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Statistical Optimization of Optical Property (Transparency) of Bleached, Oxidized Sucrose Cross-Linked Cassava Starch Film

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Abstract

The present environmental pollution caused by non-degradable polymer films has prompted researches into the development of biodegradable polymer films. This study was able to develop a bleached, cross-linked film from native cassava starch. Hydrogen peroxide (H_2O_2) was added for bleaching to improve the transparency, oxidized sucrose was added for cross-linking and further oxidation while a moderated concentration of glycerol helps to improve the plasticity. In order to have a wider area of application of the film, the transparency was optimized using Box-Benkhen method under the response surface methodology (RSM) in Design Expert 11.0 software. UV-visible spectrophotometer analysis shows that the cassava starch film produced at the above process conditions retained 91.8 % of its transparency after 24 hours water immersion

Keywords: *Biodegradable, Films, Transparency, Bleached, Cross-Linked*

INTRODUCTION

Starch generally has been considered as one of the biopolymers with the highest potential of producing biodegradable films using various methods and techniques of manufacturing and processing (Teixeira et al., 2012) and has thus earned much attention especially due to its low cost and general availability. Researchers have widely studied and reported the usage of starch from different sources for films of diverse properties for numerous industrial and domestic applications (Lopez et al., (2011); Sun et al., (2014)). Cassava starch, when processed into thin films however, possesses high sensitivity to water and moisture due to the chemical structure consequently making it highly hydrophilic (Zhu, 2015). This hydrophilic nature of cassava starch film therefore affects its competition with other petroleum products in the industries (Parra et al., (2004), (Shan-Shan & Guo-Qing, 2012). There is therefore the need for modification of the film to yield the preferred mechanical, thermal and water sensitivity properties as it applies various industrial purposes. In addition, when compared to other synthetic polymers, the flexibility of starch films can be improved by the use of plasticizers such as the commonly used glycerol (possesses a relatively low molecular weight compound that can penetrate the starch film matrix more effectively than larger polyols) (Alves et al., 2007) or other polyols which reduces crystal nature of the molecules by increasing the polymer chains (Jiménez et al., 2012). Oxidized sucrose is bio-based and non-toxic. Like other poly-aldehyde cross-linkers, the oxidized sucrose crosslinks starch by forming reversible acetal bonds with the hydroxyl groups of the starch between and within the starch chains (Ali & Syed, 2009).

In this work, a three-level Box–Behnken response surface design (BBD) was applied in studying the effect of process parameters such as temperature, time and concentration on the optical properties (transparency) of the biodegradable cassava starch film cross-linked with oxidized sucrose.

The transparency of the film was determined by scanning the films using a spectrum mode of Shimadzu UV-Vis spectrophotometer where the film samples were fixed in a cuvette in such a way as to allow visible light beam pass through it and peaks were observed within the range of 200 - 214 nm. The transparency was determined by measuring the amount of light that was passed through each film in the form of percentage transmittance. The higher the percentage transmittance, the more transparent the film is.

$$Transparency = \frac{Abs\ (214\ nm)}{Thicknesss} \quad (1)$$

MATERIALS AND METHODS

Materials: Cassava starch was purchased from local farmers in Bida, Niger State, Nigeria. Other materials used were distilled water, Sodium periodate (>98 %), glycerol (99.8 %), sucrose, barium dichloride, and hydrogen peroxide (60 %) sourced from existing standard laboratories.

Experimental Methods

The methods adopted in this work are as follows;

a. Preparation of Oxidized Sucrose

The oxidized sucrose was prepared according to the method reported by Wang *et al.* (2018).

b. Bleaching of Cassava Starch with H_2O_2 and Cross-linking with Oxidized sucrose.

The starch film was prepared by measuring 50 ml of distilled water into a 250 ml beaker and heated on a hotplate magnetic stirrer until the temperature reaches 50 ^{0}C. 3g of cassava starch was measured into the beaker and the temperature was allowed to attain 70 ^{0}C before 0.5 ml of Hydrogen peroxide, H_2O_2 (30% V/V) was added for bleaching to improve the transparency, 0. 5 ml of the oxidized sucrose was added for cross-linking and further oxidation. 1.2 ml of glycerol was added to improve the plasticity. The experimental run was repeatedly conducted sixteen times by varying time, temperature and amount of glycerol to obtain the cassava starch film (see Table 1).

RESULTS

Second-order quadratic polynomial regression model and statistical analysis

Box-Behnken is a method under response surface methodology in Design-Expert 11.0 software that is used to perform regression and graphical analysis of results. The result of the design experiment was taken as the values of transparency at each of the experimental runs. Response surface regression was used to evaluate the experimental data using the second-order polynomial equation (2):

$$y = \beta_o + \sum_{i-1}^{k} \beta i x 1 + \sum_{i-1}^{k} \beta_{ii} x_i^2 + \sum_{i_{i>j}}^{k} \sum_{j}^{k} \beta_{ij} \mathcal{X}_i \mathcal{X}_j \quad (2)$$

where; y stands for the predicted variable (dependent), x_i and x_j are the uncoded independent variables, i and j are the linear and quadratic coefficients respectively, βo is the regression co-efficient, k is the number of factors studied and optimized in the experiment (Umaru *et al.,* 2016).

The lower and upper limits of each of the independent variables were selected based on the preliminary experiment. The codes, ranges, levels and the type of factor studied are presented on Table 1.

Table 1: Codes, ranges and levels of independent variables of Temperature, Time and Amount of Glycerol on Transparency

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Temperature	Degree C	Numeric	70.00	90.00	-1 ↔ 70.00	+1 ↔ 90.00	80.00	7.07
B	Time	Min	Numeric	5.00	15.00	-1 ↔ 5.00	+1 ↔ 15.00	10.00	3.54
C	Conc	Ml	Numeric	0.4000	2.00	-1 ↔ 0.40	+1 ↔ 2.00	1.20	0.5657

The actual and predicted transparency of cassava starch film from the seventeen experimental runs by Box-Behnken design are presented on Table 2. The quadratic equation 2 was used by the software to simulate the actual experimental transparency. The model equations 3 and 4 were then developed and used for the prediction of the transparency at each of the experimental runs. Residual value of zero is an indication of closeness of actual and predicted values, the total residual sum of 2.59792E-13 shows that the actuall and the predicted transparency are sufficiently closed to each other. The plot of the actual and the predicted transparency is shown in Figure 1. with a well distributed points along the line.

Table 2: Experimental and Predicted Transparency of modified cassava starch film from Box-Behnken Experimental Design

Runs	A: Tempt (^oC)	Time (Mins)	Amount of glycerol (ml)	Transparency (%)		Residual
				Actual	Predicted	
1	70	5	1.2	81	81.455	-0.455
2	90	5	1.2	88	88.72	-0.72
3	70	15	1.2	81.7	80.98	0.72
4	90	15	1.2	90	89.545	0.455
5	70	10	0.4	84	84.395	-0.395
6	90	10	0.4	91.76	91.89	-0.13
7	70	10	2	82.4	82.27	0.13
8	90	10	2	91	90.605	0.395
9	80	5	0.4	91.23	90.38	0.85
10	80	15	0.4	91.23	91.555	-0.325
11	80	5	2	90	89.675	0.325
12	80	15	2	88	88.85	-0.85
13	80	10	1.2	87	87.3	-0.3
14	80	10	1.2	87.3	87.3	0
15	80	10	1.2	87.2	87.3	-0.1
16	80	10	1.2	87	87.3	-0.3
17	80	10	1.2	88	87.3	0.7
Total						2.59792E-13

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Coded Factors:

Transparency (%) = *+87.30 +3.96* A +0.088* B -0.85* C+0.32* A * B+ 0.21* A * C -0.50* B * C -2.47* A2 +0.35 * B2 +2.47 * C2* (3)

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Final Equation in Terms of Actual Factors:

Transparency (%) = -88.49000 +4.25925* Temperature -0.63250* Time -11.15938* Conc +6.50000E-003 * Temperature * Time +0.026250* Temperature * Conc -0.12500* Time * Conc -0.024750* Temperature2 +0.01400 * Time2 +3.85156* Conc2 (4)

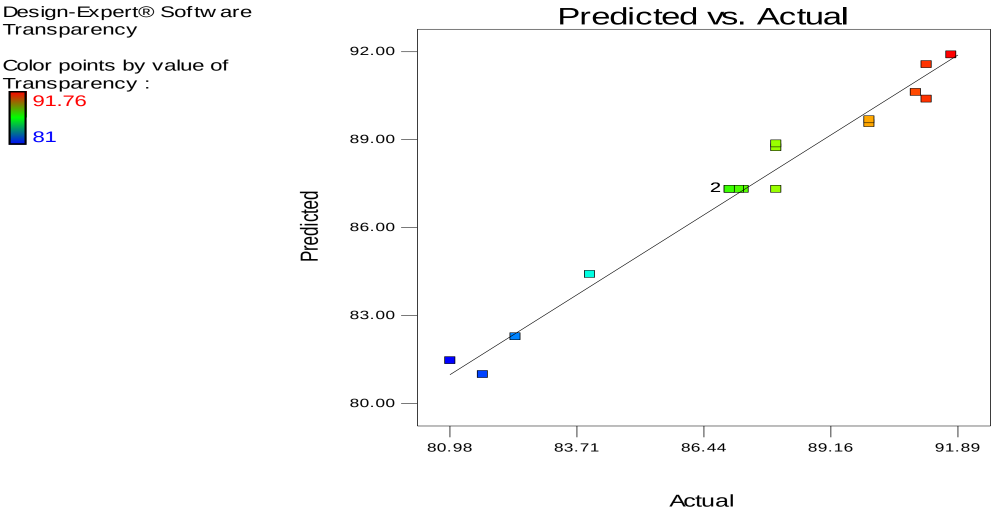

Figure 1: Plot of predicted against the actual transparency of cassava starch film

The summary statistics for the models and the lack of fit test are captured in Table 3 and 4. Coefficient of variance (R^2) and p-value of the models were tested for model significance. Model with R^2 value close to one and p-value less than 0.05 is most preferred. The four tested models are linear, two-factor interaction (2FI), quadratic, and cubic models. The cubic model has the best R^2 value of 0.9963 but it is aliased and it does not have predicted R^2 and p-value, which implies that the cubic model is not suitable for the prediction of the transparency of the cassava starch film. The quadratic model was chosen because of good R^2 value of 0.9778 and predicted value of R^2 of 0.6976 (Table 3). The linear and the 2IF models have poor values of both R^2 and predicted values of R^2, therefore both were rejected

. **Table 3: Model Summary Statistics**

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	
Linear	2.06	0.7044	0.6361	0.3853	
2FI	2.31	0.7129	0.5407	-0.4574	
Quadratic	**0.7684**	**0.9778**	**0.9493**	**0.6976**	**Suggested**
Cubic	0.4123	0.9963	0.9854		Aliased

Table 4: **Lack of Fit Tests**

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Linear	54.37645	9	6.041828	35.54016	0.0018	
2FI	52.77755	6	8.796258	51.7427	0.0010	
Quadratic	3.45295	3	1.150983	6.77049	0.0479	Suggested
Cubic	0	0				Aliased
Pure Error	0.68	4	0.17			

The Model F-value of 34.27 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, A2, C2 are significant model terms. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.The "Lack of Fit F-value" of 6.77 implies the Lack of Fit is significant. There is only a 4.79 % chance that a "Lack of Fit F-value" this large could occur due to noise. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 18.513 indicates an adequate signal. This model can be used to navigate the design space.

Table 5: ANOVA for Response Surface Quadratic Model of the Transparency

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	182.0933	9	20.23258	34.26804	< 0.0001	Significant
A-Temperature	125.2945	1	125.2945	212.2119	< 0.0001	
B-Time	0.06125	1	0.06125	0.103739	0.7568	
C-Conc	5.81405	1	5.81405	9.847288	0.0164	
AB	0.4225	1	0.4225	0.715591	0.4256	
AC	0.1764	1	0.1764	0.29877	0.6016	
BC	1	1	1	1.693705	0.2343	
A^2	25.79211	1	25.79211	43.68423	0.0003	
B^2	0.515789	1	0.515789	0.873595	0.3811	
C^2	25.58411	1	25.58411	43.33194	0.0003	
Residual	4.13295	7	0.590421			
Lack of Fit	3.45295	3	1.150983	6.77049	0.0479	Significant
Pure Error	0.68	4	0.17			
Cor Total	186.2262	16				

Adequate precision: 18.513

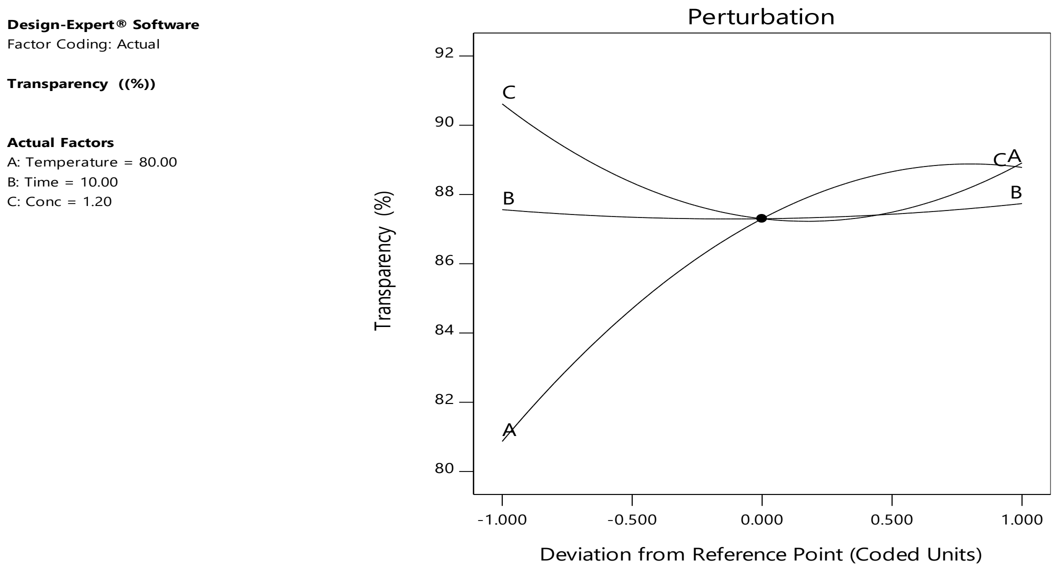

Figure 2: Single effect of Temperature, time and Concentration on Transparency

Individual Effect of Temperature, time and Concentration on Transparency

The single effect of temperature, time and glycerol concentration is presented in Figure 2. It can be clearly seen on the plot that as temperature increases from 70 ^{o}C to 90 ^{o}C, the transparency also increased from 80.87 to 88.87 %, this implies that an increase in temperature brings about increase in the kinetic energy of reactant molecules which consequently results in reduction of opaqueness and increase contact between the cassava starch and H_2O_2 in the bleaching process (Alcázar-AlayMaria & Meireles, 2015). Hence, effect of temperature has positive significant effect with P-value of < 0.0001 (Table 5) on the transparency of the cassava starch film. As time increases from 5 to 15 minutes, the transparency only has a slight increase from 88.5 % to 88.7 %, this implies that the effect of time is insignificant (P-value of 0.7568) on the transparency of cassava starch film. As the amount of glycerol increases from 0.4 to 2 ml, the transparency decreases slightly from 90.6 to 88.9 %.

Interactive Effect of Temperature, time and Concentration on Transparency

The contour plot of the interactive effect of time and temperature is presented in Figure 3. It can be clearly seen that as time increases from 5 to 15 minutes and temperature increases from 70 to 90 ^{o}C, the transparency also increases from 82.28 % to 88.13 %. This implies that increase in temperature causes an increase in the kinetic energy of the reaction and sufficient time was provided for the reactant molecules to utilize the kinetic energy for bond formation of transparent cassava starch film (Alcázar-AlayMaria & Meireles, 2015). Transparency between 85.2 to 88.1 % is more prominent and is presented in green colour as shown on the three-dimensional (3D) view of the interactive effect of temperature and time in figure 4 while 83.7 5% has the least prominent transparency.

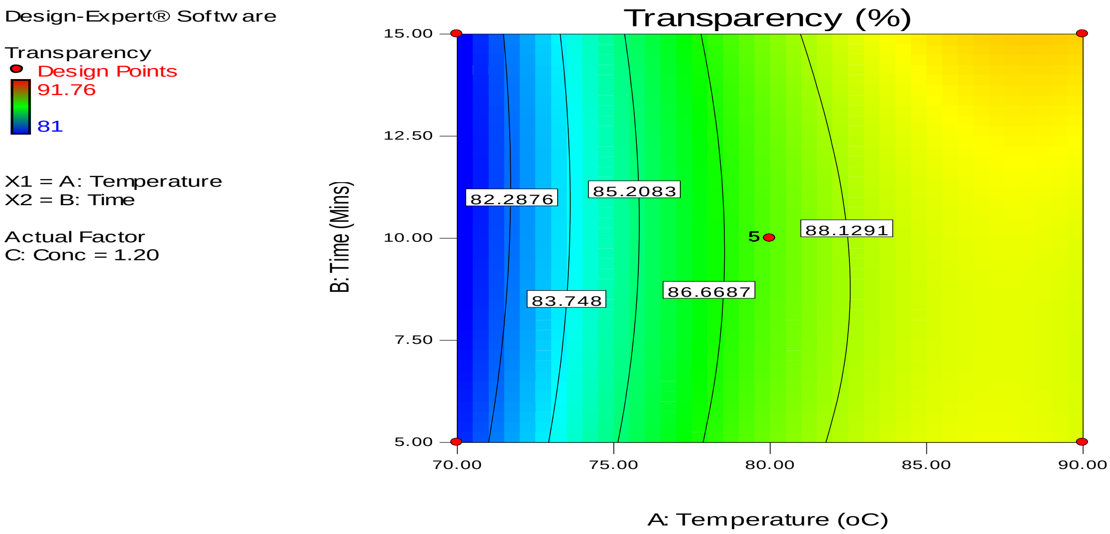

Figure 3: Contour Plot of the interactive effect of Time and Temperature of the Transparency of Cassava Starch Film.

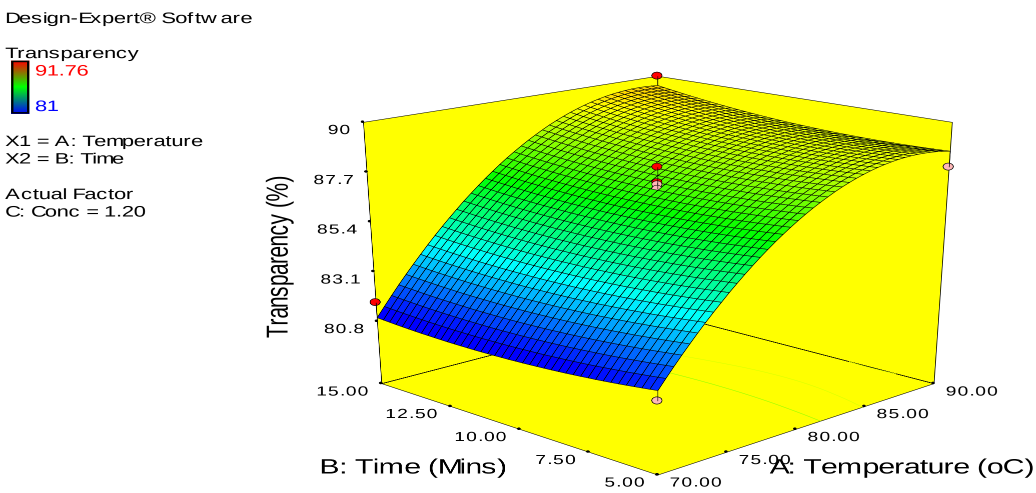

Figure 4: 3D view of the interactive effect of Time and Temperature of the Transparency of Cassava Starch Film

The contour plot of the interactive effect of glycerol concentration and temperature is presented in Figure 5. It can be clearly seen that as concentration increases from 0.4 to 2 mL and temperature increases from 70 to 90 ^{o}C, the transparency also increases from 82.6 % to 90.2 %. This implies that increase in temperature provided enough kinetic energy that enhances the binding of the glycerol to the starch molecule in other to improve on the plasticity of the film (Liang & Ludescher, 2015; Monteiro, et al., 2017). Increase in glycerol concentration without increase in temperature causes the glycerol to stay on the surface, this lead to reduction in the transparency of the starch film, transparency between 84.5 to 88.3 % is more prominent and it is represented it is the green colour while 82.3 % (blue colour) has the least prominent transparency.

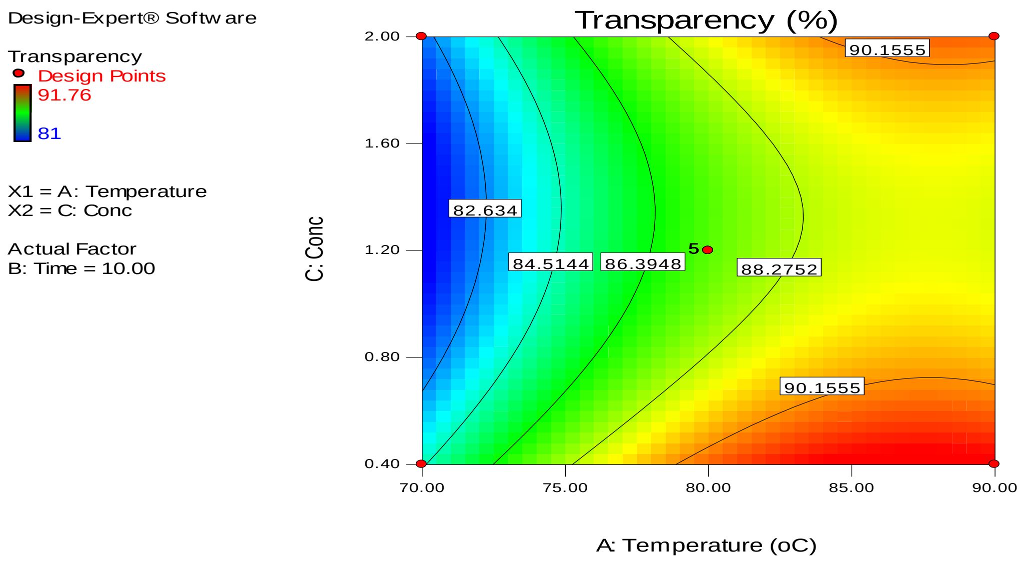

Figure 5: Contour Plot of the interactive effect of Glycerol Concentrations and Temperature on the Transparency of Cassava Starch Film.

The three dimensional (3D) view of the interactive effect of temperature and time is shown in Figure 6.

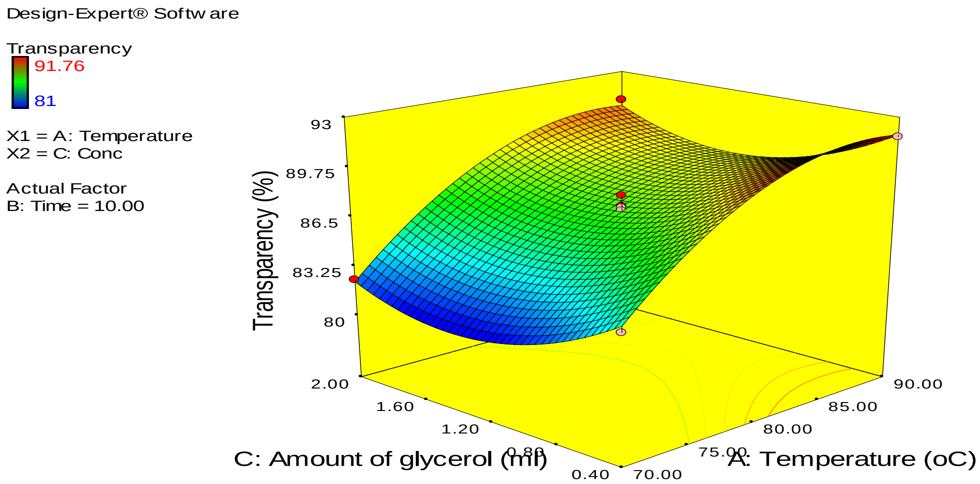

Figure 6: 3D view of the Interactive effect of Glycerol and Temperature on the Transparency of Cassava Starch Film.

The contour plot of the interactive effect of glycerol concentration and time is presented in Figure 7. It can be clearly seen that as concentration increases from 0.4 to 2 mL and time increases from 5 to 15 minutes, the transparency also increased slightly from 88.7 % to 90.8 %. This implies that increase in time and glycerol concentration does not have much effect on the transparency. Transparency between 87.9 to 88.6 % is more prominent and it is represented by the green colour while 90.8 % (red colour) has the least prominent transparency.

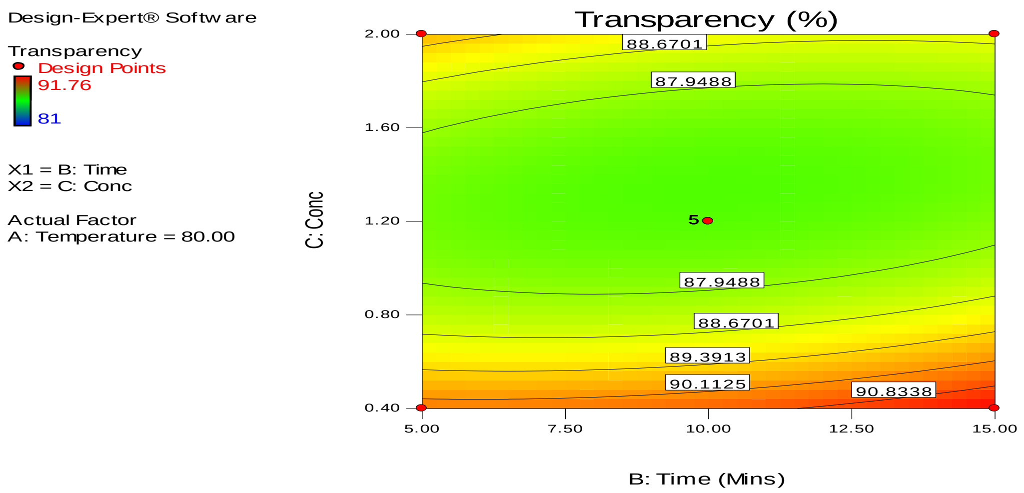

Figure 7: Contour Plot of the interactive effect of Glycerol Concentration and Temperature on the Transparency of Cassava Starch Film.

The three-dimensional (3D) view of the interactive effect of temperature and time is shown in Figure 8.

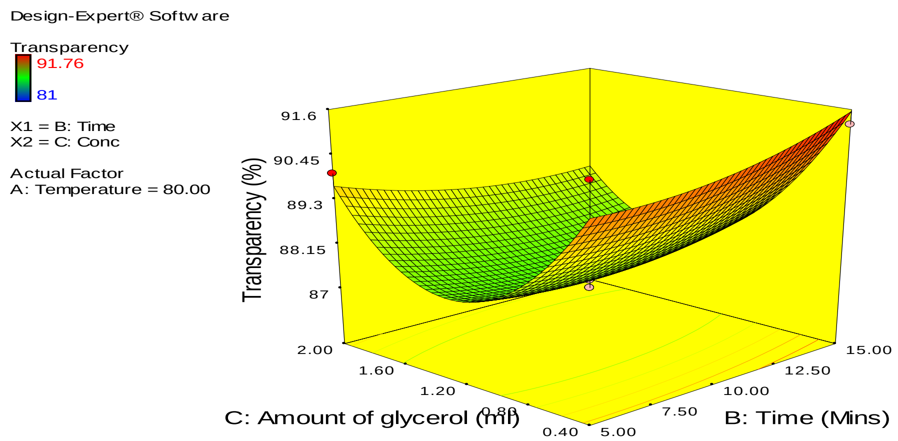

Figure 8: 3D view of the Interactive effect of Glycerol and Time on the Transparency of Cassava Starch Film.

Optimization Study of the Process Parameter on the Transparency of the Film

Numerical optimization method was used for the point prediction and the constraints is presented in Table 6. Temperature has positive significant effect on the transparency; hence its effect was maximized. Since the glycerol concentration has negative significant effect on the transparency, then its effect was minimized, while maximizing the effect of time gave the highest transparency. 93.2 % transparency was predicted at temperature of 90 ^{0}C, time of 15 Minutes and glycerol concentration of 0.4 mL (Figure 9) with desirability of 1 (Figure 10). 93.1 % transparency was validated at the predicted optimum points, this shows there is only 0.11 % err

Table 6: Optimization Constraints on Transparency of Cassava Starch Film

Name	Goal	Lower Limit	Upper Limit
A: Temperature	maximize	70	90
B: Time	maximize	5	15
C: Conc	minimize	0.4	2
Transparency	maximize	81	91.76

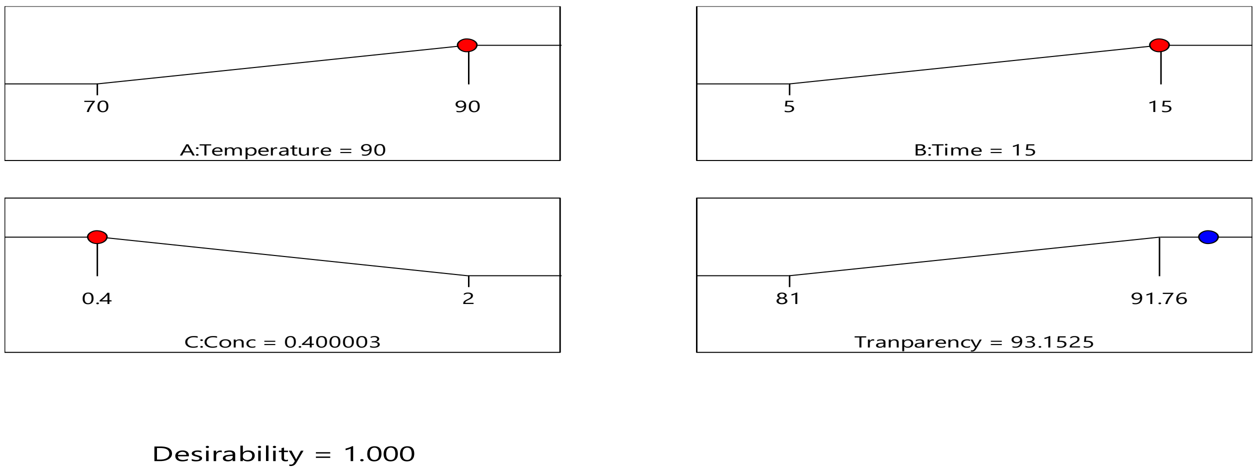

Figure 9: Optimum Point Prediction for Cassava Starch Film Transparency

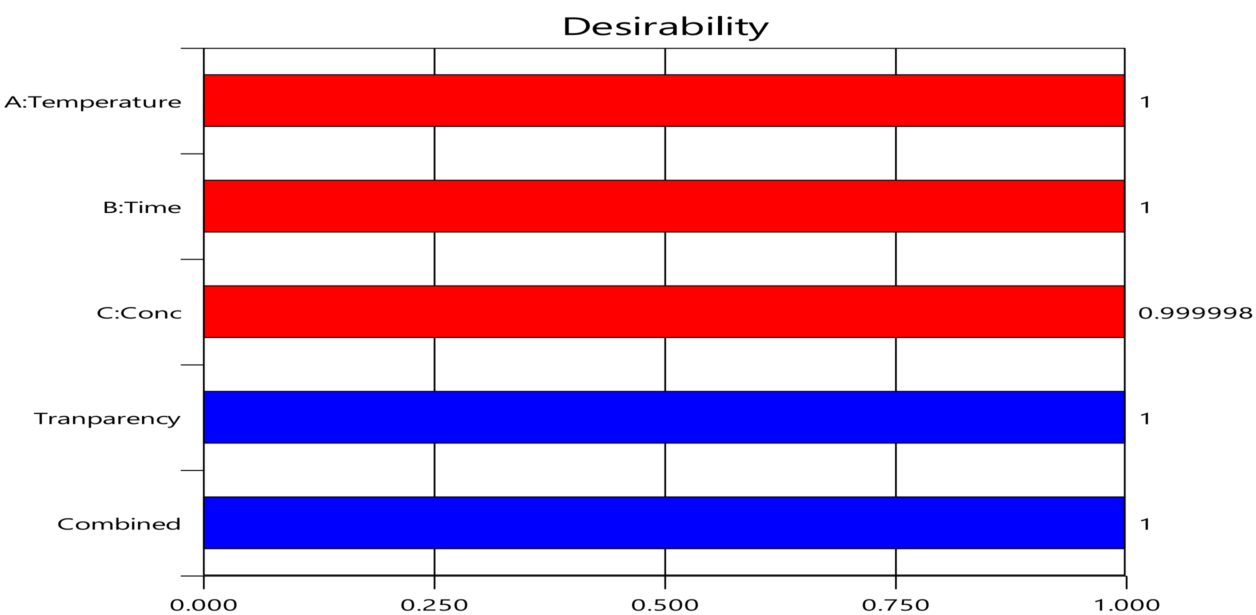

Figure 10: Desirability of Optimized Transparency

TRANSPARENCY OF THE STARCH

Table 7: Changes in Transparency of the Films at Various Hours and Intervals of 24 hours

	Process parameters				Exposure Time (Hours)					
					0	24	48	72	96	120
S/N	Sample Code	Temperature (^{0}C)	Time (Mins)	Amount of Glycerol (ml)	Degree of Transparency (%)					
1	Control	70	5	0	95.544	76.826	0	0	0	0
2	0	83	12	0.8	98.558	86.998	0	0	0	0
3	6	90	10	0.5	96.917	91.759	90.205	88.412	88.197	0
4	9	80	5	0.5	98.558	91.23	54.291	0	0	0
5	10	80	15	0.5	97.169	91.23	81.677	0	0	0

Table 7 shows the results of the transparency after the immersion in water and from 0 hours to 120 hours at intervals of 24 hours. For industrial application of the cassava starch film where transparency is important, sample 3 is considered as the best due to its ability to retain high percentage of its transparency up to 96 hours of immersion in water.

Conclusion

This study investigated the influence of temperature, time and amount of glycerol on the transparency of oxidized starch film. The Box-Behenken design method under response surface methodology of optimization gave 17 experimental runs. A second-order quadratic model suitable for the prediction of the transparency of the oxidized starch film was established. The ANOVA shows that temperature and glycerol have significant positive effect influence on the transparency. A transparency of 93.15 % was predicted at temperature of 90 ^{0}C, time of 15 minutes and 0.4 mL of glycerol with desirability of 1 using numerical optimization method. 93.1 % transparency was validated at the predicted optimum points, this shows there is only 0.11 % error.

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Conflict of Interests

There is no conflict of interest.

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