changes in thickness, solubility, water absorption capacity, moisture content, antimicrobial activity, TPC, and WVTR were 0.1–0.13, 41.8–38.2, 50–46, 6.7–4.6, 5.2–7.8, 31–35.2 mg GAE/g, and 3.42–4.6, respectively, when the starch was reduced to 2% with 25% glycerol and extract varied from 1% to 3%. For the same variation of extract, the change was well observed in thickness (0.058–0.07), solubility (52.09%–48.48%), water absorption capacity (42%–44.6%), moisture content (6%–6.3%), antimicrobial activity (6–6.5), TPC (29.6–37.4 mg GAE/g), and WVTR (1.46–2.2) for the combined reduction of both the process variables starch 2 and glycerol 10. The rise in phenolic compounds was observed with the increase in the amount of extract used in making the film, which in turn works more

efficiently in suppressing the growth of microbes and eventually increases the antioxidant activity of the film [19]. Kim, Kang, and Bin Song reported previously about the effect of extracts in increasing the antioxidant activity of the film when they worked on SBS film incorporated with berry extract.

3.3. Effect of Glycerol Concentration. For glycerol concentration increased from 4.89% to 17.5%, the variations of thickness, solubility, water absorption capacity, moisture content, antimicrobial activity, TPC, and WVTR were 0.04–0.12, 64%–33%, 51%–60%, 6.7%–9.5%, 7.2–8.7, 37.8–36 mg GAE/g, and 1.86–6.6 mg/m²s, respectively, for 4% starch and 2% guava leaf extract. The values of the response variables were further changed to 0.16 thickness, 34.16 solubility, 60% water absorption capacity, 7.57% moisture content, 7.1 antimicrobial activity, 39 mg GAE/g TPC, and 3.9 WVTR for glycerol concentration increased to 30%. Similarly, for starch (6%) and guava leaf extract (3%), the data showed variation in responses such as solubility (34.3–12.13), thickness (0.13–0.19 mm), WVTR (5.8–6.1 mg/m²s), and zone of inhibition (8.6–10.5 mm) for varied glycerol concentrations from 10% to 25%. Similarly, for the variation of glycerol from 10% to 25%, the observed response variations were thickness 0.072–0.1 mm, solubility 49.16%–23.4%, water absorption capacity 60.1%–64%, moisture content 7.1%–12.02%, antimicrobial activity 7.7–6.8, TPC 32.1–35.9 mg GAE/g, and WVTR 3.4–4.15 mg/m²s for guava leaf extract and starch concentrations of 1% and 6%, respectively. The influence of starch and glycerol on moisture

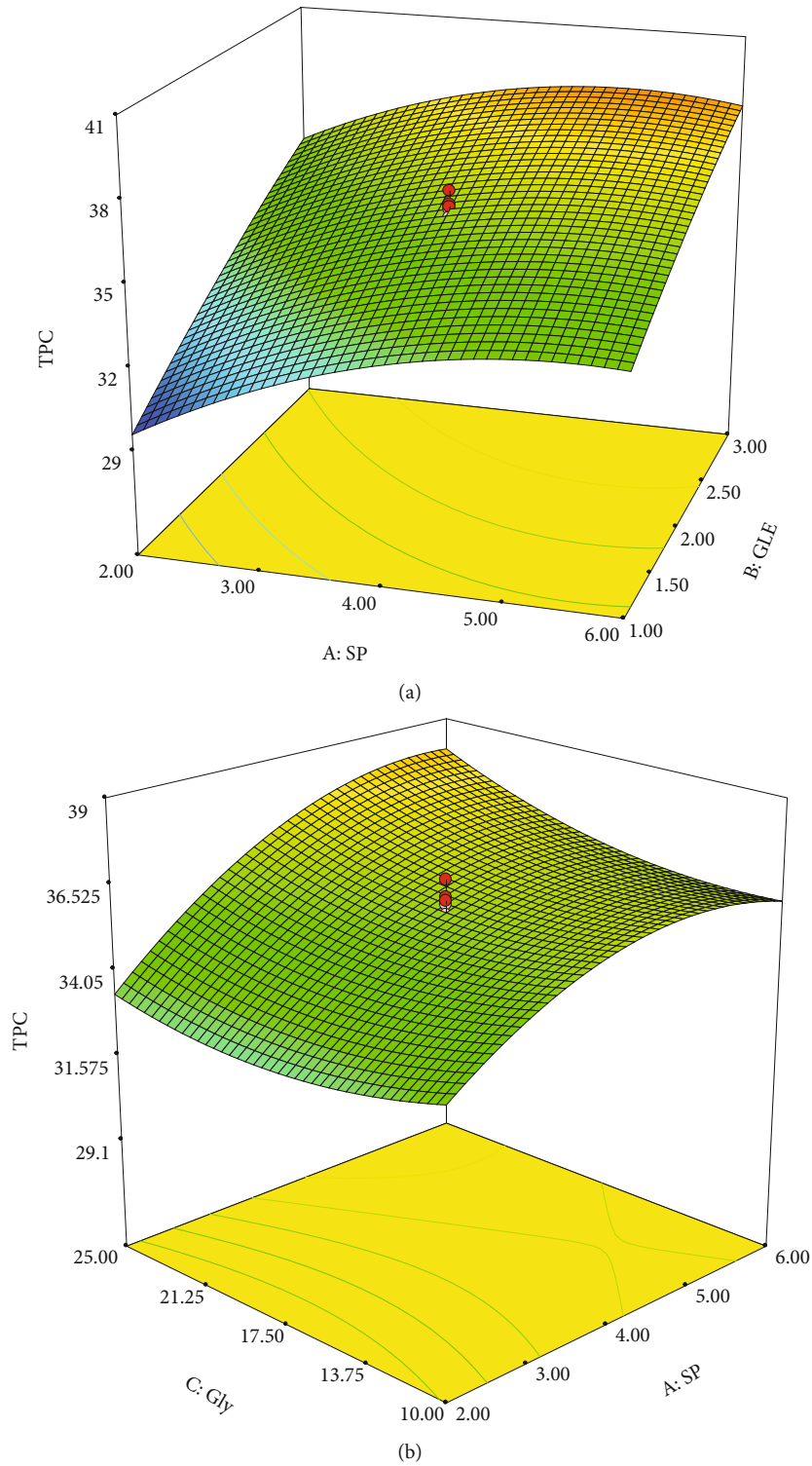


FIGURE 5: Continued.

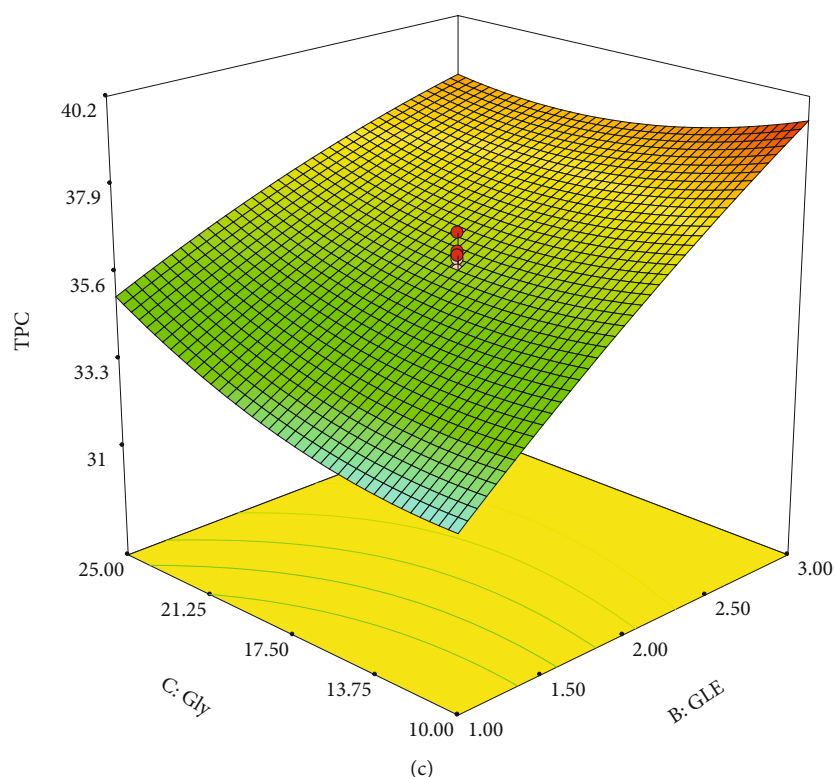


FIGURE 5: Variation of total phenolic content (TPC) with (a) guava leaf powder (GLP) and banana starch powder (SP), (b) glycerol (Gly) and banana starch powder (SP), and (c) guava leaf powder (GLP) and glycerol (Gly).

content and solubility of the film was similar to previous observation [33]. The maximum moisture absorption was witnessed for the highest starch and glycerol contents in the film. This is due to the higher capacity of starch to absorb water. Also, a similar trend was reported for taro peel starch-based biodegradable film [34]. On the other hand, for the same variation of glycerol, variations of thickness, solubility, water absorption capacity, moisture content, antimicrobial activity, TPC, and WVTR for 2% guava leaf extract and 3% starch were 0.07–0.13 mm, 48.48%–38.2%, 44.6%–46%, 6.3%–4.6%, 6.5%–7.8%, 37.4–35.2 mg GAE/g, and 2.2–4.6 mg/m²s, respectively. Further, for guava leaf extract 1% and 2% starch, variables varied as thickness (0.058–0.1 mm), solubility (52.09%–41.8%), water absorption capacity (42%–50%), moisture content (6%–6.7%), antimicrobial activity (6%–5.2%), TPC (29.6–31 mg GAE/g), and WVTR (1.46–3.42 mg/m²s) for the variation of glycerol percentage from 10% to 25%.

3.4. Model Fitting and ANOVA. Model fitness and ANOVA with their associated values of various statistical parameters for various response parameters are presented in Table 2. The relationship between various process independent and dependent variables for water absorption capacity was best expressed with a quadratic model with a high F value (110.09) and low p value (<0.0001). The significant terms for the developed model were A , B , C , AB , BC , A^2 , B^2 , and C^2 supported by other statistical parameters such as R^2 (0.9900), adj R^2 (0.9810), pred R^2 (0.9483), PRESS (82.88), CV% (2.21), and adequate precision (36.79). Lack of fit

was not significant with p value greater than 0.0001 (0.3662). The best-fit model for water absorption capacity is expressed as presented in Equation (5):

$$\begin{aligned} \text{WAC} = & 61.50 + 9.79A + 1.68B + 2.04C + 1.54AB - 0.56AC \\ & - 1.19BC - 2.03A^2 - 2.03B^2 - 2.05C^2 \end{aligned} \quad (5)$$

Moisture content was significantly influenced by the linear (A , C), interaction terms (AB , AC , and BC) and quadratic terms (A^2 , B^2 , and C^2) with higher F values ranging from 6.90 to 309.42. The F value for lack of fit was 2.40, indicating the nonsignificance of lack of fit for the quadratic model for moisture content. Similarly, other associated statistical parameters such as R^2 , adj R^2 , pred R^2 , PRESS, CV, and adequate precision values for the model were 0.9782, 0.9586, 0.8678, 15.46, 5.71%, and 25.40, respectively. The relationship of various process variables for moisture content of the film is best expressed using Equation (6):

$$\begin{aligned} \text{MC} = & 10.01 + 2.40A + 0.16B + 0.43C + 0.83AB + 0.81AC \\ & - 0.84BC - 0.34A^2 - 0.38B^2 - 0.99C^2 \end{aligned} \quad (6)$$

For antimicrobial activity, the quadratic model was the best-fit model to describe its variation with process independent variables. For such case, F and p values were 59.82 and <0.0001 with significant terms A , B , BC , A^2 , B^2 , and C^2 . F

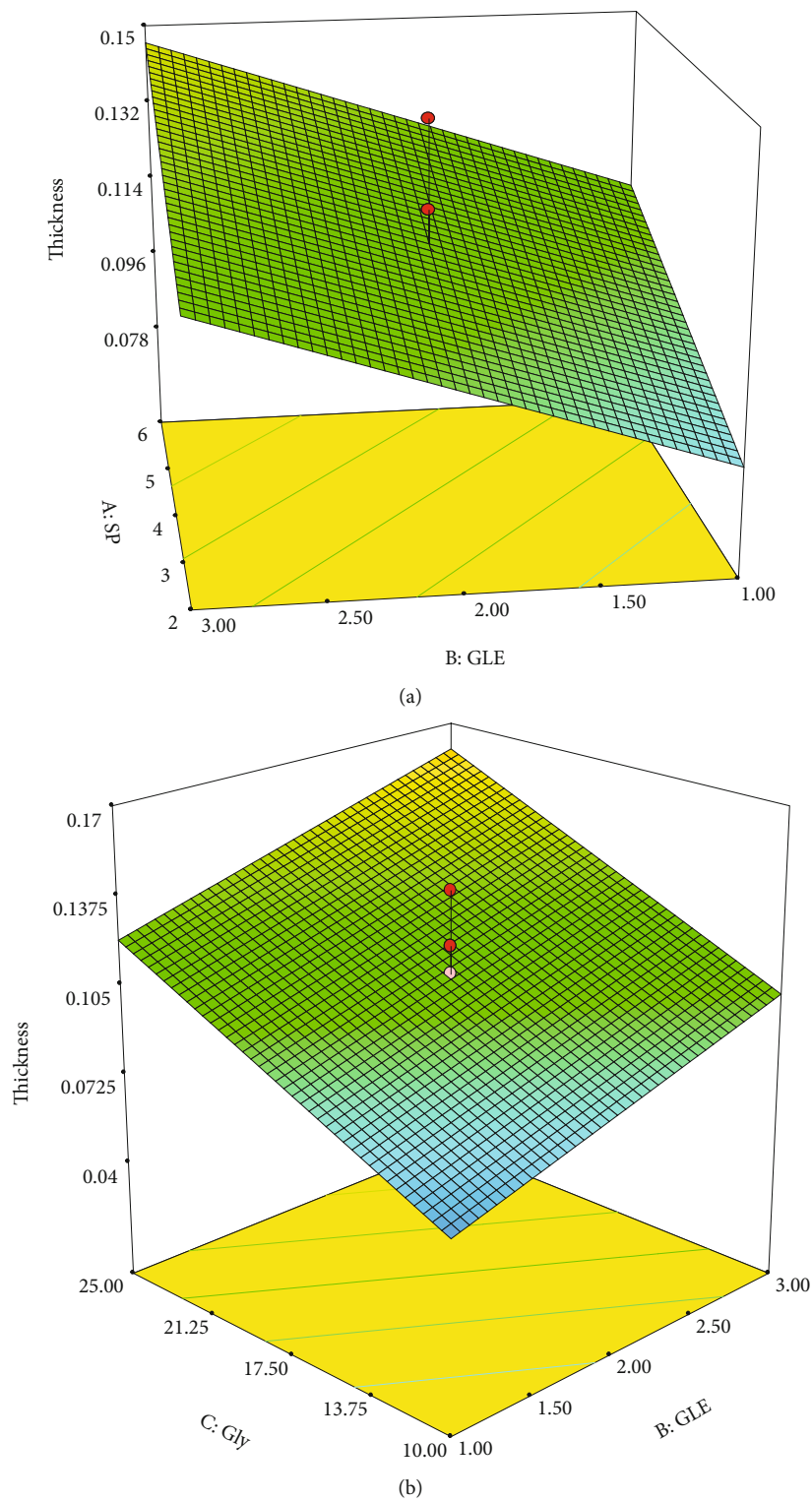


FIGURE 6: Continued.

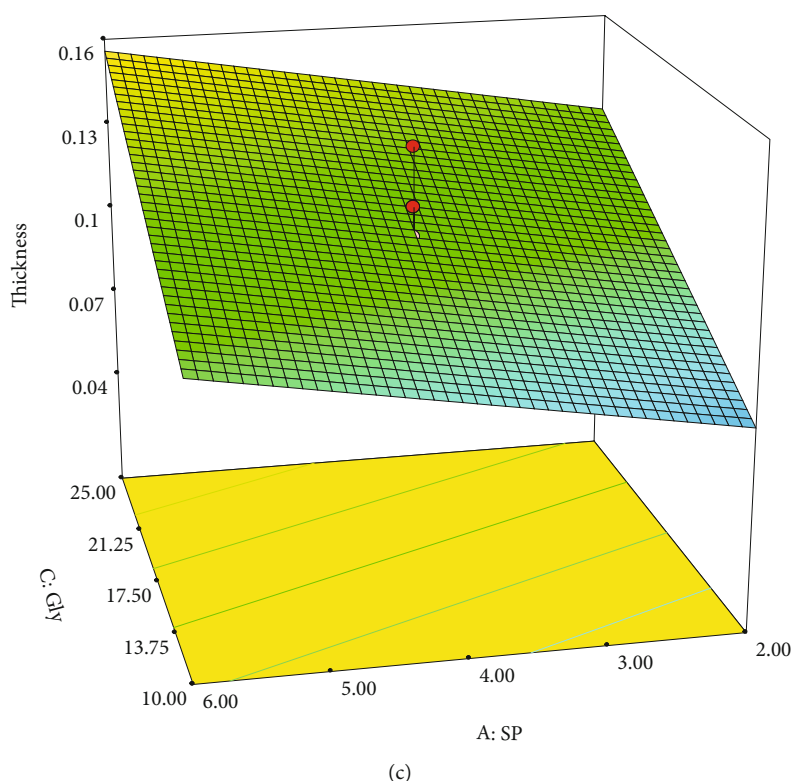


FIGURE 6: Variation of thickness with (a) guava leaf powder (GLP) and banana starch powder (SP), (b) glycerol (Gly) and banana starch powder (SP), and (c) guava leaf powder (GLP) and glycerol (Gly).

values associated with these terms ranged from 36.13 to 212.09. The lack of fit was found to have higher p values (0.4241) and lower F value (1.20). This affirmed nonsignificance of lack of fit for the model. On the other hand, the values of R^2 , adj R^2 , pred R^2 , PRESS, CV, and adequate precision were 0.9818, 0.9654, 0.9099, 3.26, 3.30%, and 28.32, respectively. The following expression (Equation (7)) represents the variation of antimicrobial activity with process-independent variables:

$$AA = 8.70 + 1.01A + 0.88B + 0.09C + 0.18AB + 0.06AC + 0.61BC - 0.40A^2 - 0.40B^2 - 0.53C^2 \quad (7)$$

Similarly, for solubility, the significant terms for the best-fit quadratic model were A , B , C , AB , AC , B^2 , and C^2 with high F value (511.73) and low p value ($p < 0.0001$). The lack of fit was nonsignificant with an F value of 0.81 and p value of 0.58. The fitness of the quadratic model was validated with other associated statistical parameters such as R^2 (0.9978), adj R^2 (0.9959), pred R^2 (0.9900), PRESS (27.94), CV (2.16%), and adequate precision (95.23). The solubility of the packaging material was best described in the form of the quadratic equation presented in Equation (8):

$$Sol = 32.82 - 7.75A - 4.36B - 8.69C - 2.36AB - 3.42AC + 0.45BC + 0.34A^2 - 1.48B^2 + 5.76C^2 \quad (8)$$

For TPC, the linear terms (A and B), interaction terms (AC and BC), and quadratic terms (A^2 and C^2) were found to be significant with F values ranging from 6.51 to 217.92. Lack-of-fit F value for the developed quadratic model was not significant with a p value of 0.1050. Similarly, other associated statistical parameters such as R^2 , adj R^2 , pred R^2 , PRESS, CV, and adequate precision values for the model were 0.9769, 0.9561, 0.8575, 27.24, 1.88%, and 21.94, respectively. The best-fit quadratic model for TPC is expressed in Equation (9):

$$TPC = 36.07 + 1.72A + 2.66B + 0.32C - 0.40AB + 0.60AC - 0.90BC - 1.32A^2 - 0.32B^2 + 0.66C^2 \quad (9)$$

The thickness of the material was influenced mostly by the linear terms (A , B , and C) with a higher model F value of 20.45 ($p < 0.0001$). Lack of fit for the developed model was not significant with the F value 3.31 ($p = 0.0984$). Associated model parameters such as R^2 , adj R^2 , pred R^2 , PRESS, CV, and adequate precision with values of 0.7932, 0.7544, 0.6461, 0.0085, 15.66%, and 15.80, respectively, justified the significance of the model. The variation of film thickness with starch, glycerol, and guava leaf extract can be expressed using following expression shown in Equation (10):

$$Thickness = 5.97 + 0.94A + 0.86B + 0.64C \quad (10)$$

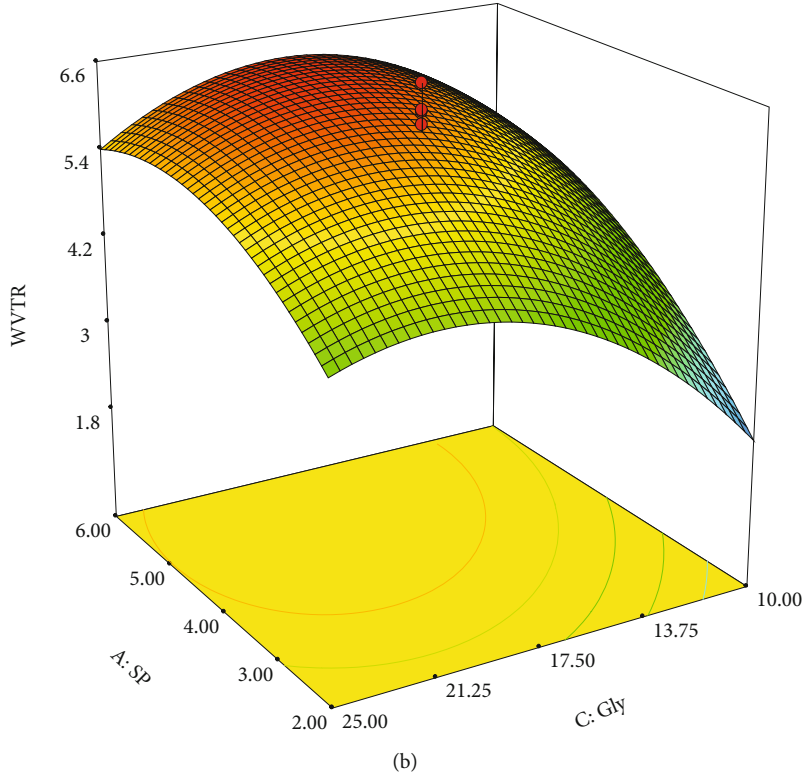
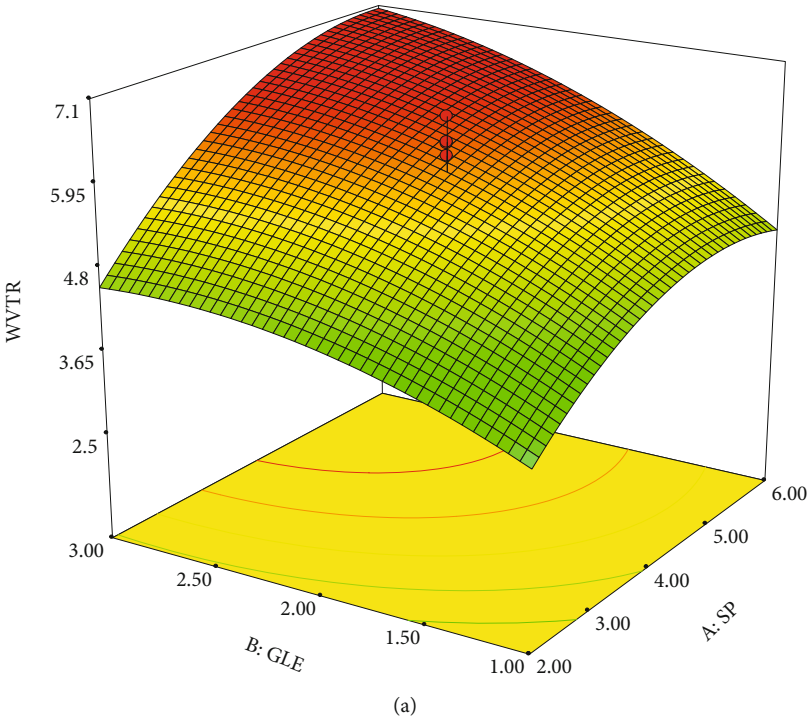


FIGURE 7: Continued.

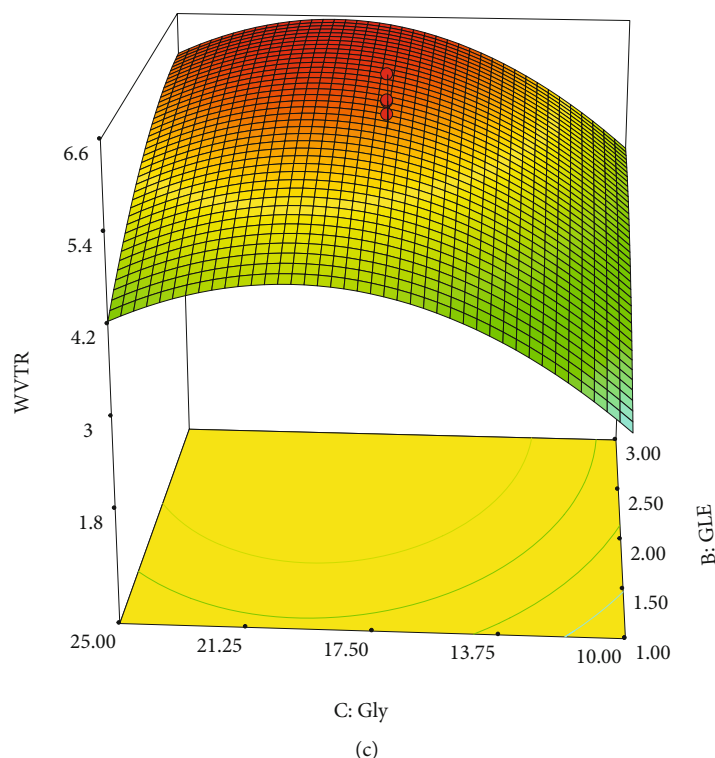


FIGURE 7: Variation of water vapour transfer rate (WVTR) with (a) guava leaf powder (GLP) and banana starch powder (SP), (b) glycerol (Gly) and banana starch powder (SP), and (c) guava leaf powder (GLP) and glycerol (Gly).

TABLE 4: Predicted and experimental values at optimum combination of various process variables.

Optimum	WAC	MC	AA	Solubility	TPC	Thickness	WVTR
Predicted	47.76	7.10	6.89	47.82	37.38	0.09	2.98
Experimental	46.98	6.38	7.53	45.42	38.44	0.085	3.25

The best-fit quadratic model describing the effect of independent variables on WVTR possesses linear terms (A , B , and C), interaction terms (AC), and quadratic terms (A^2 , B^2 , and C^2) as significant terms with a higher model F value (36.56). Lack of fit was found to be nonsignificant with p value (0.9515). Also, other statistical parameters including R^2 (0.9705), adj R^2 (0.9440), pred R^2 (0.9250), PRESS (4.01), CV (8.76), and adequate precision (17.96) support the significance of the quadratic model (Equation (11)) for the response variable:

$$\begin{aligned} \text{WVTR} = & 5.97 + 0.94A + 0.86B + 0.64C + 0.30AB - 0.41AC \\ & - 0.01BC - 0.64A^2 - 0.37B^2 - 1.08C^2 \end{aligned} \quad (11)$$

3.5. Response Surface Plot. ANOVA for various responses of packaging film is presented in Table 3. The raw material and plasticizer play a prominent role for water absorption capacity of starch-based packaging films. Graphical representations of variations of response variables such as water absorption capacity, moisture content, antimicrobial activity, solubility, TPC, thickness, and WVTR are depicted in

Figures 1, 2, 3, 4, 5, 6, and 7, respectively. All linear terms showed a positive influence on the water absorption capacity of the packaging material (Figure 1). Similarly, interaction between starch and guava leaf extract showed a positive effect, whereas other terms showed a negative effect. Starch, glycerol, and guava leaf powder are hygroscopic in nature; enhancement of these values enhances the water absorption of the film. High concentrations of plasticizer such as glycerine in the film formulation result in films with higher water absorption capacity and moisture content [35]. The moisture content of the film mainly depends on the capacity of the raw material to hold or absorb water. Starch in this case had a greater impact on determining the moisture in the film. All linear terms showed positive effects along with the interaction between starch and leaf extract and starch and glycerol. The interactive term between starch and glycerol, in addition to the quadratic terms, showed a negative effect (Figure 2). The study performed states that, due to its hydrophilic nature, starch tends to absorb large quantities of moisture at elevated RH conditions, resulting in high moisture content [36]. This eventually affects the moisture content of the film.

Antimicrobial activity followed an increasing trend for all linear and interactive terms due to the combined effect

of all the bioactive compounds present in starch and guava leaf extract (Figure 3). The higher antimicrobial activity of the film is due to strong antimicrobial activity of guava leaf extracts [37]. On the other hand, a negative effect was observed for all quadratic terms. Similarly, for solubility, all linear terms followed the negative trend along with the interactive terms of starch and guava leaf extract, starch, and glycerol. Similarly, the quadratic term of guava leaf extract possessed a negative influence on solubility. On the contrary, an increasing trend was observed for quadratic terms of starch and glycerol. Similarly, interaction between leaf extract and glycerol positively influenced the solubility of the film (Figure 4). Films with higher glycerol content have increased solubility due to the hydrophilic nature of glycerol and starch, which allows the film to easily absorb water [38]. The bioactive compounds in starch and guava leaf extract have contributed towards TPC of the film (Figure 5). All linear terms along with the interaction of starch and glycerol showed a positive trend. Further, it can be attributed to the formation of a polyphenol such as starch complex which resulted in enhanced phenolic content [39]. A negative influence was observed for interactive terms of starch extract and glycerol extract. Similarly, quadratic terms have shown a negative influence except square term of glycerol for which a positive effect was apparent. On the other hand, film thickness varied linearly with starch, guava leaf extract, and glycerol (Figure 6). This is due to enhancement of viscosity of the sample during film preparation. For WVTR, all the linear terms showed a positive influence (Figure 7). On the other hand, a negative effect was observed for other terms except interaction between starch and guava leaf extract. Similar results were reported wherein the incorporation of eucalyptus extract into tapioca starch films led to an augmentation in water vapor permeability [40]. Similar results were reported, wherein a higher glycerol concentration had a significant influence on the characteristics of the edible tapioca starch film, resulting in a decline in WVTR [41].

3.6. Optimization of the Process Parameters. The optimum values for independent parameters for the preparation of packaging film were 2.28% starch, 1.37% guava leaf extract, and 15.26% glycerol. The predicted and experimental values at optimum combination of independent variables are presented in Table 4. The corresponding response parameters such as thickness, solubility, water absorption capacity, TPC, WVTR, zone of inhibition against *E. coli*, and moisture content achieved were 0.081 mm, 42.35%, 50.66%, 31.92 mg GAE/g, 3.95 mg/m²s, 7.12 mm, and 7.94%, respectively. The obtained predicted data are in agreement with experimental data for the responses at the optimum combination of starch, guava leaf extract, and glycerol with standard deviation varied from 0.48 to 1.62.

4. Conclusion

Development and characterization of starch-based antimicrobial packaging film yield several notable insights. Firstly, the investigation provides a deeper understanding of relationship between independent and dependent variables.

Thereby, variations of relevant characteristics of the starch-based packaging film such as water absorption capacity, solubility, moisture content, thickness, TPC, and WVTR with starch, glycerol, and guava leaf concentration could be understood. Such insight facilitated better optimization of process-product characteristics. Secondly, the film's potential for commercial applications is further demonstrated by the optimal conditions found which include 2.28% starch, 1.37% guava leaf extract, 15.26% glycerol, 0.081-mm thickness, 42.35% solubility, 50.66% water absorption capacity, 31.92 mg GAE/g total polyphenol, 7.12-mm zone of inhibition against *E. coli*, and 7.94% moisture content. With potential antimicrobial properties, the packaging film would enhance the shelf life of food products. This would further foster towards commercial application of such packaging film for the packaging of various food materials. Thirdly, this study lays a groundwork for the expansion of these environmentally acceptable substitutes for industrial packaging solutions by encouraging the broader use of biopolymers, particularly starch-based materials.

Data Availability Statement

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

Ethics Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization and design: Challa Ravi Teja, Himanshu Bana, Imdadul Hoque Mondal, and Kshirod Kumar Dash; investigation and data analysis: Challa Ravi Teja, Hoque Mondal, and Kshirod Kumar Dash; preparation of draft of the manuscript: Challa Ravi Teja and Himanshu Bana; visualization, validation, resources, and funding for publication: Ayaz Mukarram Shaikh and Kovács Béla; reviewing and editing: Imdadul Hoque Mondal, Kshirod Kumar Dash, and Kovács Béla. The final draft of the manuscript was approved by all the authors.

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