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Optimization of Biodegradable Films from Avocado Seed Starch Using Response Surface Methodology

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Abstract: This study aimed to develop and optimize biodegradable films based on avocado seed starch (Persea americana) using glycerol as a plasticizer and chitosan and carrageenan as fillers. The film formulation was optimized using Response Surface Methodology (RSM) with a Box–Behnken Design to evaluate the effects of formulation variables on biodegradability and mechanical properties. The biodegradability values ranged from 31.93 % to 51.94 %, indicating that all films were biodegradable. Increasing glycerol and carrageenan concentrations significantly increased biodegradability, while higher chitosan concentration improved tensile strength but reduced biodegradability. The optimal formulation was obtained at 2.96 % glycerol, 1.56 % chitosan, and 2.85 % carrageenan, with a predicted biodegradability of 53.11 %. The results indicate a trade-off between mechanical strength and biodegradability, where higher plasticizer content enhances degradation but reduces tensile strength. This study demonstrates that RSM optimization is effective in producing biodegradable films with balanced mechanical and environmental performance, highlighting the potential of avocado seed starch as a sustainable packaging material.

Keywords: Avocado Seed Starch; Biodegradable Films; Box-Behnken Design; Optimization; Plasticizer.

1. Introduction

Packaging plays an important role in the food and beverage industry, not only for protecting products from physical and microbiological damage but also as a major source of plastic waste, since most packaging materials are made from synthetic plastics. Synthetic plastics are widely used due to their flexibility, mechanical strength, light weight, transparency, and resistance to corrosion and chemicals. However, conventional plastics are non-biodegradable and can cause long-term environmental pollution because microorganisms are unable to degrade synthetic polymer chains [1]. This environmental concern highlights the urgent need for environmentally friendly alternative packaging materials.

One promising alternative is biodegradable film, which is a thin plastic-like film produced from renewable natural materials such as polysaccharides, proteins, and lipids. Biodegradable films can degrade naturally through microbial activity, making them more environmentally friendly than conventional plastics [2], [3]. Among various natural polymers, starch is one of the most widely used raw materials due to its abundance, low cost, and good film-forming properties. Starch derived from plant sources such as cereals and tubers has shown great potential for bioplastic production. One

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locally available but underutilized starch-rich material is avocado seed (*Persea americana*). Avocado seeds contain approximately 73.61% starch and 20.56% amylose, making them a promising raw material for biodegradable film production. Previous studies reported that avocado seed starch can be processed through gelatinization to produce films with good flexibility and biodegradation rates of up to 52.85% [4]. These findings indicate that avocado seeds represent a sustainable local resource with significant potential for biodegradable packaging applications.

However, biodegradable films produced solely from starch generally have poor mechanical strength and high water permeability. Therefore, additives such as plasticizers and fillers are required to improve their physical and mechanical properties [5]. Glycerol is commonly used as a plasticizer due to its hydrophilic nature and its ability to increase flexibility by reducing intermolecular forces in polysaccharide-based films. Carrageenan, a polysaccharide extracted from red seaweed, functions as a filler and gelling agent that contributes to film structure and stability. Meanwhile, chitosan is a biodegradable and non-toxic biopolymer capable of forming transparent films with antimicrobial properties [6], [7].

Despite numerous studies on starch-based biodegradable films, most research has focused on single plasticizer systems or combinations with other polymers such as PLA, agar, or gelatin. Limited studies have investigated the combined effects of glycerol, carrageenan, and chitosan in avocado seed starch-based biodegradable films, particularly in formulation optimization using Response Surface Methodology. In addition, previous studies mainly focused on mechanical and physical properties, while optimization of biodegradability through formulation design has not been widely explored [8], [9]. Therefore, this study aims to optimize the formulation of biodegradable films based on avocado seed starch using Response Surface Methodology with a Box–Behnken Design to obtain films with balanced biodegradability and mechanical properties.

2. Research and Methodology

2.1 Materials

Avocado seeds (*Persea americana*) were obtained from a local market in Malang, East Java, Indonesia. Glycerol (analytical grade) was purchased from Merck, Darmstadt, Germany. Carrageenan was supplied by Sigma-Aldrich, St. Louis, USA. Chitosan (degree of deacetylation ≥ 85%) was obtained from Biotech Surindo, Bandung, Indonesia. Distilled water used in this study was of analytical grade.

2.2 Experiments

2.2.1 Extraction of Starch from Avocado Seeds (*Persea americana*)

Starch extraction was carried out by first cleaning and cutting the avocado seeds into small pieces, followed by grinding using a high-speed blender until a fine slurry was obtained. The resulting slurry was then allowed to settle for 24 h in a closed container to facilitate phase separation between the starch fraction and the supernatant. After sedimentation, the starch-rich layer was carefully separated and dried in an oven at 100 °C for 24 h. This drying process aimed to remove moisture content effectively, resulting in dry starch suitable for subsequent biodegradable film formulation.

2.2.2 Gelatinization Process and Film Formation

A total of 2 g of avocado seed starch was used for biodegradable film preparation. The starch was dispersed in 50 mL of solvent, followed by the addition of glycerol as a plasticizer, and chitosan and carrageenan as fillers. The mixture was heated and continuously stirred at 80 °C for 20 min until a homogeneous film-forming solution was obtained, indicating complete gelatinization of the starch.

The resulting solution was then cast onto a glass plate measuring 10 cm × 10 cm and dried at 80 °C for 48 h to allow solvent evaporation and film formation [5].

2.2.3 Optimization of Biodegradable Film Using Box–Behnken Design and Biodegradation Test

The formulation optimization of the biodegradable film and the interaction analysis among formulation components were performed using Response Surface Methodology (RSM) based on the Box–Behnken Design (BBD). Three independent variables were used: glycerol concentration (A: 1.6–3.0 % v/v), chitosan concentration (B: 1.0–2.0 % w/v), and carrageenan concentration (C: 1.6–3.0 % w/v). The selected factor ranges were determined based on preliminary experiments and literature reports indicating that these ranges produce films with good film-forming ability and mechanical stability. The biodegradation percentage was selected as the response variable. The BBD generated 17 experimental runs, which is the standard number of experiments for a three-factor Box–Behnken Design to develop a quadratic model efficiently while minimizing the number of experiments [10]. The number of experimental runs was determined according to the standard Box–Behnken Design for three variables, which provides sufficient data for developing a quadratic regression model while minimizing the total number of experiments. All experimental planning, data analysis, and development of the quadratic regression model were performed using Design-Expert® software (trial version, Stat-Ease Inc., Minneapolis, MN, USA). Biodegradation testing was carried out using the soil burial method. Film specimens measuring 2 cm × 2 cm were initially weighed to obtain their starting mass **(**W_0**)** and then buried in natural soil under ambient conditions. The samples were removed at two-day intervals over a burial period of 14 days, carefully cleaned to eliminate soil residues, dried, and reweighed to determine the remaining mass **(**W_1**).** The biodegradation percentage was subsequently calculated using the following equation:

$$\text{Biodegradation (\%)} = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

where W_0 is the initial weight of the sample and W_1 is the final weight after burial. The biodegradation values obtained from this test were used as the response data in the RSM–BBD analysis to determine the optimal formulation conditions for biodegradable film production.

2.2.4 Water Absorption Test

Water absorption of the biodegradable film was determined to evaluate its resistance to water uptake. Film samples were cut into dimensions of 3 cm × 3 cm and weighed to obtain the initial weight (W_0). The samples were then immersed in distilled water at room temperature. After a predetermined immersion period, the samples were removed, gently wiped to remove surface water, and weighed to obtain the final weight (W_1) [11]. The water absorption percentage was calculated using the following equation:

$$\text{Water absorption (\%)} = \frac{W_1 - W_0}{W_0} \times 100 \quad (2)$$

where W_0 is the initial weight of the film sample and W_1 is the final weight after immersion.

2.2.5 Tensile Strength Test

The tensile properties of the biodegradable film were evaluated using a Universal Testing Machine (MPY type). Film specimens were prepared with dimensions of 0.5 cm × 10 cm and mounted between the grips of the testing machine. The test was performed at room temperature until the film sample fractured [1]. The maximum force recorded during the test was used to calculate the tensile strength according to the following equation:

$$\sigma = \frac{F_{\max}}{A} \tag{3}$$

where σ is the tensile strength (MPa), $Fmax$ is the maximum force applied (N), and A is the cross-sectional area of the film subjected to stress (mm²).

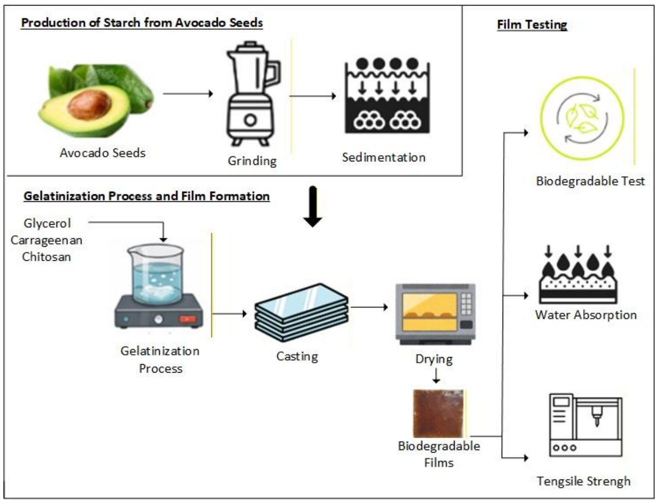


Figure 1. Schematic diagram of bioplastic film preparation.

3. Results and Discussion

3.1 Characterization of Avocado Seed Starch

The initial stage of bioplastic production involved starch extraction from avocado seeds (*Persea americana*). The starch sediment exhibited a reddish-brown color due to enzymatic browning caused by the oxidation of phenolic compounds, such as dopamine, catalyzed by polyphenol oxidase, a phenomenon commonly observed in phenolic-rich materials [12]. Avocado seeds contain various phenolic compounds, including catechins, tyrosine, caffeic acid, and leucoanthocyanins, which may promote this oxidation process [13]. Avocado seed starch contains approximately 78.66 % starch with an amylose content of about 23.11 %, as confirmed by the iodine test through a characteristic blue–purple color change. The presence of amylose is essential for starch gelatinization and contributes to the formation of bioplastic films with improved elasticity and tensile strength [10].

3.2 Effect of Glycerol, Chitosan, and Carrageenan on the Biodegradability of Bioplastic Films

The biodegradation test was conducted to evaluate the impact of varying glycerol, chitosan, and carrageenan concentrations on the biodegradability of bioplastic films. Based on the results in Table 1, the biodegradation of the bioplastic films ranged from 31.93 % to 51.94 %, indicating that all formulations are biodegradable. The concentration variations of glycerol, chitosan, and carrageenan significantly influenced the biodegradation rates. Increasing the glycerol concentration generally enhanced biodegradation [14]. This can be attributed to the role of glycerol as a plasticizer, which increases the hydrophilic properties and flexibility of the film matrix, facilitating water penetration and microbial activity, which are essential for degradation. The highest biodegradation (51.94 %) was observed in the formulation with 3.0 % (v/v) glycerol, 1.5 % (w/v) chitosan, and 3.0 % (w/v) carrageenan, which reflects the positive contribution of both glycerol and carrageenan in enhancing biodegradation [15]. On the other hand, increasing the chitosan concentration to 2.0 % w/v led to a decrease in biodegradability, as shown by the lowest biodegradation value of 31.93 %. This can be attributed to the relatively rigid structure of chitosan and its antimicrobial properties, which may hinder the biological degradation process [16]. Carrageenan, being a hydrophilic polysaccharide, positively contributes to biodegradation due to its ability to facilitate microbial activity and water absorption [5], [17]. The center point formulation showed a relatively stable biodegradation value, suggesting the effectiveness of the Box–Behnken Design in modeling the biodegradation response. The results of the biodegradation tests for the different formulations are presented in Table 1**.**

Table 1. Effect of Glycerol, Chitosan, and Carrageenan Concentrations on the Biodegradability

No	Glycerol (% v/v)	Chitosan (% w/v)	Carrageenan (% w/v)	Biodegradability (%)
1	1.6	2.0	2.3	37.03
2	2.3	1.0	3.0	41.28
3	1.6	1.5	1.6	34.05
4	3.0	1.0	2.3	39.98
5	2.3	2.0	1.6	31.93
6	3.0	2.0	2.3	48.89
7	2.3	2.0	3.0	41.11
8	2.3	1.5	2.3	48.24
9	2.3	1.5	2.3	47.55
10	1.6	1.0	2.3	34.58
11	3.0	1.5	3.0	51.94
12	2.3	1.0	1.6	37.08
13	1.6	1.5	3.0	42.78
14	2.3	1.5	2.3	49.78
15	3.0	1.5	1.6	43.39
16	2.3	1.5	2.3	47.80
17	2.3	1.5	2.3	51.31

In order to investigate the effects of different formulation components on the properties of biodegradable films, three formulations were selected for testing based on varying glycerol, chitosan, and carrageenan concentrations. The formulations represent the center point, high biodegradability, and low biodegradability conditions. These formulations were characterized by three key properties: biodegradation**,** water absorption, and tensile strength. The center point formulation represents a baseline with intermediate concentrations of glycerol (2.3 % v/v), chitosan (1.5 % w/v), and carrageenan (2.3 % w/v). The table below summarizes the results of the biodegradation, water absorption, and tensile strength tests for these formulations.

Table 2. Effect of Glycerol, Chitosan, and Carrageenan Concentrations on the Biodegradability, Water Absorption, and Tensile Properties

Formulation	Glycerol (% v/v)	Chitosan (% w/v)	Carrageenan (% w/v)	Bio-degradability (%)	Water Absorption (%)	Tensile Strength (MPa)
Center point	2.3	1.5	2.3	49.78	26.40	5
High biodegradability	3.0	1.5	3.0	51.94	35.70	3
Low biodegradability	2.3	2.0	1.6	31.93	23.40	6

The results presented in Table 2 demonstrate the effects of formulation composition on biodegradation, water absorption, and tensile strength of the biodegradable films. The center point formulation (2.3 % glycerol, 1.5 % chitosan, 2.3 % carrageenan) exhibited balanced properties, with a biodegradation rate of 49.78 %, water absorption of 26.40 %, and tensile strength of 5 MPa, indicating a moderate mechanical strength suitable for biodegradable applications. The formulation with high biodegradability (3.0% (v/v) glycerol, 1.5 % (w/v) chitosan, 3.0 % (w/v) carrageenan) showed the highest biodegradation rate (51.94%) but lower tensile strength (3 MPa), indicating that higher glycerol content improved biodegradability but reduced mechanical strength. Conversely, the formulation with higher chitosan content (2.0% chitosan) exhibited the lowest biodegradation rate (31.93 %) but the highest tensile strength (6 MPa), indicating improved mechanical properties but slower degradation [18]. These results indicate a trade-off between biodegradability and mechanical strength, where increasing glycerol and carrageenan enhances biodegradation while increasing chitosan improves tensile strength but reduces biodegradability [14], [19]. This behavior can be explained by polymer interaction mechanisms. Glycerol acts as a plasticizer that reduces intermolecular forces between starch chains, increasing flexibility and water permeability, which accelerates biodegradation. Carrageenan contributes to film matrix formation and increases hydrophilicity, facilitating water diffusion into the film matrix. In contrast, chitosan forms a more rigid polymer network through hydrogen bonding interactions, improving tensile strength but limiting water penetration and slowing biodegradation. These findings are consistent with previous studies on starch–chitosan biodegradable films, which reported that increasing chitosan concentration improves mechanical strength but reduces biodegradation rate.

3.3 Optimization Using Response Surface Methodology (Box–Behnken Design)

Among the experimental approaches used in Response Surface Methodology (RSM), the Box–Behnken Design (BBD) is widely recognized for its ability to evaluate variable effects using a limited number of experiments. In this work, BBD was chosen because it allows efficient optimization while reducing the total number of experimental runs required. The selected factor levels were processed using the BBD feature in Design-Expert® software (trial version), producing 17 experimental formulations for optimizing the biodegradable film composition. The statistical analysis revealed that interactions among the studied variables played an important role in influencing the biodegradation behavior of the bioplastic film [20], [21] . To represent this relationship, a quadratic regression model was applied, as it provided a suitable fit and demonstrated good agreement between predicted and experimental values. Accordingly, the dependence of the biodegradation response on the formulation variables is described by the quadratic equation presented in Equation (2):

$$\begin{aligned}\text{Biodegradatio} &= 48.936 + 5.18875A + 0.755B + 3.11375C + 1.615AB + 1.3925AC + 1.245BC \\ &\quad - 2.53175A^2 - 6.28425B^2 - 4.80175C^2\end{aligned}$$

In this study, A, B, and C denote the concentrations of glycerol, chitosan, and carrageenan, respectively. An analysis of variance (ANOVA) was carried out to determine the relative importance of each variable and their possible interactions on the biodegradability of the bioplastic film. This approach was used not only to evaluate the significance of the regression model, but also to identify which formulation parameters contributed most to the observed response [22]. The influence of each term was assessed using p-values at a 95 % confidence level. Generally, variables associated with higher F-values and lower p-values indicate a stronger contribution to the biodegradation response. Parameters with p-values lower than 0.05 were therefore considered statistically significant, suggesting that the developed model is capable of describing the experimental behavior with acceptable accuracy. Furthermore, the ANOVA results provide useful insight into the sensitivity of the biodegradation response toward changes in formulation variables, which is important for guiding optimization and formulation design. The complete ANOVA summary is presented in Table 3.

Table 3. ANOVA for the Quadratic Response Surface Model (RSM)

Parameter	Sum of Squares	df	Mean Square	F-Value	p-Value
Model	641,37	9	71,26	9,71	0,0034*
A - Glycerol	215,39	1	215,39	29,36	0,0010*
B - Chitosan	4,56	1	4,56	0,62	0,4563**
C - Carrageenan	77,56	1	77,56	10,57	0,0140*
AB	10,43	1	10,43	1,42	0,2719**
AC	7,76	1	7,76	1,06	0,3381**
BC	6,20	1	6,20	0,85	0,3885**
A^2	26,99	1	26,99	3,68	0,0966**
B^2	166,28	1	166,28	22,66	0,0021*
C^2	97,08	1	97,08	13,23	0,0083*
Residual	51,36	7	7,34		
Lack of Fit	41,31	3	13,77	5,48	0,0669**
Pure Error	10,04	4	2,51		
Cor Total	692,72	16			

**significant **not significant*

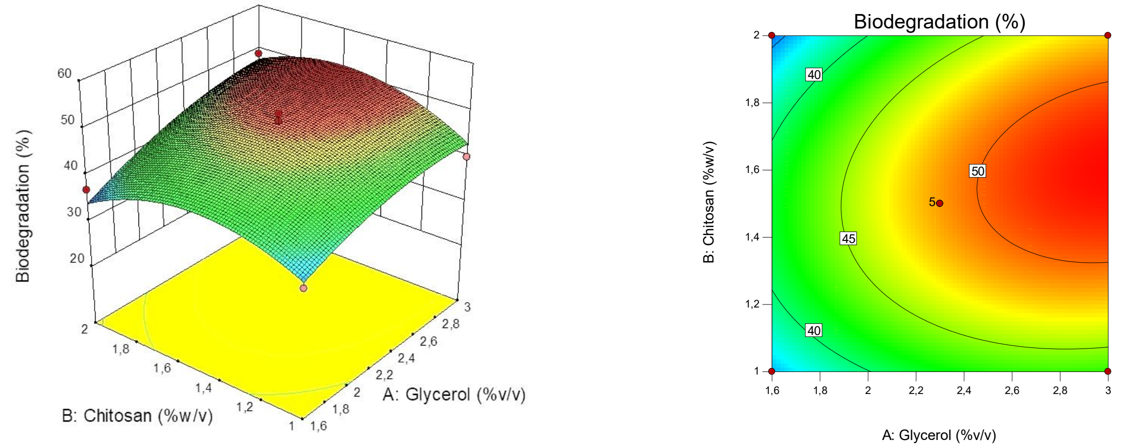
Table 4. Summary and Relevant Statistics of ANOVA

Statistic	Result
Standard Deviation	2.71
Mean	42.53
R^2	0.9259
Adjusted R^2	0.8305
Predicted R^2	0.0231
PRESS	676.70

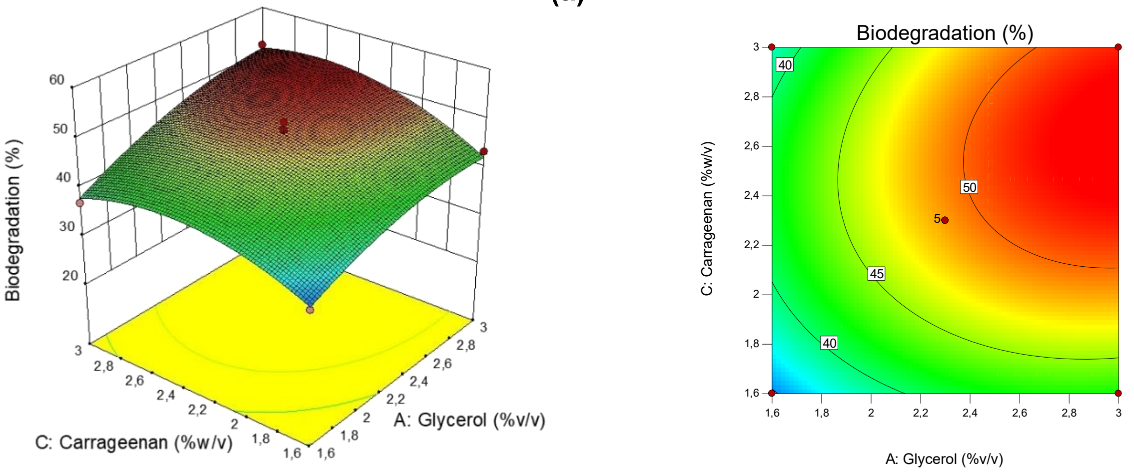
Based on the ANOVA results presented in Table 3, the quadratic response surface model exhibits a statistically significant performance, as reflected by an F-value of 9.71 and a p-value of 0.0034, which is lower than the 0.05 significance level. This indicates that the model is valid and that the likelihood of the observed response being caused by random variation is very small [23]. Among the individual factors, glycerol (A) and carrageenan (C) showed a significant effect on film biodegradability, with p-values of 0.0010 and 0.0140, respectively. Conversely, chitosan (B) did not present a significant linear effect, as its p-value (0.4563) exceeded the accepted significance threshold. Furthermore, the interaction terms (AB, AC, and BC) were not statistically significant ($p > 0.05$), suggesting that the combined influence of the formulation variables did not markedly affect the biodegradation response [23]. In contrast, the quadratic components contributed substantially to the model, particularly B^2 (chitosan^2) with a p-value of 0.0021 and C^2 (carrageenan^2) with a p-value of 0.0083, while A^2 (glycerol^2) showed a moderate but noticeable effect ($p = 0.0966$). These outcomes imply that the biodegradation characteristics of the bioplastic film are primarily controlled by nonlinear relationships, especially those associated with chitosan and carrageenan concentrations [24].

The adequacy of the model was further examined using a lack-of-fit analysis. An F-value of 5.48 accompanied by a p-value of 0.0669 (> 0.05) indicates that the lack of fit is not significant, demonstrating good conformity between the predicted and experimental data. However, the Predicted

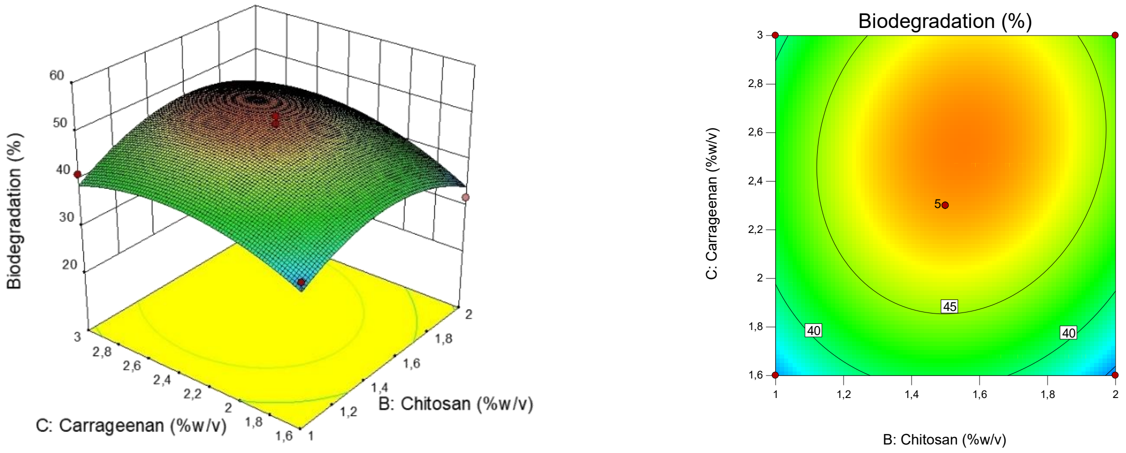
R² value (0.0231) is considerably lower than the Adjusted R² (0.8305), indicating that the model has limited predictive capability for new observations. This discrepancy may be attributed to the relatively small number of experimental runs and the variability of biodegradation data obtained from soil burial tests, which are strongly influenced by environmental factors such as soil moisture, microbial activity, and temperature. Despite this limitation, the model remains statistically significant based on the ANOVA results and is still adequate for describing the relationship between formulation variables and biodegradability within the studied experimental range. Further experiments with additional data points are recommended to improve the predictive accuracy of the model. Overall, the statistical parameters summarized in Table 3 confirm that the developed quadratic model is sufficiently reliable for describing the relationship between formulation variables and biodegradability and can be effectively applied for trend analysis and optimization within the studied experimental range [24].



(a)



(b)



(c)

Figure 2. 3-D and 2-D Surface Plots Showing the Interaction Effects on Biodegradability: (a) Effect of glycerol concentration on biodegradability, (b) effect of chitosan concentration, and (c) effect of carrageenan concentration.

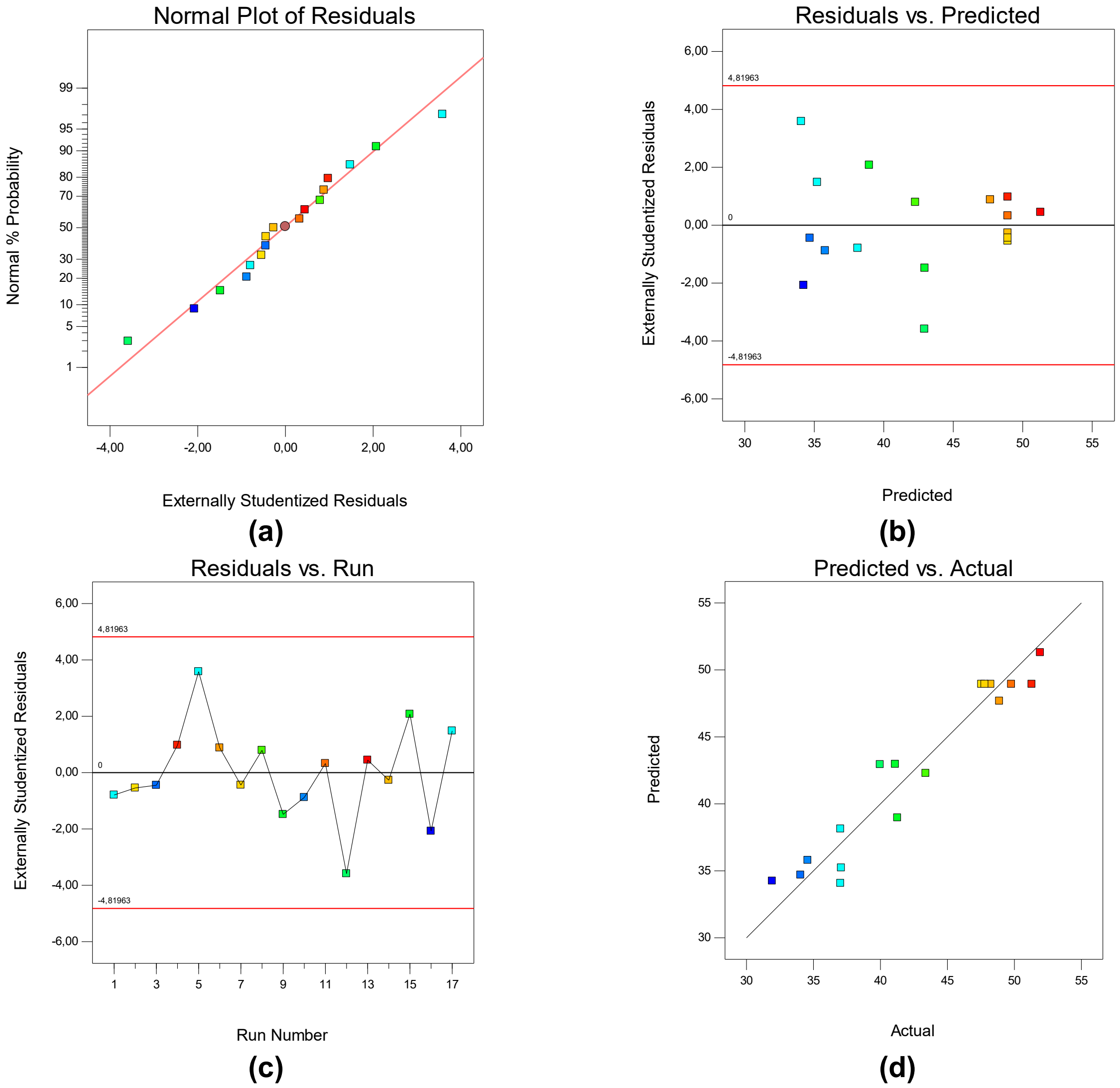
In this study, the effects of various formulation components on the biodegradable film properties were evaluated using 2-D and 3-D surface and contour plots. These plots were obtained from the regression model derived from Equation (3) and are shown in Figure 2**.** Figure 2 illustrates the 2-D and 3-D models of the relationship between the three independent factors: glycerol (A)**,** chitosan (B)**,** and carrageenan (C), which affect the biodegradation of the bioplastic film.

In Figure 2(a), the 2-D and 3-D surface plots developed for the biodegradation response with varying ratios of glycerol and chitosan, at a fixed concentration of 2.3 % carrageenan (w/v), demonstrate that increasing the glycerol concentration results in a higher biodegradation percentage of the film. The highest biodegradation percentage (51.94 %) was observed when glycerol was at 3% (v/v) and chitosan at 1.5% (w/v), with carrageenan at 3% (w/v). This formulation demonstrated the highest biodegradation among all combinations. On the other hand, the lowest biodegradation (31.93 %) occurred when glycerol was at 2.3 % (v/v), chitosan at 2 % (w/v), and carrageenan at 1.6 % (w/v). Additionally, the interaction between glycerol and chitosan (AB) significantly affected the biodegradation of the film, with a p-value of 0.0259, indicating that this interaction is crucial for enhancing biodegradation. Figure 2(b) shows that increasing both glycerol and carrageenan concentrations enhances biodegradation. This suggests a direct correlation between increasing the concentrations of glycerol and carrageenan and the biodegradability of the film. The highest biodegradation observed for glycerol and carrageenan interactions was 51.31 %, which is consistent with the optimal formulation. In Figure 2(c)**,** increasing the chitosan concentration did not significantly impact biodegradation. However, the addition of carrageenan at 1.5 % chitosan (w/v) significantly improved the biodegradability of the bioplastic film, as indicated by a biodegradation percentage of 49.78% at this combination. This suggests that chitosan alone has limited influence on biodegradation, but its combination with carrageenan positively contributes to the film's degradation properties.

The optimization of biodegradable film production from avocado seed starch using the Box–Behnken Design (BBD) indicated that the best formulation was achieved at 2.964 % (v/v) glycerol, 1.560 % (w/v) chitosan, and 2.849 % (w/v) carrageenan, yielding a predicted biodegradation value of 53.113 %. These results suggest that the biodegradation behavior of starch-based bioplastics is closely related to the formulation composition, particularly the interaction between plasticizers and biopolymer reinforcements. Studies on avocado seed starch films have shown that the presence of glycerol and chitosan increases the hydrophilic nature of the matrix, which facilitates microbial degradation in soil environments. In comparison, biodegradable films derived from other plant-based materials, such as corn or butterfly pea, generally exhibit biodegradation levels between 40% and 66%, indicating that biodegradation efficiency is strongly affected by the botanical origin of the raw material [20].

3.3 Verification and Validation of Response Surface Optimization

The results of the validation confirm that the quadratic regression model performs reliably and satisfies the basic requirements of analysis of variance. To verify the consistency of the experimental data and assess the predictive capability of the model, an adequacy evaluation was conducted. A mathematical model that passes this evaluation can be considered to represent the actual biodegradation process with sufficient accuracy. The relationship between the experimental design and the residual distribution for the biodegradation response is illustrated in Figure 3.



(a) (b) (c) (d)

Figure 3. Diagnostic plots for the quadratic model of biodegradation response: (a) Normal probability plot of studentized residuals; (b) Externally studentized residuals versus predicted values; (c) Externally studentized residuals versus run number; (d) Predicted versus actual values.

Figure 3 presents the diagnostic plots used to assess the adequacy and validity of the model developed for predicting the biodegradability of the bioplastic film. Figure 3a shows the normal probability plot versus internally studentized residuals. This plot is used to evaluate whether the residuals follow a normal distribution, a key assumption in regression analysis. As the experimental values align closely along the straight line, this confirms that the residuals are normally distributed [25]. The absence of deviations indicates that the model meets the normality assumption, which reinforces the model's reliability in predicting biodegradability. Figure 3b displays the residuals vs. predicted values plot. The points are scattered around the horizontal line at zero, with no clear patterns. This indicates that the model does not exhibit any bias or systematic errors in predicting the biodegradability, further supporting the adequacy of the model. Figure 3c shows the residuals vs. run number plot. The residuals are consistently within the range of 0 to ±3, suggesting that there are no outliers or unexpected errors affecting the model. This indicates that the experimental setup is stable and the residuals fall within acceptable limits, further demonstrating the model's suitability for the data lastly, Figure 3d presents the leverage vs. run number plot. All data points fall within the range of 0 to 1 for leverage values, indicating that no individual experiment has an undue influence on the model [4], [26]–[28]. This suggests that no influential data points exist that could distort the model's predictions, confirming the robustness of the regression model [29], [30]. Together, these plots provide a satisfactory evaluation of the model's adequacy and fit. Based on these results, it can be concluded that the empirical model is suitable for accurately representing and optimizing the process of biodegradable film production using the Box-Behnken Design (BBD). The random scatter of points within the acceptable range of residuals further supports the adequacy of the model for this application.

4. Conclusion

The study demonstrates that biodegradable films can be effectively produced from avocado seed starch, with glycerol, chitosan, and carrageenan playing key roles in their properties. The optimization of the film formulation using Box–Behnken Design showed that glycerol and carrageenan concentrations positively influence biodegradability, while chitosan enhances mechanical strength. The optimal film formulation, with predicted maximum biodegradation of 53.11 %, highlights the potential of avocado seed starch as a sustainable material for bioplastic production. The findings suggest that this formulation could be further explored for practical applications in the food packaging industry, and future research could focus on refining the properties of biodegradable films for specific uses.

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