Research Article

Process Development for Edible Film Preparation Using Avocado Seed Starch: Response Surface Modeling and Analysis for Water-Vapor Permeability

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Starch-based edible films are gaining huge interest in food packaging industries. In the present work, avocado seed starch (ASS) was extracted and used to develop an edible film. The influence of four important process factors, starch, agar, sorbitol, and Tween-20, was studied on one of the important barrier properties, water-vapor permeability (WP), of developed edible film. The three-level RSM design with Box–Behnken approach was carried out to investigate the film property, WP. ASS-based edible films were prepared by the casting method. The results revealed that the increment in the contents of Tween-20 and sorbitol reduces the WP of the film. Using the response surface analysis, the effect of the aforementioned factors was analyzed; they showed significant impact on WP. To predict the influence of the selected process parameters, a second-order polynomial equation was constructed. Additionally, Pareto analysis of variance was employed over the obtained results to investigate the significance of the developed process model.

1. Introduction

Petroleum-based non-biodegradable polymeric wastes are significantly increasing the ecological impacts and creating huge global concern. Hence, there is an imperative requirement to develop eco-friendly bio-based materials to alternate the nonrenewable plastic materials [1, 2]. Cellulose derivatives, proteins, gums, lipids, and starch are well-known biopolymers that are commonly used as raw material for biodegradable films. Specifically, for packaging processed or fresh foods, these biopolymers offer significant contribution of preparing thin edible films [3]. One of the abundant, nontoxic, and renewable resources is starch that has the potential capability of forming continuous matrix [4–6]. Starch has the potential advantage of being comparatively low cost. It is able to form films that have a similar characteristic to synthetic polymers such as being odorless, transparent, and tasteless and having oxygen permeability resistance. However, the films prepared using starch generally require the amalgamation of plasticizer for reducing their brittleness. Another important ingredient for preparing eco-friendly packaging films is agar, which is a biodegradable polysaccharide [7–9]. It is used as a cohesive mediator in the polymeric chain and provides better improvisation in properties related to mechanical and barrier possessions [10, 11]. During the preparation of edible films, hydrophobic constituents (surfactants) are added to decrease the hygroscopicity of the films that can provide appreciable desirability [12]. Different studies have reported about the investigation of behavior of water-vapor permeability (WP) on edible film with respect to different factors [13]. The combined effect of starch extracted from avocado

seed, agar, Tween-80, and sorbitol has not been described elsewhere. Keeping this view, it is important to study about the barrier properties with respect to different proportions of process variables to gain appropriate knowledge about the possibilities of applications for food packing materials. Hence, this investigation was aimed to investigate the effect of selected process factors, namely, avocado seed starch, agar, Tween-20, and sorbitol, on the important barrier property, WP, of an edible film. The combination of the aforementioned parameters was designed using the three-level Box–Behnken method; further, the WP of the film developed from each commination was determined and analyzed by response surface approach.

2. Materials and Methods

2.1. Materials. Avocado seeds were collected from the fruit market of Addis Ababa, Ethiopia (Figure 1). Tween-20 (food grade with 99% purity) and sorbitol (food grade with 98% purity) were obtained from Hi-Media Chemicals, Addis Ababa. Agar was procured from Merck Chemicals, Addis Ababa.

2.2. Extraction of Avocado Seed Starch (ASS). Avocado seeds were grounded well using the laboratory mixer-grinder. The powder was well saturated by soaking with large amounts of distilled water. Prior to this process, sodium metabisulphite was dissolved (0.075% (w/v)) with the distilled water. Further, the soaked content of material was kept to settle for 4 h at room temperature, approximately, $28 \pm 2°C$. Then, the clear supernatant liquid was carefully transferred to a clean beaker. The sedimented starch was washed repeatedly using sodium metabisulphite solution till the clear suspension was observed. In order to remove the cell debris, the obtained sediment was further filtered using a fine muslin cloth. From the resultant filtration, the clear suspension was collected. Again, it was filtered using $224 \mu m$ fine sieve. Subsequently, it was subjected to keep deposit. The starch was sedimented and then it was repeatedly washed using distilled water until the wash water was observed to be free of impurities and clear. As a result, the starch was isolated, then sieved, and dried in air at room temperature. In the extracted starch, the amylopectin and amylose concentrations were determined using the method explained by Nogueira et al. [14]. Presence of moisture in the starch was determined by the AOAC procedure [15].

2.3. Preparation of Edible Films. The formulation solutions to prepare the film were formulated with different combinations of agar (0.5–1.0 g), sorbitol (0.5–1.0 ml), Tween-20 (0.1–0.5 ml), and ASS (1–3 g). Casting is one of the well-recognized methods for edible film preparation. This method was adopted to develop the films by following the method specified by Sundramurthy et al. [16]. The developed clear films were carefully detached from the Petri dishes; further, they were equilibrated at 25°C with 57% relative humidity for 3 days before the experimental investigations.

FIGURE 1: Avocado (*Persea americana*) seed.

2.4. Determination of Water-Vapor Permeability (WP). For food packaging purpose, one of the important barrier properties, water-vapor permeability, is a huge concern for edible films. WP of the developed film was assessed using the method defined by Mali et al. [17]. As per the standard, ASTM D6701, WP was considered. The film thickness was assessed using an appropriate digital micrometer. WP (g/m s Pa) was detected using

$$WP = \frac{WVTR}{K(P_1 - P_1)l},\tag{1}$$

where *l* refers to the thickness of the film (m), *K* refers to the saturation vapor pressure of water (Pa) at the test temperature (25°C), P_1 refers to relative humidity of the desiccator, and P_2 refers to the relative humidity in the permeation cell. The average value of triplicate experiments was recorded as response.

2.5. Experimental Design and Statistical Analysis. In this investigation, the effect of selected factors (ASS content (F1), 1–3 g; sorbitol concentration (F2), 0.5–1.0 ml; concentration of agar (F3), 0.5–1.0 g; and Tween-20 (F4) contentment, 0.1–0.5 ml) on the WP of the edible films was studied. The Box–Behnken design (BBD) can be more effective than other design approaches. Moreover, it also needs fewer runs compared to other response surface designs. Hence, the BBD design was considered for carrying out the combination of different concentrations of selected factors. Using a

nonlinear regression technique, a second-order polynomial equation was constructed using the experimental results. This model can be used to analyze the true functional relationship between WP and chosen independent factors. This equation can be written as follows:

$$\text{WP} = \beta_0 + \sum_{j=1}^{k} \beta_j F + \sum_{j=1}^{k} \beta_{jj} F_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ji} F_i F_j + e_i, \quad (2)$$

where WP is water-vapor permeability (in the present study, as response); F_i and F_j are process factors (i and j range from 1 to k); β_0 implies model intercept coefficient; β_j, β_{jj}, and β_{ij} are refer to interaction coefficients; k refers to the number of selected independent factors (where k is 4 in this work); and e_i is the error function [18]. Results from experimentations were subjected to be analyzed by a statistical software application, Design-Expert 12.0.7.1 (Stat-Ease Inc., USA). To determine the coefficients, the multiple regression investigation was executed. These coefficients can be taken to determine the response (WP). In order to examine the compatibility of the different models constructed to the experimental results, the model summary statistics and sequential model sum of squares were tested. Pareto analysis of variance was carried out to assess the attained experimental results. Different statistical analyses such as F value, p value, sum of squares, degrees of freedom, determination of coefficient, coefficient of variation, predicted determination of coefficient, and adjusted determination of coefficient were employed to obtain the significance of the statistical status of the developed model. Further, the model was used to generate 3D plots for response surfaces to correlate the pure relation between WP and selected four independent factors.

3. Results and Discussion

3.1. Chemical Composition of ASS.
Figures 2(a) and 2(b) show the starch extracted from the avocado seed and edible film prepared using ASS, respectively. As per the standard procedure, the chemical composition of the ASS was determined. The results showed that the presence of the isolated starch was observed to be $97.32 \pm 0.51\%$. Furthermore, the presence of moisture content of $10.45 \pm 0.54\%$ and ash content of $0.23 \pm 0.02\%$ was also observed. It was found that the content of amylopectin and amylose in the ASS was determined to be 71% and 29%, respectively. Generally,

edible film is formed by the involvement of two different process, namely, retrogradation and gelatinization. In retrogradation, solubility of dissolved starch was decreased. During the gelatinization process, leaching of amylose present in the starch, disruption, and swelling are involved. Hence, amylose is a significant liability during edible film preparation using starch because of its capability to establish the continuous viscous mass on the film forming solutions. Additionally, amylose shows an appreciable rate in the retrogradation progression. In this study, a substantial content of the amylose was found in the ASS as reported by Bastarrachea et al. [19].

3.2. Experimental Design Analysis.
Different combinations of process variable according to the Box–Behnken experimental design with obtained responses are presented in Table 1. Each edible film prepared from the BBD design combination was subjected to examine WP. These data were examined. Different models, namely, cubic, quadratic, interactive (2FI), and linear models, were used to get regression correlation equation. Based on the tests, namely, model summary statistics and the sequential model sum of squares, the appropriateness of equation among various models to express the effect of chosen process factors over the WP was carried out, and it is presented in Table 2.

From the results of the model adequacy test, it was observed that interactive (2FI) and linear models have comparatively lower value of R^2, predicted R^2, and adjusted R^2. It was found that the cubic model can be aliased. Thus, the quadratic model for including linear, interactive, and quadratic terms was selected to express the effect of process factors on the WP of ASS-based bio-films. Additionally, adequacy of obtained quadratic model was investigated by Pareto analysis of variance.

3.3. Model Development and Statistical Analysis.
The mathematical model of second-order polynomial equation (equation (3)) can be used to fit the experimental results to generate the empirical models. This equation of the model can exhibit the true correlation between WP and the selected independent factors. The obtained empirical model of quadratic form in terms of actual parameters is presented as follows:

$$
\begin{aligned}
\text{WP} = {}& 1.00588 - 0.300167\,\text{Starch} - 1.30517\,\text{Sorbitol} \\
& + 0.107833\,\text{Agar} + 0.370833\,\text{Tween 20} + 0.127000\,\text{Starch} * \text{Sorbitol} \\
& + 0.034000\,\text{Starch} * \text{Agar} - 0.015000\,\text{Starch} * \text{Tween 20} + 0.068000\,\text{Sorbitol} * \text{Agar} \\
& - 0.135000\,\text{Sorbitol} * \text{Tween 20} - 0.445000\,\text{Agar} * \text{Tween 20} + 0.047708\left(\text{Starch}^2\right) \\
& + 0.579333\left(\text{Sorbitol}^2\right) + 0.025333\left(\text{Agar}^2\right) - 0.266667\left(\text{Tween 20}^2\right).
\end{aligned}
\quad (3)
$$

Fisher's F-test was used to examine the statistical significance of the obtained polynomial model using ANOVA analysis. From the analysis, it was observed that the

predicted model was very good in being statistically significant (Table 3). The model F-value was found to be higher ($F_{\text{Model}} = 4102.45$), and acceptable low probability value
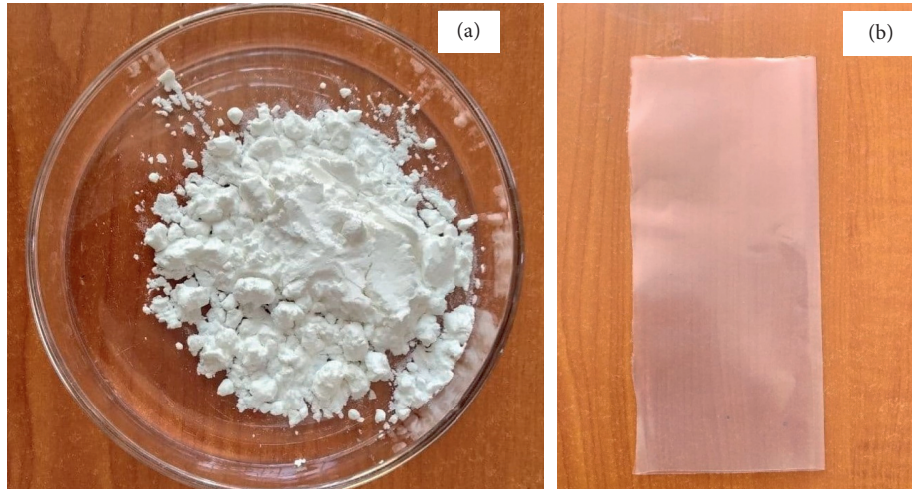
FIGURE 2: The starch extracted from ASS (a) and the developed edible film prepared from ASS (b).

TABLE 1: Design combination of process variables and obtained responses (WP).

Run no.	ASS content (g)	Sorbitol (ml)	Agar (g)	Tween-20 (ml)	WP of film (g/m·s·Pa) $\times 10^{-10}$
1	3	1	0.75	0.3	0.345
2	2	0.75	0.75	0.3	0.266
3	2	0.75	0.5	0.1	0.252
4	2	1	1	0.3	0.297
5	2	0.75	1	0.1	0.363
6	2	0.75	0.75	0.3	0.266
7	3	0.75	0.75	0.5	0.255
8	2	0.75	1	0.5	0.217
9	2	0.5	0.5	0.3	0.319
10	1	1	0.75.	0.3	0.269
11	2	1	0.5	0.3	0.225
12	3	0.5	0.75	0.3	0.367
13	1	0.75	0.75	0.5	0.249
14	2	1	0.75	0.5	0.191
15	2	0.5	1	0.3	0.374
16	2	0.75	0.75	0.3	0.266
17	2	0.75	0.5	0.5	0.195
18	1	0.75	1	0.3	0.332
19	2	1	0.75	0.1	0.306
20	1	0.5	0.75	0.3	0.418
21	2	0.75	0.75	0.3	0.266
22	3	0.75	1	0.3	0.366
23	2	0.75	0.75	0.3	0.266
24	2	0.5	0.75	0.1	0.379
25	3	0.75	0.5	0.3	0.282
26	1	0.75	0.75	0.1	0.345
27	3	0.75	0.75	0.1	0.363
28	1	0.75	0.5	0.3	0.282
29	2	0.5	0.75	0.5	0.291

($p < 0.0001$) showed that the predicted model was statistically significant to express WP of the films. The high value of R^2 (0.9998) specified that correlation between experimental and predicted values was found to be high. The values of predicted R^2 (0.9986) and the adjusted R^2 (0.9995) were found to be very close to the value of R^2 which is in good agreement.

3.4. Response Surface Analysis for Water-Vapor Permeability. BBD coupled with RSM was used to assess the effect of four selected independent factors such as ASS (1–3 g), Tween-20 (0.1–0.5 ml), agar (0.5–1.0 g), and sorbitol (0.5–1.0 ml) on WP of the ASS-based edible films. Using a generated mathematical equation (equation (2)), plots for 3D response surface were drawn as a function of two independent factors, while

TABLE 2: Adequacy of tested models for WP as response.

Source	Std. dev	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.0358	0.6836	0.6309	0.5285	0.0460	
2FI	0.0367	0.7512	0.6130	0.2847	0.0697	
Quadratic	0.0013	0.9998	0.9995	0.9986	0.0001	Suggested
Cubic	0.0003	1.000	1.000	0.9991	0.0001	Aliased

PRESS: predicted residual error sum of squares.

TABLE 3: ANOVA analysis for predicted second-order polynomial equation.

| Source	Sum of squares	df	Mean square	F value	p value	
Model	0.0974	14	0.0070	4102.45	<0.0001	Significant
A-starch	0.0006	1	0.0006	338.41	<0.0001	
B-sorbitol	0.0221	1	0.0221	13028.60	<0.0001	
C-agar	0.0129	1	0.0129	7625.63	<0.0001	
D-Tween-20	0.0310	1	0.0310	18278.60	<0.0001	
AB	0.0040	1	0.0040	2376.91	<0.0001	
AC	0.0003	1	0.0003	170.36	<0.0001	
AD	0.0000	1	0.0000	21.22	0.0004	
BC	0.0001	1	0.0001	42.59	<0.0001	
BD	0.0002	1	0.0002	107.43	<0.0001	
CD	0.0020	1	0.0020	1167.31	<0.0001	
A^2	0.0148	1	0.0148	8702.87	<0.0001	
B^2	0.0085	1	0.0085	5012.92	<0.0001	
C^2	0.0000	1	0.0000	9.59	0.0079	
D^2	0.0007	1	0.0007	435.04	<0.0001	
Residual	0.0000	14	$1.696E-06$			
Lack of fit	0.0000	10	$2.375E-06$			Not significant
Pure error	0.0000	4	0.0000			
Cor total	0.0975	28				

(a)

(b)
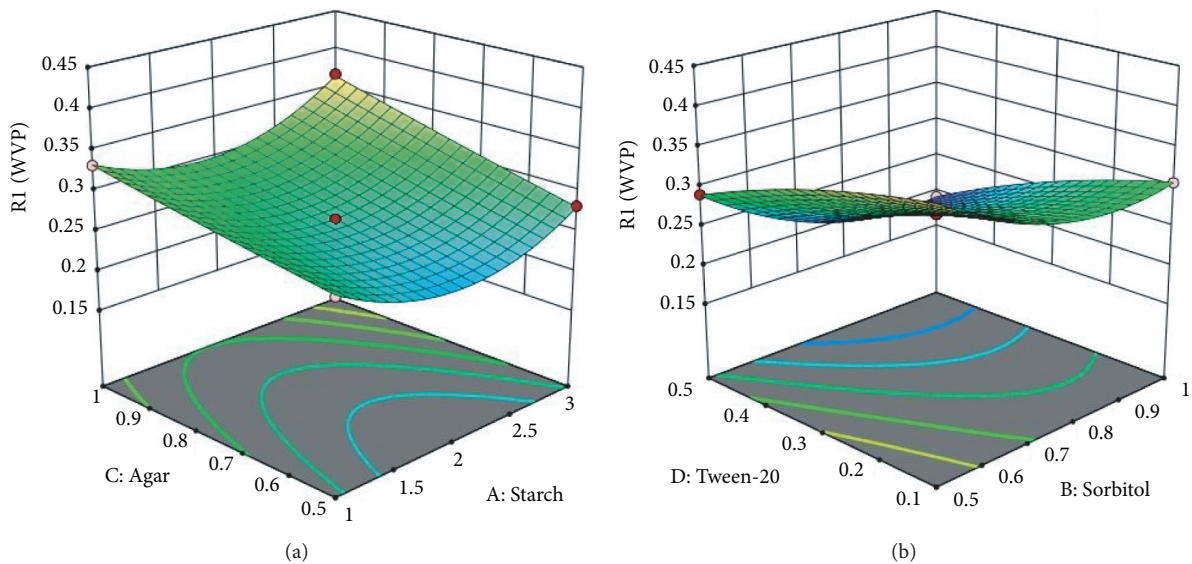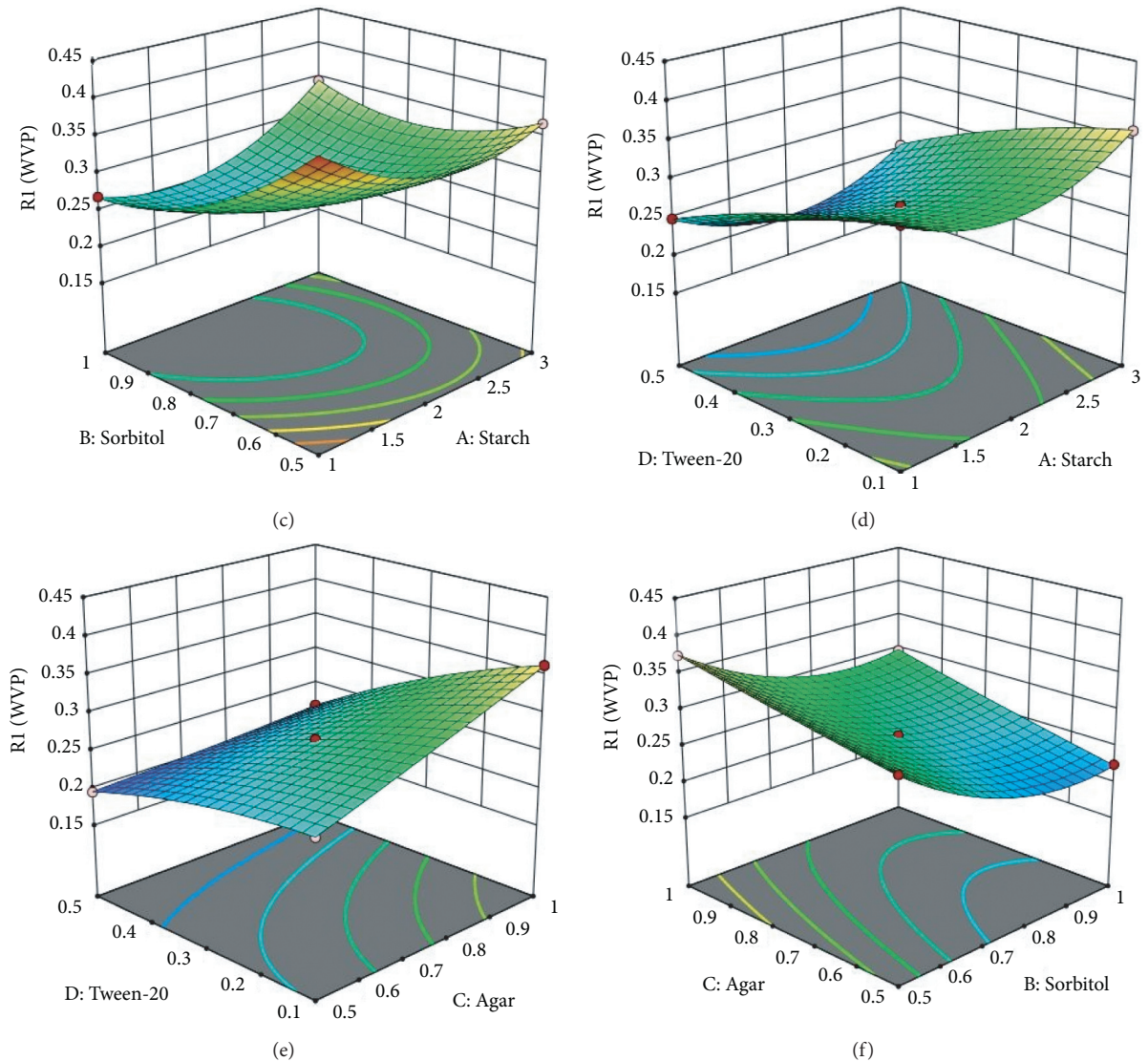
FIGURE 3: Continued.

(c)

(d)

(e)

(f)

FIGURE 3: 3D surface showing the interaction impacts of process variables on WP.

the other two parameters were kept at constant. Experimental results revealed that the increase in concentration of starch and agar led to the increase in WP (Figure 3(a)).

This is because of intermolecular connections becoming high between starch and agar at high concentrations. In addition, high concentrations of starch and agar increase the free space that led to extending the intermolecular distance. Normally, increment in plasticizer concentration reduces the intermolecular bonds among the polymer chains [20]. But in this case, WP of ASS films was slowly decremented with sorbitol concentration increment (Figure 3(b)). This happened due to the ineffectiveness in reducing intermolecular hydrogen bonding between amylopectin and amylose [21–23]. Additional increment in Tween-20 concentration reduces the affinity water and available number of polar groups. Hence, WP of the films were getting decremented (Figure 3(b)). From the developed model, it was observed that the concentration of starch and sorbitol had the negative effect on WP. Since the WP needs to be minimized, these factors are

considered as most effective factors. Steric-hinderance structure of the Tween-20 is also responsible for the hydrophilic/lipophilic balance (HLB) ratio and hydrophobicity [24–26].

4. Conclusion

The edible film was developed using the starch extracted from avocado seeds (ASS). The combinations of different concentrations of ASS (1-3g), Tween-20 (0.1-0.5ml), agar (0.5-1.0g), and sorbitol (0.5-1.0ml) were examined for the WP. The effect of the four important process factors on the water-vapor permeability (WP) of the developed films was investigated using the three-level Box–Behnken design coupled with RSM. The developed edible films seemed to be homogenous and transparent. From the experimental results, it was found that WP of the ASS-based films decreased with increment in Tween-20 and sorbitol. It is apparent that WP is well controlled by surfactant and plasticizer

concentrations. A second-order equation was constructed for WP with high value of determination coefficient ($R^2 = 0.99$). 3D response surface plots were constructed using the experimental data that can predict the relationship between process factors and WP. From the analysis, the results showed that sorbitol and Tween-20 significantly affect the WP property of ASS-based edible film.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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