

RESEARCH ARTICLE

Preparation of bioplastic film from chitosan and mango (*Mangifera Indica L. Anacardiaceae*) kernel starch by casting method

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ABSTRACT

The accumulation of plastics in landfills and oceans has encouraged the development of biodegradable plastic products from renewable sources. Natural polymers are excellent candidates that need further modification of their functional and structural properties comparable to conventional plastics. This study aims to fabricate and optimize the formulation of bioplastic films from chitosan and mango kernel starch with glycerol as a plasticizer using response surface methodology (RSM). The chitosan-to-starch mass ratio (1:0.17 to 1:5.83) and glycerol concentration per gram of dry polymer (15.86% to 44.14%) were assigned as the independent variables to design an empirical model that describes the films' elastic modulus as the sole response. The results yielded an optimal formulation of 1:0.17 chitosan-to-starch mass ratio (2% weight by volume chitosan solution blend) with 15.86% glycerol (per gram of dry chitosan and starch). Reproduction of the optimized film was carried out to validate the empirical model. Characterization of the films' mechanical and barrier properties, surface morphology, and biodegradability were also investigated in this work. The results suggest that the functional properties of the bioplastic film surpass other chitosan-based bioplastic film blends and can be developed further to become a more sustainable alternative to conventional plastic packaging products.

Keywords: biodegradable; bioplastic film; chitosan; mango kernel starch; RSM (response surface methodology)

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1. Introduction

Recent trends in the bioplastic industry lean towards the use of starch-based biopolymers due to their abundance and film-forming properties^[1]. Biofilms made from starches obtained from typical sources (e.g., corn starch) were found to exhibit similar desirable physical and functional properties as conventional plastics in terms of color, texture, and impermeability to oxygen^[2]. However, drawbacks in terms of solubility in water, sensitivity to humid environments, and brittleness limit its applications^[3].

Chitosan (CH) is a polycationic linear polysaccharide that is sourced from the deacetylation of chitin—a naturally abundant and renewable biopolymer that is mainly recovered from seafood by-products (e.g., heads, tails, shells) from crustacean-processing industries^[4]. Several studies have explored the use of both starch and chitosan to form a composite film that exhibits improved mechanical properties and lower water permeability compared to starch-alone films^[5,6]. The degree of improvement for these properties is highly

dependent on the ratios of the components, as different starch sources have varied amylose to amylopectin ratios which play a big role in the final product's mechanical and functional properties. Most of the studies concerning chitosan-starch blends have utilized starch from sources that have agricultural value and thus are consistently in high demand due to its extensive use in the food industry (e.g., corn starch). Mango kernel starch (MKS) is a value-added product that can be derived from waste mango seed kernels generated by processing facilities. MKS can be extracted for use in non-food applications, making it a sustainable alternative to starch extracted from conventional sources that have increasing industrial demand, thus promoting circular economy. The utilization of MKS in the fabrication of chitosan-starch bioplastic films have not yet been explored by recent research efforts, thus the optimal formulation for the chitosan-mango kernel starch blend have not yet been investigated. Further improvements on the mechanical properties of chitosan-starch films were observed by previous studies^[7,8] with the addition of glycerol as plasticizer. Provided that the addition of glycerol also affects the tensile properties of the bioplastic, an overlapping effect that could be linked to the interaction between the plasticizer and the polymers was suspected^[9,10]. However, as far as these studies are concerned, the optimization of the CH-S-G bioplastic formulation with respect to a particular parameter has not yet been explored.

The present study was carried out to develop a sustainable alternative to conventional single-use conventional plastic by using chitosan and mango kernel starch with the addition of glycerol as plasticizer. The study aimed to determine optimal CH-MKS-G bioplastic formulation using response surface methodology with the corresponding component ratios as the independent variables and the elastic modulus as the sole response. The preparation of chitosan-mango kernel starch bioplastic film with glycerol as plasticizer was carried out via solution casting method. Moreover, the characterization of the optimized bioplastic film by their nominal tensile strength, elongation-at-break, water barrier property, surface morphology, and biodegradability were also done in this study.

2. Materials and methods

Mango seeds were obtained from a local mango processing company in Mandaue City, Cebu. The chemical reagents used in the experiment include high molecular weight ($100 \times 10^4 - 200 \times 10^4$ Da) chitosan powder at 75% deacetylation rate (Sigma-Aldrich SA-419419), >80% by volume glacial acetic acid (Ajax Finechem, Auckland, Australia), glycerol >99.5% by weight (Ajax Finechem, Auckland, Australia), anhydrous calcium chloride powder (Scharlau, Spain), sodium hydroxide pellets (Scharlau, Spain), and amylose from potato (Sigma-Aldrich, USA). Sodium bisulphite, undenatured ethanol 95% by volume, and potassium iodide, and other analytical grade reagents were obtained from local chemical suppliers. All chemicals were used without further purification.

2.1. Mango starch extraction and characterization

The preparation and extraction of starch from mango kernels were done by steeping cleaned and cut kernels in 0.2% by weight NaHSO₃ solution for 48 hours in a refrigerated environment as reported by Silva et al.^[11]. The mixture was later strained and weighed to determine the total mango kernel starch yield. These were washed with water and the kernel-water mixture was further liquefied in an electric blender until visibly homogenous. The slurry was strained through a cheesecloth and the filtrate was set aside to settle. This was decanted and 0.05 M NaOH solution was added. Repetitive decantation steps were done until the supernatant was no longer cloudy. The starch was recovered and spread onto a tray to air-dry for ~24 hours. This was weighed and stored in a polypropylene container and refrigerated for subsequent use. The total MKS yield was calculated at 8.75%.

The moisture content of the extracted mango kernel starch (MKS) was also determined using the oven-dry test. Crucibles with crucible covers were pre-heated at 105 ± 2 °C until constant weight. ~1 gram of MKS

samples were added into the crucibles and dried at 105 ± 2 °C for 2 hours then cooled in a desiccator for an hour. These were then weighed. The heating, cooling, and weighing of the MKS samples was repeated until constant weight. This was done in triplicate and the moisture content was found to be at $30.33 \pm 0.63\%$.

The apparent amylose content of the extracted MKS was determined by iodine colorimetry using the methods presented in a previous study^[12]. This test was done to confirm the amylose content of MKS as reported in literature^[13,14]. The maximum absorbance was measured using a UV-1700 UV Vis Spectrophotometer (Shimadzu Corp. PharmaSpec UV-1700 Spectrophotometer) and a calibration curve was generated at 620 nm. Sample solution with 0.1 g MKS was prepared, and an aliquot was tested in triplicate. The apparent amylose content was determined to be at $35.39 \pm 0.05\%$.

2.2. Optimization of bioplastic composition

2.2.1. Experimental research design

Response surface methodology based central composite design (RSM-CCD) was used to optimize the elastic modulus (Y) of the bioplastic film with respect to changes in the independent variables CH:MKS ratio (X_1) and glycerol concentration (X_2). For this study, two independent variables (X_1 , X_2) were involved, thus an alpha value of 1.414 was used. The actual factor levels are within the variable ranges set at a CH:MKS ratio of 1:1 to 1:5 and a glycerol concentration of 20 to 40%. The software that was used in this study was Minitab Statistical Software^[15] which generated the proposed factor combinations for the experiment (refer to **Table 1**). The experimental design required thirteen experimental runs which were done in triplicate.

2.2.2. CH-MKS-G film preparation

CH solution of 2% by dry weight CH per volume was prepared by dissolving 20 grams of CH 1000 mL of 1% by volume aqueous acetic acid solution. The mixture was allowed to homogenize at 600 rpm and 40 °C until a fine and clear solution was obtained. The prepared CH solution was stored in a 1-L Erlenmeyer flask with stopper and refrigerated (~ 4 °C) for subsequent use.

Starch solution was prepared with the extracted MKS. The gelatinization process of the MKS solution was adapted from the methods presented by Silva et al.^[11]. Raw MKS was dissolved in water and the resulting solution was gelatinized at 600 rpm and 73.1 ± 4.8 °C within the range of gelatinization temperatures^[13,16] for at least an hour. The gelatinized MKS solution was then set aside to cool.

The preparation of the various CH-MKS-G blends was done using separate flasks. To each flask, 90 mL of the CH solution and 90 mL of MKS solution of varying concentrations was added to make varying CH-MKS ratio (1:0.17; 1:1; 1:3; 1:5; 1:5.83). Glycerol was then added at 15.86, 20, 30, 40, and 44.14% $G/CH + MKS$. These were homogenized at 600 RPM and degassed using a vacuum pump.

Finally, 150 mL of the blends were cast on custom-made glass molds. These were set aside to dry at ambient conditions for at least four days. The dried films were gently peeled off the molds and kept on metal mesh screens with parchment paper lining and adequate air circulation. These were conditioned for at least 40 hours at laboratory conditions (27.0 ± 1 °C and $58 \pm 7\%$ RH) monitored by a handheld humidity and temperature meter. A schematic diagram of the fabrication process is shown in **Figure 1**.

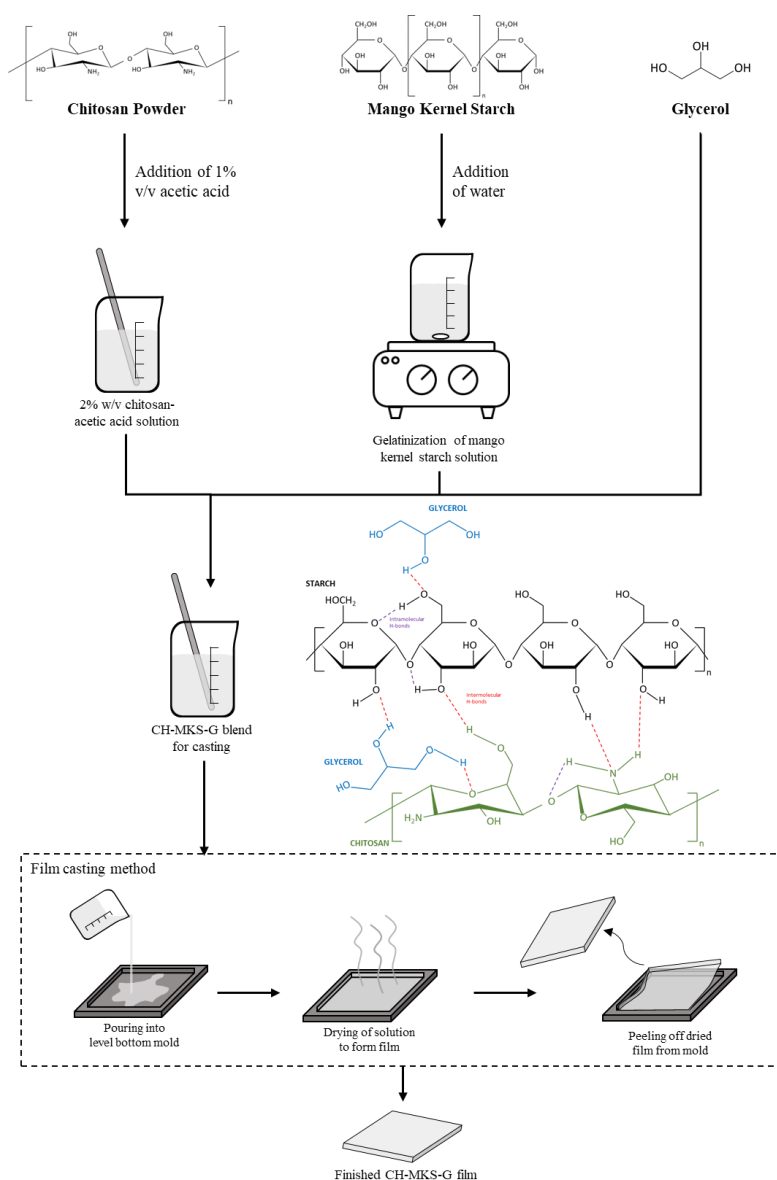


Figure 1. Schematic diagram of the production of CH-MKS-G bioplastic film.

2.2.3. Determination of elastic modulus

The thickness of the film samples was measured with a Mitutoyo 7301 Dial Type thickness gauge according to the American Society for Testing and Materials (ASTM) standard method D6988-13^[17]. The elastic modulus of the CH-MKS-G films were evaluated using a PARAM XLW (PC) Auto Tensile Tester according to ASTM standard method D882-18^[18]. The test films consisted of 100-mm-by-25-mm uniform strips. The rate of grip separation was 25 mm/min and an initial grip separation of 50 mm. The force (N) and extension (mm) values were recorded to calculate the tensile stress (TS) and the strain (ϵ). A stress-strain curve was constructed, and the elastic modulus (λ) was determined. These values were also used to determine the nominal tensile strength (TS_n) and elongation at break (ϵ_0) for the characterization of the film. A minimum of 3 pre-conditioned specimens were tested.

2.2.4. Statistical analysis

The gathered data from the experimental runs were subjected to multiple regression analysis. This analysis of the RSM-CCD model was done using the Minitab Statistical Software^[15]. A second-order polynomial equation, shown in Equation (1), was fitted to the response values by least squares.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2 \quad (1)$$

where Y is the elastic modulus (λ), X_1 is the MKS value of the normalized CH:MKS ratio, X_2 is the glycerol concentration (%G/CH+MKS), and β_i is the regression coefficients (β_0 : intercept; β_1 and β_2 : linear coefficients; β_3 and β_4 : quadratic coefficients; β_5 : coefficient of interaction term). The coefficient values were determined using the Analyze Response Surface Design function of Minitab. The significance of each term was evaluated based on their p -values.

Analysis of variance (ANOVA), the coefficient of determination (R^2), the adjusted and predicted coefficients of determination ($adj - R^2$; $pred - R^2$) and lack-of-fit were also determined by Minitab. These values were used to assess the quality of the statistical model fitting. The Response Optimizer function of Minitab was used to determine the optimum setting of the independent variables. All hypotheses were tested at 95% confidence interval.

2.2.5. Validation of response model

The determined “optimal” CH-MKS-G blend composition was used to validate the response model. The elastic modulus of the film sample with the “optimal” blend was determined in a separate experimental run. The resulting response was compared to the predicted values from the model and the relative difference percentage was calculated.

2.3. Characterization of CH-MKS-G films

The resulting bioplastic film with the “optimal” CH-MKS-G blend composition was characterized in terms of its mechanical properties, water vapor barrier property, morphological properties, and biodegradability.

2.3.1. Water vapor transmittance rate

The rate at which water vapor permeate through the film was determined by desiccant method using test dishes filled with anhydrous CaCl_2 and sealed with the film samples according to ASTM standard method E96/E96M^[19]. The setup was placed in laboratory conditions where the humidity and temperature were monitored and maintained at $73 \pm 2\%$ RH and 25.6 ± 0.5 °C. The test dishes were weighed every hour for a total of nine data points. The recorded weights of the dishes were plotted against time and least squares regression was done to obtain the slope of the line which represented the average change of weight over time (G/t). The water vapor transmission ($WVTR$) of the film sample was determined using Equation (2) where A refers to the test area.

$$WVTR = \left(\frac{G}{t}\right) \left(\frac{1}{A}\right) \quad (2)$$

2.3.2. Morphological properties

A 5 mm by 5 mm film sample was deposited on an aluminum holder and coated with gold (coating thickness, 60–100 Å) using a Quorum Q150RS Sputter Coater. The morphological properties of the film sample were studied using a Zeiss Sigma 500 VP Analytical Field Emission Scanning Electron Microscope (FE-SEM) at ambient conditions (25.5 °C and 55% RH). FE-SEM photographs were taken of the surface of the film sample at 5.00 kV accelerating voltage, a working distance of 15.5 mm at low magnifications of 30× and 1,000×. FE-SEM photographs were also taken for the cross-section of the film sample at 5.00 accelerating voltage, a working distance of 13.3 mm, and low magnifications of 30× and 501×.

2.3.3. Biodegradability

The biodegradability of the films was investigated using the soil-burial test method where 3-cm-by-3-cm sample pieces were buried in potted soil as presented in a study by Mantia et al.^[20]. Samples were cut pieces and dried at 50 °C until a constant weight was obtained as the initial sample weight W_0 . Modifications to the method involve the use of commercial pumice stones in place of milled perlite to aid aeration in the soil and

minimize water retention. A mixture of 1:4 commercial pumice to soil was prepared and moistened with 400 mL tap water per kilogram of dry mixture. In plastic pots, soil mixture was placed at a depth of ~2 cm. The pre-cut film sample was then laid flat on the soil mixture and an additional 10-cm depth of soil mixture was placed. These were allowed to incubate at laboratory conditions. Samples were then dried at 50 °C until constant weight of W_i to remove any excess moisture. The weight of the oven-dried films was measured and the weight loss in percentage was calculated using Equation (3). Regular sampling was done until the total average weight loss reached 90%.

$$\%weight\ loss = \frac{W_0 - W_i}{W_0} \times 100\% \quad (3)$$

3. Results and discussion

3.1. Regression model for elastic modulus

The experimental elastic modulus (Y) obtained from the runs with various combinations of the independent variables (X_1 , MKS value; X_2 , G conc.) are presented in **Table 1** along with other mechanical properties.

Table 1. Experimental values of response variable (elastic modulus) for various combinations of factors (MKS term of CH-MKS ratio and glycerol concentration) of the CH-MKS-G blends.

Run order	Factor levels		Composition by mass				Dependent variables		
	^a X_1	^b X_2	x_{CH}	x_{MKS}	x_G	x_{others}	$\lambda \pm SD$ [MPa], Y	$TS_n \pm SD$ [MPa]	$\epsilon\% \pm SD$ [%]
1*	3.00	30.00	0.1819	0.5361	0.2158	0.0661	288 ± 68	10.4 ± 0.7	23 ± 9
2	5.00	40.00	0.1110	0.4894	0.2653	0.1343	7.95 ± 0.72	1.60 ± 0.52	35 ± 4
3	5.83	30.00	0.1226	0.5297	0.2435	0.1042	34.3 ± 28.1	3.38 ± 1.38	29 ± 4
4*	3.00	30.00	0.1756	0.5251	0.2157	0.0836	201 ± 10	10.6 ± 1.1	27 ± 2
5*	3.00	30.00	0.1819	0.5361	0.2158	0.0661	369 ± 22	11.3 ± 1.2	25 ± 2
6	5.00	20.00	0.1307	0.6123	0.1563	0.1007	234 ± 8	8.35 ± 0.95	13 ± 9
7*	3.00	30.00	0.1819	0.5361	0.2158	0.0661	119 ± 25	8.35 ± 2.89	28 ± 10
8	3.00	44.14	0.1430	0.4298	0.2517	0.1755	32.7 ± 7.4	4.66 ± 0.62	38 ± 4
9*	3.00	30.00	0.1756	0.5251	0.2157	0.0836	480 ± 6	13.5 ± 0.1	27 ± 7
10	1.00	20.00	0.3265	0.3205	0.1327	0.2204	514 ± 54	16.5 ± 1.8	8.7 ± 5.6
11	3.00	15.86	0.2195	0.6420	0.1367	0.0018	996 ± 148	24.5 ± 2.2	5.1 ± 0.9
12	0.17	30.00	0.4350	0.0703	0.1534	0.3413	681 ± 158	13.8 ± 1.5	11 ± 4
13	1.00	40.00	0.2296	0.2224	0.1840	0.3640	235 ± 187	10.9 ± 3.3	27 ± 16

Experimental runs were done in triplicates ($n = 3$) and the reported results are the average with its standard deviation;*, center points; ^a, MKS term of CH:MKS ratio [g MKS/g CH]; ^b, Glycerol concentration [% G/CH+MKS]; ^c, composition of CH-MKS-G film expressed in mass fractions; MKS, mango kernel starch; CH, chitosan; λ , elastic modulus; TS_n , nominal tensile strength; $\epsilon\%$, elongation-at-break; SD , standard deviation

As not all terms contribute significantly to the empirical model, the model was reduced. This was done by evaluation of the probability value (p -value) at a significance level of 0.05. Terms whose p -value is less than that of the set significance level (p -value < 0.05) have a statistically significant contribution to the response behavior^[15]. From the initial empirical model equation, only the linear terms X_1 and X_2 were statistically significant. This reduces the model equation from a second-order polynomial to a linear equation shown in Equation (5). The ANOVA results of the reduced model as well as other statistical parameters is shown in **Table 2**.

$$Y = 1289 - 88.8X_1 - 23.34X_2 \quad (4)$$

Table 2. ANOVA and model summary results for the reduced empirical model developed by Minitab in the optimization of CH-MKS-G bioplastic film composition.

Source	DF	Adj SS	Adj MS	F-value	p-value
Model	2	688,279	344,140	11.62	0.002*
Linear	2	688,279	344,140	11.62	0.002*
X_1	1	252,615	252,615	8.53	0.015*
X_2	1	435,664	435,664	14.71	0.003*
Error	10	296,284	29,608	-	-
Lack-of-Fit	6	216,534	36,089	1.81	0.294**
Pure error	4	79,549	19,887	-	-
Total	12	984,363	-	-	-
Model summary					
Coefficient of determination (R^2 , %)					69.92
Adjusted coefficient of determination ($adj-R^2$, %)					63.91
Predicted coefficient of determination ($pred-R^2$, %)					42.46
X_1 , MKS value of the normalized CH:MKS ratio					
X_2 , glycerol concentration (% weight of glycerol per total dry weight of biopolymer)					
*, Significant at 5% (p -value < 0.05)					
**, Insignificant at 10% (p -value > 0.10)					

The established empirical model shown in Equation (4) was assessed based on the statistical parameters summarized in **Table 2**. ANOVA is a commonly used statistical method to summarize the fitted model's significance and adequacy to explain the data in the region of experimentation. The R^2 -value is the percentage of the variations of the dependent variable that can be explained by the independent variables and their interactions. The model established in this study has an R^2 of 69.92% which predicts 69.92% of the variance of the elastic modulus is accounted for by the predictors of the model. A modification of the R^2 which accounts for the number of independent variables present in a model is preferred. The $adj-R^2$ provides a correction to the goodness-of-fit of the model by only incorporating the number of predictors/independent variables which influence the model's performance. For the reduced model, the predicted R^2 is 63.91%, which is lower than R^2 -value. A lower $adj-R^2$ indicates that some of the independent variables are insignificant to the response or outcome. On the other hand, the $pred-R^2$ determines predictive ability of the model or how well the established model predicts the response value for new observations other than the existing data set. The empirical model of this study has a $pred-R^2$ of 42.46% which is distinctly less than that of the $adj-R^2$ value of the model which suggests that the previous model may be over-fit. An overfitted model becomes tailored to the sample data and therefore is unsuitable in predicting new observations of the population due to an excessive number of terms or high variations in the response^[21]. In this study, the significant difference between the adjusted and predicted R^2 is an effect of the variances in the response elastic modulus from the replicates of the center point (refer to **Table 1**; $X_1 = 3.00$; $X_2 = 30.00$).

The lack-of-fit is used as a supporting measure to determine the adequacy of the fitted model^[22]. Since the p -value of the lack-of-fit error is large compared to the confidence interval (p -value = 0.294 > 0.05) then it can be said that the model is adequate based on the lack-of-fit error. Regardless of the low R^2 value due to high variability of the experimental data, the trend observed in the data set still provides information about the behavior of the dependent variable in response to change in the independent variables^[23]. This relationship is observed in the plotted contour and surface plots presented in **Figure 2**.

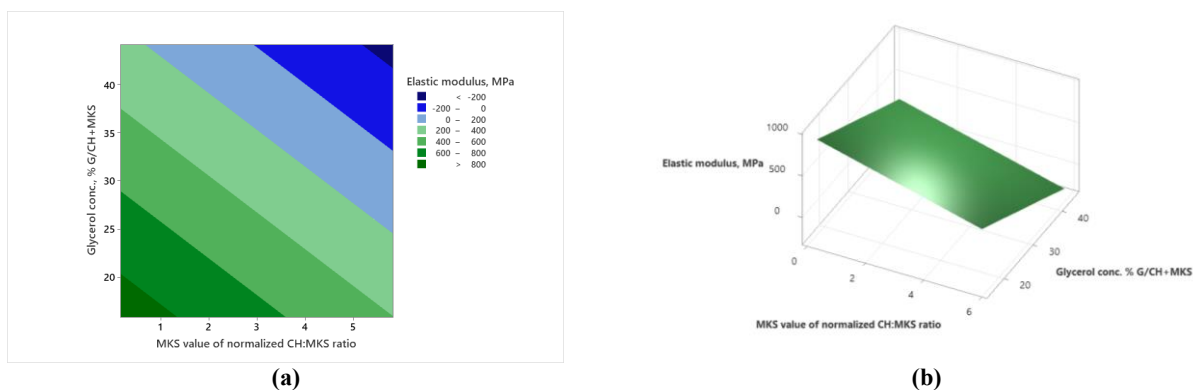


Figure 2. Minitab generated contour plot (a) and surface plot (b) of the response variable elastic modulus (MPa) as a function of MKS value of the normalized MKS:CH ratio and glycerol concentration (%weight glycerol per total dry weight of biopolymer) of the CH-MKS-G bioplastic film tested.

The removed interaction term (X_1X_2) between MKS value and glycerol concentration is considered statistically insignificant thus their effect on the elastic modulus is independent of each other. That said, the major factors are statistically significant based on their p -value (X_1 : p -value = 0.015 < 0.05; X_2 : p -value = 0.003 < 0.05).

From the regression coefficients in Equation (4), one unit increase in the MKS value is associated with an 88.8 decrease in elastic modulus while a unit increase in the glycerol concentration decreases the elastic modulus of the film by 23.34. Contrary to the absolute value of the regression coefficients, the contribution to the change in elastic modulus by the glycerol concentration is greater than that of the MKS value based on the F -value shown in **Table 2**. Both the MKS and glycerol concentration in the formulation of the CH-MKS-G bioplastic film affect the elastic modulus in a similar trend—where the elastic modulus decreases as the concentration of either component increases. This trend is observable in the data gathered. The highest elastic modulus of 996 ± 148 MPa was obtained at the central value of the MKS value (CH:MKS ratio of 1:3) and low glycerol concentration (15.86 %G/CH+MKS). On the other hand, the lowest response of 7.95 ± 0.72 MPa was obtained at a high MKS value and glycerol concentration (CH:MKS ratio of 1:5; 40 %G/CH+MKS). It can also be observed that at a low concentration of MKS (CH:MKS ratio of 1:0.17) is at its highest elastic modulus value of 681 ± 158 MPa in comparison to the other MKS values when glycerol concentration is held at central value (30 %G/CH + MKS). This descending trend of the response variable implies that the maximum response will be observed at a low value of the independent variables. Thus, lower MKS and glycerol concentrations would produce CH-MKS-G bioplastic film of higher elastic modulus within the boundaries of the experimental region.

The plots presented in **Figure 2** do not show any curvature nor an enclosed region that corresponds to the presence of an optimum response. This entails that the range of independent variables in this study is unable to produce an optimal condition to maximize the elastic modulus for a CH-MKS-G bioplastic film within the boundaries of the experimental region. Thus, the optimum response may be observed beyond the range considered in this study. This optimum elastic modulus is expected to lie in a region with low MKS and low glycerol concentration.

3.2. Validation of the response model

The empirical model produced from the regression analysis is linear which does not yield an optimum setting within the set range of parameters. Nonetheless, a maximum elastic modulus can still be determined from the established model. Using the built-in Response Optimizer function of Minitab with the goal of maximizing the response variable within the region of experimentation. The maximum elastic modulus of 904 MPa is achieved at a CH:MKS ratio of 1:0.17 and a glycerol concentration of 15.86 grams of glycerol per 100

grams of total dry biopolymer (CH and MKS). The combination of low MKS and low G concentrations coincide with the observed trend of the elastic modulus where the highest value of the response variable in the experimental runs was observed to be found at the lower MKS and G concentrations. This CH-MKS-G film composition was determined to have a composite desirability of 0.9069 which is close to unity. This measure is an indication on how well the optimal setting achieve favorable results for the aimed response—a desirability of one indicates complete satisfaction, while a desirability of zero indicates unacceptable response^[24].

To validate the response model, experimental runs were performed in triplicates using films with an MKS value of 0.17 and glycerol concentration of 15.86%. This resulted in an actual elastic modulus value of 381 ± 40 MPa. There is a relative error of 57.76% when comparing the actual and predicted values of the response. The significantly large difference between the actual and predicted values implies the low predictive power of the fitted empirical model which coincides with the low *pred-R*² of the model as previously stated. This suggests that the model can only describe the correlation of the independent variables and the response within the given set of experimental data but is not sufficient to provide valid predictions. The validation further proved that the variances in the response of the original data set were not accounted for in the model. The variances in the responses of the experimental runs can be attributed to the factors affecting the film fabrication process, which may include the drying conditions (e.g., humidity) which were observed to have fluctuated within the drying period. During the fabrication of the selected formulation for the model validation, the humidity of the drying environment was recorded at 77% RH during the conditioning step which is beyond the set conditions ($58 \pm 7\%$ RH). It was observed in related studies that the relative humidity of which the films are subjected to during conditioning also have an effect in the tensile properties of the bioplastic film. In a study by Ren et al.^[9], a drastic change in the tensile properties of the CH-S films was observed when subjected to different humidities (75% and 95% RH). This effect was linked to the hydrophilic nature of the polymers used in the bioplastic films which allowed the film to absorb moisture from the atmosphere.

3.3. Characterization of CH-MKS-G film

Characterization was done to the CH-MKS-G film with a CH:MKS ratio of 1:0.17 and a glycerol concentration of 15.86 grams of glycerol per 100 grams of total dry biopolymer (CH and MKS).

3.3.1. Mechanical properties

The bioplastic film blend was tested in triplicate for its tensile strength and elongation at break, both of which are vital mechanical properties for its application as a packaging product. The test yielded 27.1 ± 1.9 MPa for the tensile strength and $41 \pm 8\%$ elongation. The variances among the runs were within acceptable range, which suggests that the samples exhibited similar tensile properties regardless of how each sample film was cut, and further implying that the material is homogenous and isotropic. These values coincide with other studies that have investigated the effects of chitosan and starch in terms of enhancing the bioplastic films' tensile properties. Mollah et al.^[25] reported that the tensile strength of CH-S films increased with the addition of chitosan, from 27 MPa (20% CH) to 53 MPa (80% CH). However, increasing the chitosan content also subsequently decreased its elongation from 23% to 12% for the same CH-S composition. This implies that the interaction between chitosan and starch improves the overall mechanical strength of the film as compared to pure chitosan or starch films, but the addition of either component in excess can lead to undesirable changes such as increased brittleness and lesser elongation in higher chitosan concentrations. Zhong et al.^[26], presented similar observations where CH-S films with high starch concentrations were observed to be heterogenous when viewed under a microscope, which resulted in weak interactions between the two polymers. The weak CH-S interaction is commonly evident in high-amylose starches in high concentrations because of its tendency to retrograde—the recrystallization of disaggregated amylose chains, which reduces the surface area available for contact between the two polymers^[27].

In another study by Xu et al.^[10], it was reported that for glycerol-plasticized CH-S films, the range of TS and EB values at several concentrations are within 22–40.25 MPa and 25–57%, respectively. The findings of this current study closely correspond to the TS and EB values of the films consisting of 1:0.5 chitosan to starch ratio with the addition of 0.75 grams of glycerin at 27 MPa and 47%, respectively. In comparison to commercially used plastic packaging films, the film produced in this study has a comparable tensile strength to LDPE (8.3–31.4 MPa) and HDPE (22.1–31.0 MPa) films^[28]. On the other hand, the elongation-at-break of the produced CH-MKS-G film is comparable to that of PET (30–300%), HDPE (10–1200%), and PVC (40–80%) plastic films^[28]. This denotes that the use of MKS and chitosan with glycerol can still perform as well as starches from conventional sources and exhibit similar mechanical properties to common plastics.

3.3.2. Water vapor transmittance rate

Good barrier properties of a film are crucial in preventing early deterioration of packaged items especially due to external factors such as moisture. The water vapor transmission rate of the bioplastic film resulted in an average of $50.42 \pm 2.68 \frac{\text{g}}{\text{m}^2 \cdot \text{h}}$ ($1,210 \pm 64 \frac{\text{g}}{\text{day} \cdot \text{m}^2}$) among three trials at 25.6 ± 0.5 °C and $73 \pm 2\%$ relative humidity. This value is lower than the water vapor transmission rate (WVTR) of pure chitosan films investigated in other studies investigating the same CH-S bioplastic composition. Zhong et al.^[26] and Xu et al.^[10] presented in their studies that pure chitosan-based films had a WVTR of $68.67 \frac{\text{g}}{\text{m}^2 \cdot \text{h}}$ and $52.73 \frac{\text{g}}{\text{m}^2 \cdot \text{h}}$, respectively. Both studies further expounded that the addition of starch significantly reduces the WVTR of chitosan-based films and related this effect to the CH-S interaction, arguing that although both components are hydrophilic, such interaction due to thermal mixing reduced the number of functional groups that interact with water vapor from the external environment^[10,26]. The water barrier property of the films may also be significantly affected by the molecular weight and degree of deacetylation of the chitosan component and the amylose content of the starch.

The WVTR of films is related to the water vapor permeability (WVP), thickness, and pressure gradient which is mathematically described as

$$WVP = WVTR \left(\frac{t}{\Delta P} \right) \quad (5)$$

where ΔP is the water vapor differential across the film and t is the film thickness. ΔP is affected by changes in temperature and humidity in the surrounding environment. In a study by Basha et al., the WVTR of biomass-based films materials were found to increase proportionally with increasing temperature and relative humidity^[29]. This observation is especially evident in hydrophilic films due to the interaction of water molecules with the polar groups in the film matrix^[26]. Thus, the addition of chitosan to starch films reduces the availability of hydrophilic groups due to the formation of intermolecular bonds between the protonated amino groups from chitosan and the OH⁻ groups from starch^[9]. In terms of film thickness, hydrophilic films often exhibit a positive proportional relationship between water vapor permeability (WVP) and thickness^[30]. This denotes that an increase in the thickness of the films will result in a lower WVTR. For chitosan films, moisture sorption increases at high water vapor pressures which consequently causes swelling in the films. The swelling causes a disruption in the polymer microstructure and allows for an increase in permeance, but overtime reduces the transmission rate towards the other side of the film due to moisture absorption in the film^[31].

The WVTR of common plastic, such as PET ($16\text{--}20 \frac{\text{g}}{\text{m}^2 \cdot \text{day}}$), HDPE ($5\text{--}12 \frac{\text{g}}{\text{m}^2 \cdot \text{day}}$), and PP ($3\text{--}7 \frac{\text{g}}{\text{m}^2 \cdot \text{day}}$) films^[32], are significantly lower than the bioplastic film produced in this study. This indicates that the CH-MKS-G film produced requires further improvements to compete with the water barrier properties of commercially available plastic packaging films. From this observation, the WVTR of the bioplastic film being investigated in this study permits lower permeance of water vapor relative to other chitosan-starch composite

films while further developments are required to be able to be considered suitable for commercial packaging applications.

3.3.3. Surface morphology

Figures 3a and **3b** shows the field emission scanning electron microscopy images of the bioplastic film surface from low to high magnification. At 30× magnification (a), a smooth surface with no evident traces of phase separation is observed. The smooth and homogenous surface indicates good solubility of the polymers in the aqueous medium, thus indicates good mechanical properties. There is also the presence of solid aggregates characterized by the white, near-spherical protrusions. Focusing on these irregularities at a higher magnification rate (1,010×), white, irregularly shaped patches on the surface indicate separation of some solid particles from the surface. Additionally, striations on the surface indicated by rough streaks are also evident in the image. This is attributed to the repeated stresses that the material is exposed to during the peeling of the film from its cast. Although the force applied does not exceed the ultimate tensile strength, this procedure causes the material to undergo polymer fatigue—which is localized plastic deformation that causes microscopic crazes and damages its surface^[33]. The interface (cross-sectional) morphology of the bioplastic film was also observed shown in **Figures 3c** and **3d**. The images show a rough edge wherein strands of the material seem to be stretched out, forming crevices parallel to the edge's length. These crevices are formed by fibrils which is the result of plastic deformation that occurred when the film was cut.

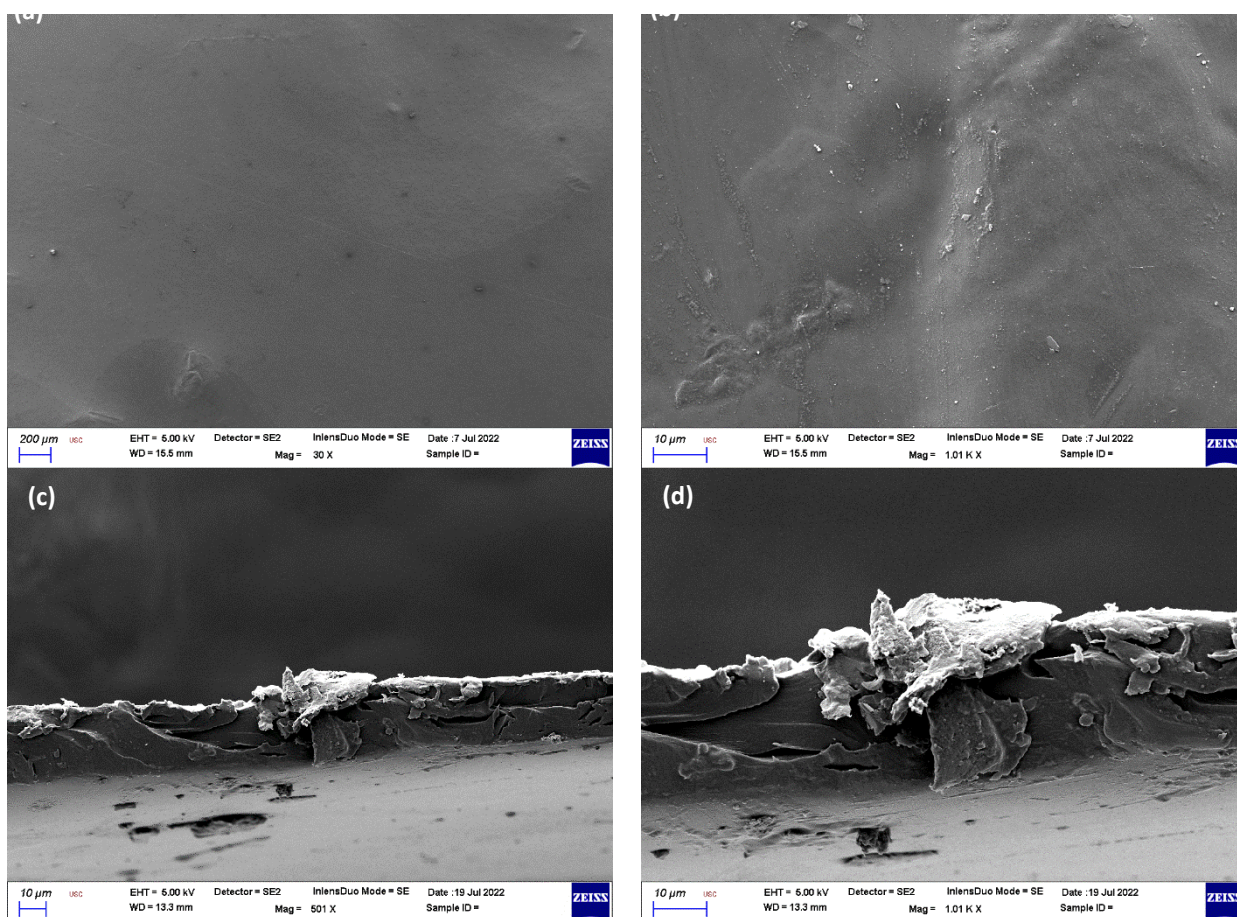


Figure 3. Surface morphology of the CH-MKS-G film (1:0.17 CH:MKS ratio; 15.86 g glycerol per 100 g dry chitosan and starch) at (a) 30× and (b) 1,010× magnification at 5.00 kV accelerating voltage and 15.5 mm working distance and cross-sectional view at (c) 501× and (d) 1,010× magnification at 5.00 kV accelerating voltage and 13.3 mm working distance.

3.3.4. Biodegradability

Test schemes for biodegradability require that a material loses 90% of its mass within a span of 90 days^[34]. The bioplastic films subjected to the soil burial test showed significant weight loss within a span of two weeks. After 4 days of incubation at room temperature, the recovered films were dried to constant weight and showed $83.29 \pm 5.49\%$ weight loss, which is close to the weight loss criterion for biodegradability. During sampling, a large portion of the films can still be recovered. Visual observation of the remnants, as shown in **Figure 4**, showed discoloration and the appearance of large holes or pores in most of the bioplastic surface. The appearance of color spots and patches may be linked to the decomposing action of microorganisms in the soil^[35]. After 6 more days from the first sampling, the films lost $89.09 \pm 1.74\%$ of their original mass. At this point, although discernible portions of the bioplastic material can be recovered from the substrate, the remnants no longer reflect the original texture nor color of the original film. Moreover, the films appear to be swelled due to moisture absorption. After 15 days, the bioplastic films have lost $96.05 \pm 1.43\%$ of their original mass. The remaining parts of the bioplastic films were difficult to recover but can still be distinguished from other matter in the substrate. At this point, the soil burial test was concluded as the films already demonstrated almost complete degradation. Thus, the produced bioplastic film is considered biodegradable according to the European Standard EN 13432:2000 for packaging materials^[34].

In comparison to a similar study by Mutmainna et al.^[36], which produced chitosan-potato starch composites with glycerin as plasticizer, the optimized bioplastic film produced in this study achieved a higher weight loss with a difference of $\sim 15\%$ in the same time frame. On the other hand, commercially available plastics made from corn, polylactic acid, potato, and sugarcane was reported to fully degrade in 7 to 20 weeks^[37]. This implies that the bioplastic film produced in this study degrades at a higher rate than most common bioplastics.

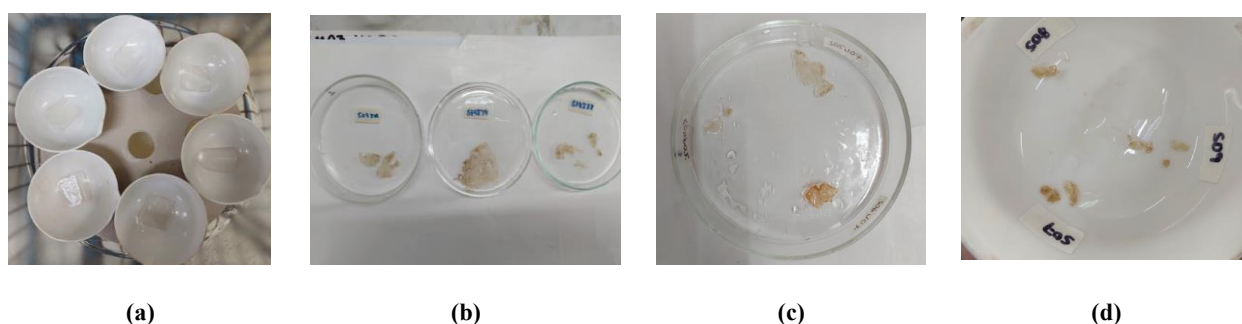


Figure 4. Images of the CH-MKS-G bioplastic films during the soil-burial test. (a) The films after oven-drying and before soil-burial test and the recovered film after (b) day 4, (c) day 10, and (d) day 15.

4. Conclusion

Bioplastic films made from high molecular weight chitosan and mango kernel starch with glycerol as plasticizer were successfully prepared and characterized. Through this study, it was found that the mango kernel starch and glycerol concentrations have significant effects on the elastic modulus of the film. Model fitting by regression produced a linear relationship between the variables which indicated that there is no optimal formulation in the experimental region (1:0.17 to 1:5.83 CH:MKS ratio and 15.86 to 44.14% weight of glycerol per total dry weight of chitosan and starch). The results posit that low MKS, and glycerol concentrations would produce a film with higher elastic modulus. The CH-MKS-G film with a CH:MKS ratio of 1:0.17 and a glycerol concentration of 15.86% was selected based on the trend displayed in the model and characterized in terms of its mechanical properties, water vapor transmittance rate, surface morphology, and biodegradability. The results show that the bioplastic film exhibited properties that are desirable for packaging applications and that the formulation had better characteristics than that of CH-S composite films investigated in previous studies.

The authors of this work recommend the incorporation of factors influencing the films' characteristics (i.e., drying time, humidity, and temperature) in a multivariable experiment design to obtain a better-fitting predictive model for the determination of an optimal blend formulation. It is also recommended to consider a new range of material ratios that includes lower starch and glycerol concentration. Other additives should also be considered which would improve the overall film's properties, especially its barrier properties.

Author contributions

Conceptualization, ASA and KCV; methodology, ASA and KCV; software, ASA; validation, ASA; formal analysis, ASA and KCV; investigation, KCV; resources, ASA and KCV.; data curation, ASA; writing—original draft preparation, ASA and KCV; writing—review and editing, ASA and KCV; visualization, ASA; supervision, KCV, NPT, FDS, and MKP; project administration, KCV, NPT, FDS, and MKP; funding acquisition, NPT, FDS, and MKP. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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