Mathematical Modelling and Optimization of Chitosan Based Polymer Film Quality Parameters.

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ABSTRACT
Modelling and optimization is a form of mathematical expression that attempts to determine the optimal value of a complex equation. The characterization, modelling and optimization of chitosan-based edible film was investigated. Formulated matrix from chitosan percentage compositions of 2, 3 and 4 % w/v plasticized with glycerol:sorbitol ratio of 100:0, 80:20, and 60:40 % v/v. Pure chitosan (without plasticizer) of the three levels of treatment served as control experiment. Casting method of film production was adopted. The films mechanical properties (tensile strength, elongation at break, young modulus, film thickness, and puncture strength) and Barrier properties (water vapour permeability, solubility in water, degree of swelling and film moisture content) were evaluated. The barrier and mechanical properties of the films were modelled and optimized using Design-Expert 13 software. All the edible film properties fitted adequately into quadratic model equations with the exception of film solubility that fitted best into linear model based on highest values of R^2, adjusted R^2 and $P < 0.05$. Mathematical equation for each parameter was developed as generated through statistical surface response methodology (SSRM). Optimized result generated gave 2 % chitosan plasticized with 70 % glycerol and 30 % sorbitol having a desirable value of 0.51. At this optimized simulation, the surface responses were determined as tensile strength 12.32MPa, puncture strength 11.67N, elongation at break 4.7%, Young's Modulus 2.63MPa, water vapour permeability $10.38gm^{-1}s^{-1}KPa^{-1}$, moisture content 9.11%, film solubility 27.86% and degree of swelling 124.70%.

I.   INTRODUCTION

Food packaging is the enclosure of food materials or products to protect and preserve their nutritional quality and increase their shelf-life for sustainable food value chains (Lindh *et al*. 2016, United Nations, 2015). The problems of packaging have led to huge post-harvest losses of food materials the world over. The primary purpose of packaging is to keep the food safe for human and animal consumption, be affordable, meet consumers' preference and be environmentally friendly. The commonly used synthetic food packaging materials such as polyethylene, terephthalate, polyvinylchloride, polyethylene, polypropylene, polystyrene, and polyamide are petrochemical-based (Jacob *et al*. 2020, Patricia and Manuel 2019, and Muncke *et al*. 2016). Though these synthetic materials have the benefits of efficient mechanical and barrier properties at a low cost, the disadvantages of non-degradable far outweighed their benefits. They all ended up as plastics polluting the environment, which can take hundreds of years for a complete breakdown of these materials (Lindani *et al.* 2020)**.** Life in the seas and oceans are affected by the toxic released from these plastics and can be cancerous to human health as seafoods are consumed by man along the food chain. Depletion of the ozone layers is another resultant effect of non-degradable waste product from synthetic food packaging materials. In addition to environmental problems, consumers globally are requesting from industries a healthy food without the use of chemical preservatives at the same time with extended shelf-life (Campos-Requena *et al.* 2017, Miquel *et al*., 2011 Chien *et al*., 2007).

 As a result of these problems, various food regulatory bodies of nations are now encouraging the food industries to reduce if not totally eliminate the use of petrochemical-based food packaging materials (Miguel *et al*. 2011).

Present developments in the food packaging industries is now replacing synthetic materials with bio-degradable, non-toxic, environmentally friendly, and edible materials based on the outcome of several scientific researches. Ashok, et al. (2016) and Lindani et al. (2020) reported that a bio-degradable food packaging material is a material that can disintegrate from its longer molecular chains into smaller molecular components

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parts when acted upon by agents of disintegration such as temperature, moisture, bio-enzymes. Edible, bio-degradable polymers being used for packaging are products from polysaccharides, proteins (both plants and animals), gums, lipids, or their combinations (Kamaldeen *et al*., 2019, and Campos-Requena *et al*., 2017). Patricial and Manuel (2019) reported that the application of these materials or their combinations depend on their ability to prevent moisture and aroma loss, solute transport, water absorption within the food matrix, or oxygen penetration. Thin edible films and coatings are obtained from these materials to provide a covering for fresh and processed food products to prolong their shelf-life beyond normal.

The main objective of this work is to mathematically optimize the modelling of plasticizer (glycerol:sorbitol) and chitosan-based polymer's quality parameters using Statistical Surface Response Methodology (SSRM).

Optimization modelling is a form of mathematical expression that attempts to determine the optimal value of a complex equation. It is the design formulation required to minimize the number of trials and analyze the effect of the different compositions using the response surfaces for each quality parameter to obtain the appropriate formulations for the optimum value of each quality parameters (Kamaldeen *et al.* 2019; Malakar *et al.* 2013). This work went further to carry out an experimental validation of the appropriate formulation's quality parameters.

II.   Materials and Methods

2.1 Development of a Chitosan-Based Edible Coatings

2.1.1 Sources of Raw Materials

Food grade Chitosan powder >88% purity was purchased through online marketing from a Canada-based manufacturing company-Chitolytic Chitosan Company and delivered through courier service. Glycerol, Sorbitol and other reagents such as acetic acid and sodium hydroxide of analytical grade were purchased from a chemical supplier in Kano, Nigeria.

2.1.2 Formulation of Edible Coating Solution

The formulation was in three different concentrations of Chitosan (C_1 = 2 % w/v, C_2 = 3 % w/v and C_3 = 4 % w/v). Plasticizers used were Glycerol and Sorbitol in four different combinations (P_1 = 100 % glycerol:0 % sorbitol, P_2 = 80 % glycerol:20 % sorbitol, P3 = 60 % glycerol:40 % sorbitol), and Po (without plasticizer) to serve as control experiment.

2.1.3 Experimental Design

A completely Randomized Design (CRD) in a 3x4 factorial was adopted for the production of edible films. Twelve (12) treatments were generated to be replicated three (3) times each giving a total of thirty-six (36) experiments

Table 1: Experimental Design of Edible Film Production

C_1P_0	C_1P_1	C_1P_2	C_1P_3
C_2P_0	C_2P_1	C_2P_2	C_2P_3
C_3P_0	C_3P_1	C3P2	C_3P_3

NOTE: C_1- 2 % Chitosan, C_2- 3 % Chitosan, C_3- 4 % Chitosan
   P_0- Gly 0 %: Sorb 0%, P_1- Gly 100 %: Sorb 0 %, P_2- Gly 80 %: Sorb.20 %,
   P_3- Gly 60 %: Sorb 40 %

Table 2 Full Factorial Experimental Design using Design Expert (version-13) Software

Run	Chitosan %	Glycerol %	Sorbitol %
1	2	60	40
2	3	60	40
3	3	0	0
4	4	80	20
5	4	60	40
6	3	80	20
7	2	0	0
8	3	100	0
9	4	0	0
10	2	100	0
11	2	80	20
12	4	100	0

2.1.4 Production of Edible Films

The method of production of edible film solution as described by Butler et al. (1996) with modifications by Nazan, et al. (2004) was adopted. The casting method of film application as described by Prakoso et al., 2023 was also applied. 200ml of distilled water was measured with a measuring cylinder into a 500ml glass beaker, 1% acetic acid was added and the solution was heated on a Bunsen burner. At 60°C, chitosan powder of appropriate weight corresponding to different proportions (2 %, 3 %, and 4 % w/v) was added gradually with continuous stirring with a stirring rod until the powder was completely dissolved. This was then followed by the addition of 5 % v/v 0.1 M NaOH solution to neutralize the film-forming solution by adjusting its pH to 8.5. Different percentages of glycerol and sorbitol (100% gly: 0% sorb, 80% gly:20% sorb, 60% gly:40% sorb) as plasticizers were added, thereafter they were placed inside a water bath at 85°C to be heat-denatured for 5minutes for cross-linking of the molecular chains. The solution was cooled to 50°C. For thickness uniformity, 25ml of the film forming solution was cast in a sterilized petri dish of 8.5cm diameter and allowed to set for 24 h. Already set film was dried overnight inside an oven at 35°C (Prakoso *et al*., 2023). Dried polymer matrix films were detached from the Petri dishes and preserved.

2.2 Mechanical Properties of Edible Films

2.2.1   Tensile Strength

The method used was according to Saiful *et al.* (2013). Samples were initially pre-conditioned for 72 h inside a desiccator containing a saturated solution of magnesium nitrate salt at 25°C and 52% ±1 RH. The test was carried out at the National Centre for Agricultural Mechanization (NCAM) Ilorin, Kwara State, Nigeria using the method described by Dursun and Erkan (2014). The instrument used was Testometric Materials Testing Machine No. 0500-10080 Product of United Kingdom. Film samples were cut into strips. The width of each sample was 15 mm and the length after clamping between the lower and upper jaws was 30 mm. The thickness of each polymer film sample was taken and recorded using a digital Vernier caliper. The test speed of the machine was 5.000 mm/min. Loading was done steadily under monitoring on the system attached to the machine until the film extends to the breaking point. Tensile strength was calculated from:

$$\text{Tensile Strength} = \frac{F}{A} \ (N/M^2) \hspace{3cm} (1)$$

Where; F is the maximum force applied at the point of rupture of the film matrix under test,

A is the cross-sectional area of the membrane matrix (m^2)

2.2.2   Elongation at Break (EAB)

This was determined by calculation. The maximum extension of the membrane matrix at the point of breaking was divided by the original length. EAB was determined using equation (2):

$$\text{EAB} = \frac{\Delta L}{L1} \times 100 \hspace{3cm} (2)$$

Where: ΔL = Change in Length (L_2-L_1), L_1 = Initial length, L_2 = Final length after membrane rupture.

2.2.3 Puncture Test

The puncture test is to determine the amount of force to puncture the membrane matrix under compressional force. This mechanical property test was conducted using Testometric testing machine No. 0500-10080 at NCAM Ilorin. The method described by Acharya, (2016) was adopted. The sample holding jaw having a hole diameter of 15 mm was modified to suit this test. Samples of 50 mm diameter were cut out from the film membrane and placed centrally on the hollow jaw. The thickness of each sample was taken and using digital Vernier caliper. This formed their height on the machine. The test speed and preload forces were 25.000 mm/min and 0.5000 N respectively. The cylindrical probe of diameter 2 mm attached to a digital force gauge of the machine was then gradually dropped perpendicular to the membrane surface and forced to pass through it. The maximum force that was able to rupture the membrane was then recorded. The experiment was repeated twice for each sample and the mean value was calculated.

2.2.4   Young's Modulus

The Young Modulus (E) is a mechanical property of an engineering material that determines its ability to easily stretch and deform under loading. It is defined as the ratio between tensile strength and percentage strain.

$$\text{Young Modulus (E)} = \frac{\text{Tensile Stress}}{\text{\%Elongation}} \ (N/m^2 \text{ or Pa}) \hspace{2cm} (3)$$

2.3 Barrier Properties

2.3.1 Water Vapour Permeability (WVP)

The water vapour permeability of the formulated and produced edible film was determined by the Gravitational method at 25 ± 1°C using a modified ASTM E96-80 procedure as described by Zhang et al., (2016). The procedure for the desiccant method of American Standard for Testing Materials was adopted for the analysis. The film was sealed over the open surface of a glass crucible with adhesive gum containing anhydrous calcium chloride filled up to 1cm from the top level to create a vacuum condition. The initial weight of the crucible plus $CaCl_2$ was measured with an analytical weighing scale and recorded as $W_{o.}$ The crucible was now placed inside the desiccator containing a saturated solution of $Mg_2(NO_3)$. The water vapor transferred through the film membrane and absorbed by the desiccant was then determined by measuring the weight gained after every hour. Readings were taken for 6 h. WVP was calculated from the following equation:

WVP = $\frac{CX}{A \Delta P}$ ` (4)

Where:

WVP = Water Vapour Permeability in g/m s Pa,

X= the film thickness (m),

A = area of the exposed film (m^2),

ΔP =the water vapor pressure differential across the film (Pa),

C = the slope of the weight gain of the dish against time to the nearest 0.0001g. The slope was calculated by linear regression and correlation coefficient (r^2). Readings for each polymer films were replicated three times.

2.3.2 Solubility of Film Membrane

Film solubility determines the biodegradability of edible films. To evaluate the solubility capacity of the developed film, the method used by Saifu, et al. (2013) was adopted in which seven 5.5 cm square pieces was cut from the films and weigh to the nearest 0.0001g using electronic scale. One of the samples was then placed inside a perforated sample holder and immersed in a beaker containing 100 ml of phosphate buffer solution at $25℃$ with continuous stirring using a magnetic stirrer at 500 rpm. After 10mins of stirring the sample holder was removed and its content filled up to 100ml in a measuring flask. The solution and film piece was poured into a filter paper, rinsed with 10 ml distilled water and dried at 70 ^{o}C in a vacuum oven for 24 h to determine the dry mass of the film. Total soluble matter was calculated from the initial gross mass and the final dry mass using equation (5)

% Water Solubility = $\frac{\text{Initial film mass} - \text{final film mass}}{\text{Initial film mass}} \times 100$ (5)

2.3.3 Degree of Swelling (DoS)

The swelling test was performed using the method described by (Kamaldeen, *et al.* 2019) with little modifications. Samples of the produced films was dried in an oven at 105°C for 24 h. They were weighed on an electronic scale after cooling in a desiccator and recorded as $W_;$ they were then immersed in 250ml beaker containing 100 ml of distilled water and placed under a room temperature for another 24 h. Swelled films were removed from the solution and surface water was sucked out using filter paper and final weight taken as W_F. Degree of swelling in percentage was calculated using equation (6)

Degree of Swelling (%) = $\frac{W_F - W}{W} \times 100$ (6)

Where: W_F = Final weight after swelling, W= Initial weight before swelling.

2.3.4 Film Moisture Content

The gravimetrical method of measuring moisture content as described by Guanjie *et. al.* (2019) was used to determine the moisture content of films that was initially preserved inside a desiccator at 25°C and 52 $\pm$1 RH. Initial weight was measured on an analytical weighing balance and recorded as W_1. It was then placed inside a laboratory oven dryer set at 105°C for 24 h. The final weight after 24 h was taken and recorded as W_2. Percentage moisture content was calculated using equation (7):

Film Moisture Content (%) = $\frac{W_1 - W_2}{W_1} \times 100$

(7)

2.4 Modelling Film Quality Parameters

The modelling equation of edible films produced from 3 different percentage compositions of chitosan and 4 different percentages plasticizer as factors with their corresponding surface responses (quality parameters) were determined using design expert version-13 software. An experimental design of 3x4 factorial were generated given a total of 12 trial formulations as independent factors. The dependent variables evaluated were: water vapour permeability (g/m.s.KPa), Film Solubility (%), Degree of Swelling (%), Film Moisture Content (%), Tensile Strength (MPa), Young's Modulus (MPa), Puncture Strength (N), Elongation at Break (%). The mean value of all the responses were fitted to get their corresponding equation. The best fitted model equations were determined based on highest values of adjusted multiple correlation coefficient (Adjusted- R^2) and predicted multiple correlation coefficients (Predicted-R^2).

2.5 Optimization of Film Quality Parameters

Design expert version-13 software application was used to optimize the quality parameters of the developed edible films. The parameters include the followings: tensile strength, film elongation at break, puncture strength, young's modulus, water vapour permeability, membrane solubility in water, degree of swelling and film moisture content. Upper and lower limit values were set for the chitosan and plasticizer based on their degree of importance to the preservation of the food products. Tensile stress, puncture strength, elongation at break, young's modulus, and solubility were all set at maximum level while water vapour permeability, moisture content, and degree of swelling were set at minimum level.

III. Result and Discussion

Table 3: Adjusted R^2 and Predicted R^2 of the Polymer Film quality parameters.

Response	Model	Adjusted R^2 (%)	Predicted R^2 (%)
Puncture Test	Linear	75.19	65.22
	Linear + Square	72.96	40.20
	Quadratic	94.54	83.61
Tensile Strength	Linear	91.65	87.24
	Linear + Square	91.64	78.55
	Quadratic	99.65	99.20
Elongation	Linear	89.75	86.02
	Linear + Square	88.48	84.02
	Quadratic	94.67	89.76
Young's Modulus	Linear	93.64	89.06
	Linear + Square	95.62	86.67
	Quadratic	99.70	99.72
Water Vapour Permeability	Linear	61.51	34.57
	Linear + Square	70.00	54.76
	Quadratic	90.61	53.17
Film Solubility	Linear	87.55	82.47
	Linear + Square	86.14	73.98
	Quadratic	85.35	61.35
Moisture Content	Linear	81.61	70.63
	Linear + Square	87.42	83.48
	Quadratic	95.66	84.27
Degree of Swelling	Linear	66.35	41.07
	Linear + Square	75.76	46.46
	Quadratic	94.77	82.40

From Table 3 the result of adjusted R^2 and predicted R^2 were displayed. Based on highest values of adjusted and predicted R^2(s) it was observed from the result that film tensile stress, elongation at break, puncture strength, young's modulus, water vapour permeability, degree of swelling and moisture content were best fitted into quadratic modelling equations while only solubility was best fitted into linear model equation.

Table 4 Statistical Analysis for Modelling Quality Parameters of Percentage Compositions   of Chitosan and Plasticizers Based Films.

		Puncture Strength (N)			
Source	**Degree of Freedom (DF)**	**Sum of Squares (SS)**	**Mean Squares (SS)**	**F-Value**	**P-Value**
Model	5	571.82	114.36	39.12	0.0002
A	1	411.76	411.76	140.83	0.0001
B	1	0.3724	0.3724	0.1274	0.7334
AB	1	3.74	3.74	1.28	0.3014
A^2	1	13.13	13.13	4.49	0.0784
B^2	1	85.23	85.23	29.15	0.0017
		TENSILE STRENGTH (MPa)			
Source	Degree of Freedom (DF)	Sum of Squares (SS)	Mean Squares (MS)	F-Value	P-Value
Model	5	611.74	122.35	630.65	0.0001
A	1	100.27	100.27	516.87	0.0001
B	1	404.90	404.90	2087.06	0.0001
AB	1	4.63	4.63	23.88	0.0027
A^2	1	1.50	1.50	7.71	0.0322
B^2	1	34.59	34.59	178.31	0.0001
		ELONGATION @ BREAK %			
Model	5	5.24	1.05	40.11	0.0002
A	1	4.11	4.11	157.33	0.0001
B	1	0.7137	0.7137	27.32	0.0020
AB	1	0.0004	0.0004	0.0165	0.9019
A^2	1	0.0048	0.0048	0.1844	0.6826
B^2	1	0.297	0.2907	11.13	0.0157
		YOUNG'S MODULUS (MPa)			
Model	5	42.05	8.41	724.00	0.0001
A	1	12.78	12.78	1100.43	0.0001
B	1	24.70	24.70	2126.56	0.0001
AB	1	0.8505	0.8505	73.22	0.0001
A^2	1	0.8505	0.8505	73.22	0.0001
B^2	1	1.02	1.02	87.87	0.0001
		WVP x10^{-7} (g/m.s.KPa)			
Model	5	290.79	58.16	22.24	0.0008
A	1	31.59	31.59	12.08	0.0132
B	1	146.22	146.22	55.92	0.0003
AB	1	29.64	29.64	11.34	0.0151
A^2	1	2.86	2.86	1.09	0.3362
B^2	1	48.32	48.32	18.48	0.0051
		Moisture Content %			
Model	5	571.51	114.30	49.49	0.0001
A	1	199.96	199.96	86.57	0.0001
B	1	234.38	234.38	101.47	0.0001
AB	1	34.50	34.50	14.94	0.0083
A^2	1	37.30	37.30	16.15	0.0070
B^2	1	2.41	2.41	1.04	0.3463
		Film Solubility %			
Model	2	110.53	55.26	39.69	0.0001
A	1	12.70	12.70	9.12	0.0145
B	1	97.83	97.83	70.26	0.0001
		Degree of Swelling %			
Model	5	1.365E+05	27299.03	40.85	0.0001
A	1	25489.66	25489.66	38.15	0.0008
B	1	64526.07	64526.07	96.57	0.0001
AB	1	13912.08	13912.08	20.82	0.0038
A^2	1	188.61	188.61	0.2823	0.6143
B^2	1	20569.06	20569.06	30.78	0.0014

3.1 Modelling Film Quality Parameter Equations.

3.1.1 **Tensile Strength:** The p-value of quadratic model was 0.0001, (Table 4) which is less than 5 %. The implication of this is that the chance of the model going into error was less than 5 %. The quadratic equation was suitable to predict the tensile stress of film membrane. The final equation in terms of coded factors was:

Y= 4.17B^2+0.75A^2-1.02AB -7.93B+3.66A+11.03 (8)

Where Y = Tensile Stress

A= Chitosan Concentration

B= Plasticizer Blend (glycerol: Sorbitol)

it was observed that the square of chitosan concentration (A^2), square of plasticizer blend (B^2), interaction between chitosan and plasticizers (AB), chitosan (A) and plasticizer (B) all have significant ($p<0.05$) effect on the tensile stress of the film membrane. The negative coefficient of plasticizer (B) and that of chitosan-plasticizer (AB) interaction indicated that as their concentrations were increasing the value of Tensile strength was reducing. Figure 1 showed a graphical presentation of the effect of different concentrations of chitosan and plasticizers on the tensile stress of the edible film. It can be seen that as chitosan solute increases from 2 to 4% concentration, the tensile stress was also increasing. This behaviour is in line with the findings of Miquel et al. (2011) where the increase in chitosan led to increase in tensile stress of polymer films. The effect of plasticizer on polymer film as shown from figure 1 indicated that as glycerol was decreasing from 100 to 60 % and inclusion rate of sorbitol was increasing from 0 to 40 % tensile stress was increasing. The reason for this could be because glycerol has a light molecular weight and it increased the intermolecular spacing along the chitosan molecular inter-chain thereby increasing the ductility and flexibility but reducing the strength of the film. However, sorbitol on the other hand has heavier molecular weight and closes the intermolecular spacing along the chitosan inter-chain which increased the plasticity of the polymer thereby increasing the film tensile strength than glycerol. The implication of this was that sorbitol enhance preservation of coated food product under tensile stress. Hafnimardiyanti et. al. (2016) and Kamaldeen et. al. (2019) came up with similar results that sorbitol increases tensile strength of edible polymer films.
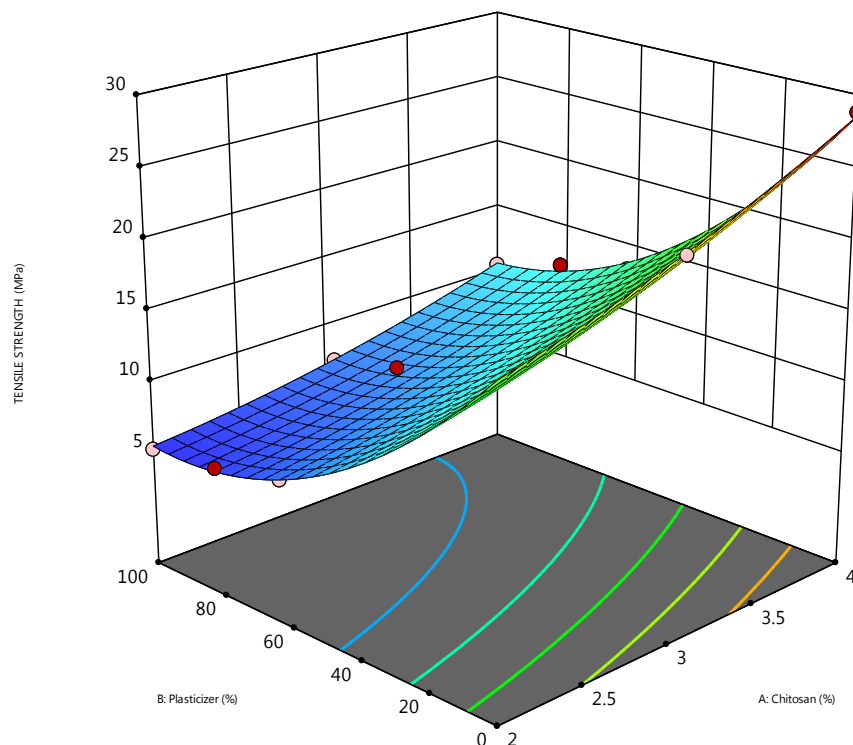

Figure 1. Effect of Chitosan and Plasticizers Concentrations on Tensile Stress of   Polymer Films.

3.1.2 Elongation at Break

It's a mechanical property to measure the ductility of material i.e., the ability of such material to stretch beyond the limit before breaking. It also determines the reliability of the material when applied to coated food products (Borges *et. al.*, 2015; Ewelina *et. al.*, 2014). The result of Elongation at break from Table 4 followed a quadratic model with p-value of 0.016 that is less than 0.05. The significant of this result showed that the

chances of model going into error was less than 5 % (p<0.05). The final equation in terms of coded factors for this parameter was:

$Y = 4.02 - 0.74A + 0.33B + 0.38B^2$ (9)

Where Y= Elongation at Break

A = Chitosan concentration %

B = Plasticizer (glycerol: sorbitol) %

From table 4 interaction between chitosan and plasticizer (AB) and square of chitosan (A^2) were not significant (p> 0.05). Figure 2 was a graphical representation of the effect of chitosan and plasticizer compositions on elongation at break of developed polymer films. From the equation negative coefficient of chitosan (A) indicated that as chitosan concentration increased elongation at break was decreasing given a minimum quadratic curve. There was a steady decease in film elongation with increase in sorbitol inclusion rate in glycerol: sorbitol interaction with chitosan molecules. This showed that glycerol increases the ductility and flexibility of the developed films. Positive coefficients of plasticizer indicated that film elongation increases as plasticizer increases. Reason for this could be that chitosan as reported in literatures (Utami et. al., 2021; Jasor et. al., 2014, Piermaria *et al*., 2015, Sharma *et al*., 2015, Campos-Requena *et al*., 2017) to be hard and brittle which makes the films to be fragile and breaks easily under tensile load. As the solute increases, the bond between chitosan molecules becomes more compact and shorter. The surface pores of films are closed with additional solutes blocking space for extension. Sanyang et al. (2015), Bakry et al. (2017), and Jouki et al. (2013) also reported similar results with the interaction of sorbitol and glycerol on elongation of films.
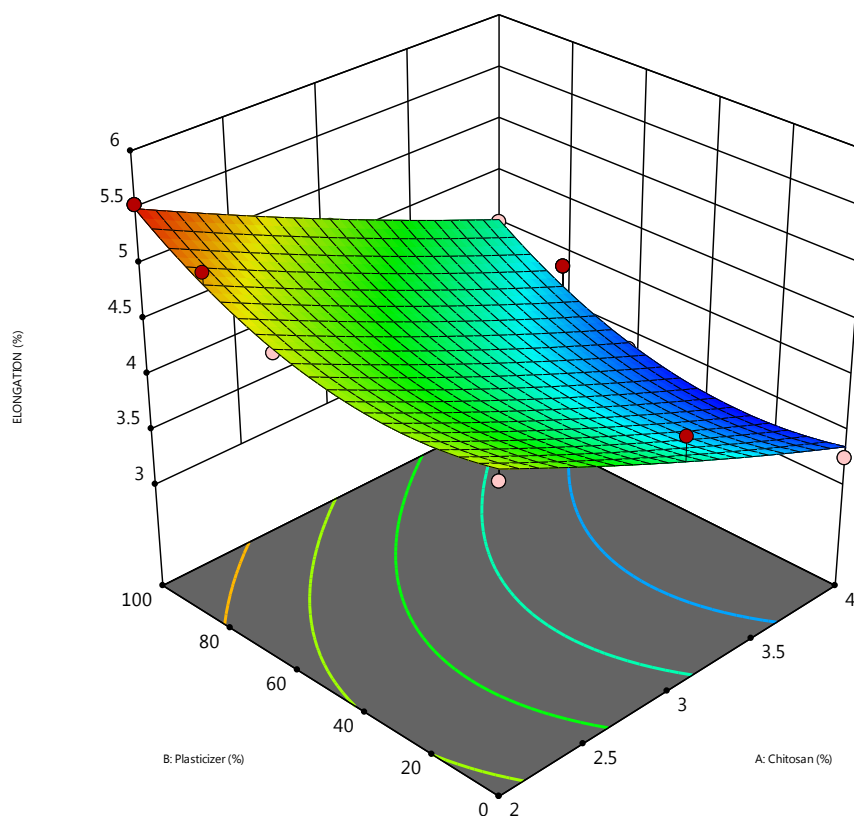

Figure 2: Effect of Chitosan and Plasticizers on Film's Elongation at Break

3.1.3 Puncture strength

Measures the amount of force required by a probe to puncture food products coated with membrane matrix when subjected to a compressional force. The result of puncture test from Table 4 shows a quadratic equation model with p-value of 0.0002 which was less than 0.05 and it simply implies that the chances of the equation failing was less than 5%. The final
equation based on coded factors was:

$Y = 22.47 + 7.43A - 6.55B^2$ (10)

Where Y = Puncture Strength

A= Chitosan Composition

B= Plasticizer (glycerol: sorbitol)

From Table 4 it could be observed that, interaction between chitosan and plasticizer (AB), plasticizer (B), and square of (B) were not significant (p>0.05) to puncture strength parameter. From equation 10, it was observed that chitosan concentration was the major factor for the puncture strength of polymer coating of coated products. Figure 4 gave a maximum quadratic curve behaviour of the puncture strength of the film. The curve was on the increase attaining its peak value at maximum chitosan concentration. The higher the chitosan composition the higher the mechanical properties of the polymer film (Razavi et. al., 2015). This could be because as the chitosan solute was increasing, the film matrix becomes denser and the molecules are more closely bonded together which reduced the tension over the molecules, hence the increase in puncture strength. From the response surface result in figure 3, it can be observed that puncture strength reduced with 100 % glycerol inclusion. This could be because of the hydrophilic nature of glycerol that increases the molecular mobility of the dense matrix, thereby weaking the inter- molecular forces between component compounds, hence offering a weak resistance to probe puncture. Plasticizing effect as observed from figure 3 showed that an inclusion rate of sorbitol increased puncture strength to maximum. This can be as a result of plastic nature of sorbitol offering more resistance to compressional force of the probe. This result is similar to findings by Ballesteros-Mártinez, et al. (2020) while working on the effect of glycerol and sorbitol on potato starch properties as edible coating.
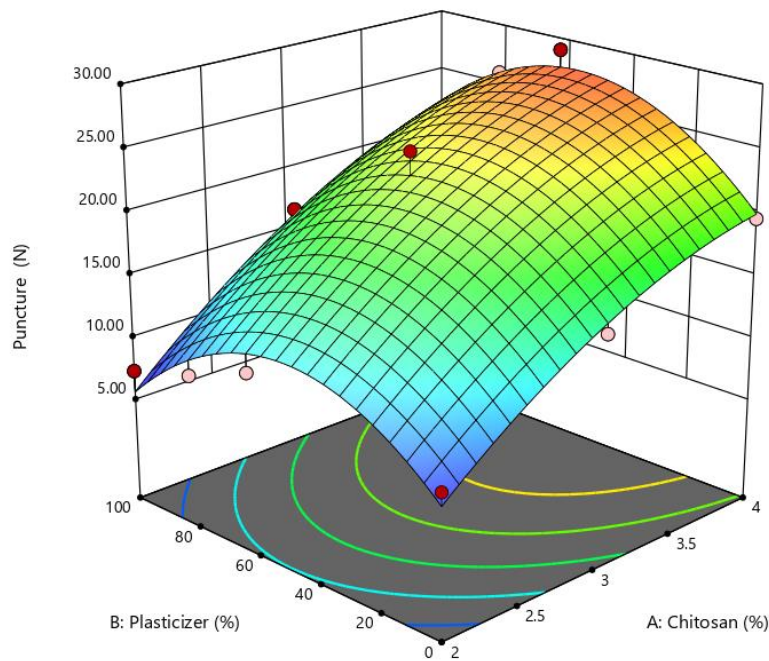

Figure 3: Effect of Chitosan and Plasticizer on Film Puncture Strenght

3.1.4 Young Modulus

It is the mechanical property of polymer film that determines how easily it can be stretched and deformed beyond limit before failing. The result of statistical analysis for Young Modulus in Table 4 of a minimum quadratic model was p = 0.0001. This was less than 0.05, (p<0.05). It simply implies that the chances of the equation failing was less than 5% and its final equation (11) based on the coded factors was:

$Y = 2.75 + 1.31A - 1.96B - 0.44AB + 0.31A^2 + 0.72B^2$ (11)

Where: Y= Young Modulus
        A = Chitosan Composition
        B = Plasticizer (glycerol: sorbitol)

From Table 4 it was observed that chitosan (A), plasticizer (B), interaction between (AB), square of (A) and square of (B) were all significant to Young Modulus parameter. Negative coefficients of plasticizer (B) and that of chitosan:plasticizer (AB) interaction from the equation indicated that as these two factors were increasing, Young Modulus was decreasing. The equation showed a direct relationship between Young Modulus and chitosan factor, as chitosan concentration was increasing, there was a steady increase in Young Modulus as

observed from Figure 4. It is required of food packaging material to possess maximum ductility and ability to stretch to the limit before failing so as to give maximum preservation to the coated food products. The plasticizer effect showed that glycerol reduces Modulus at 100 % inclusion rate and was increasing significantly as sorbitol increases from 20 to 40 % inclusion rate. This result was supported by earlier researchers.
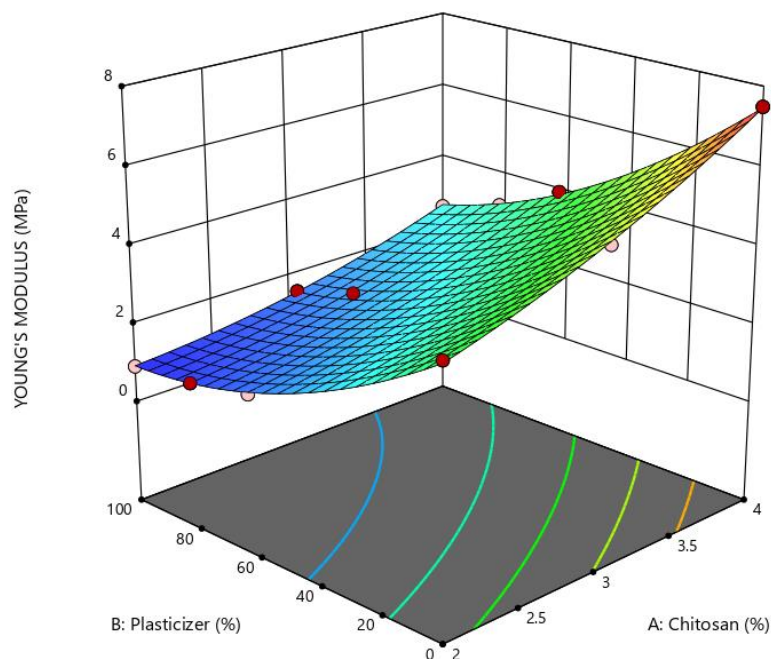

Figure 4 Effect of Plasticized Chitosan-Based Polymer Film Membrane on Young Modulus

3.1.5 Water vapour permeability (WVP)

It is a measure of rate of moisture movement between food products and its immediate environment through a semi permeable membrane polymer. It is an important property that determines rate of deterioration and eventual shelf-life of the product. The more moisture that diffuses into the food product is directly proportional to a destructive reaction within the food product and hence a reduced shelf-life. For a good food packaging polymer film water vapour permeability must be as low as possible (Pankaj 2020, Kamaldeen *et al*., 2019, Shima *et al*., 2017). According to Pankaj (2020), water vapour permeability depends on the following factors: temperature and humidity of the surrounding atmosphere, film structure, type of plasticizer used, and mobility of the polymeric chain.

The statistical result of WVP given in Table 4 of quadratic model with a p value less than 0.05. This implies that the chance of the model failing was less than 5%. The mathematical equation (12) based on coded factors was

$$Y = 4.93B^2 + 2.57AB - 4.77B - 2.06A + 4.78 \qquad (12)$$
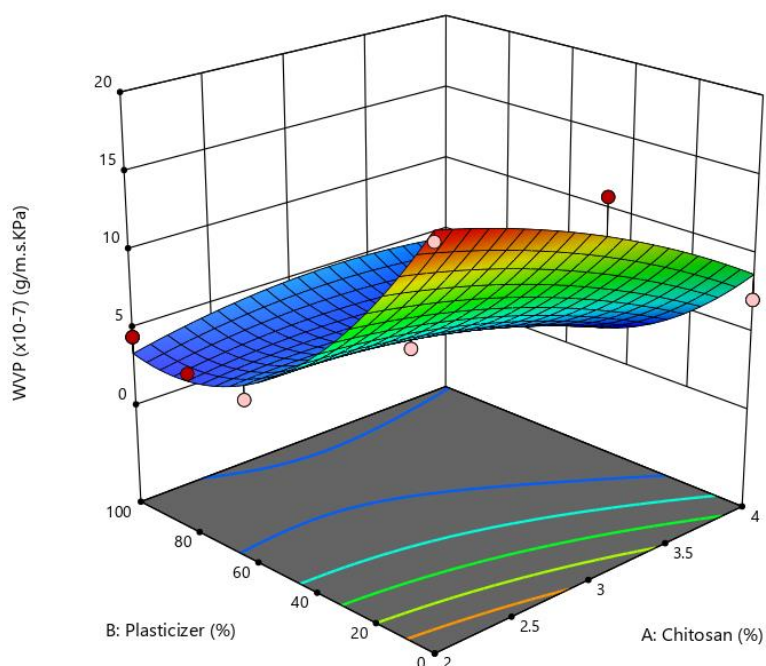
Where Y = Water vapour permeability
      A = Chitosan concentration
      B = Plasticizers (glycerol:sorbitol)

Table 4 indicated that only the square of chitosan (A^2) was not significant to WVP with p>0.05, all others were significant at p<0.05. From the equation negative coefficient of plasticizer (B) and coefficient of chitosan (B) implies more concentration of these factors will reduce WVP. Graphical presentation of statistical analysis for WVP was as presented in Figure 5. It was observed from the figure that as the concentration of chitosan increased, there was decline in the WVP. This observation could be explained that at low level of concentration there were more inter-chain spaces between molecules that could allow passage of water molecules and as the concentration increases, chitosan solute were filling-up the voids, film matrix increased in viscosity and density, film becomes more plastic in nature thereby reducing permeability of the polymer matrix. Addition of glycerol increased WVP but was reducing as sorbitol inclusion was increasing attaining a minimum level at 60:40 ratio. The graph showed a sharp increase in WVP below 60 % glycerol inclusion. Sorbitol is hydrophobic and crystallizes at room temperature with high molecular weight. The result of increased sorbitol inclusion in the dense matrix led to an increase in plasticity and a reduction in the WVP of the polymer film.

Coated food product with this formulation will archive a better preservation (Ballesteros-Mártinez *et. al.* 2020; Yang *et al.* 2019; Sanyang *et al.* 2015).

Figure 5 Effect of Plasticized Chitosan-Based Polymer Film Membrane on Water Vapour   Permeability

3.1.6 Film Moisture content

The level of moisture contained in the biofilm determines the hydrophilicity and hygroscopicity of film, a condition that determines which film or coating formulation and plasticizer best applied to what product. Statistical analysis of the membrane moisture content in Table 4 was of quadratic model $p < 0.0001$ (less than 0.05). The implication was that the probability of the equation failing was less than 5 %. The final equation based on the coded factors was as given in equation (13):

$$Y = 19.32 - 3.74A^2 + 2.78AB + 6.03B + 5.17A \qquad (13)$$

Where:

Y = Film moisture content

A = Chitosan concentration

B = Plasticizers (glycerol: sorbitol)

From Table 4, it can be observed that the square of plasticizer (B^2) was not significant ($p > 0.05$) to film moisture content. The negative coefficient of the square of chitosan factor (A) implies an increase in chitosan concentration results decrease film moisture content.

The graphical presentation of the statistical analysis of the effect of plasticizer and chitosan on film moisture content was as presented in Figure 6. From the figure it can be seen that the higher the chitosan concentration, the higher the film moisture content. As the solute increased in the film matrix, viscosity was increased and the water holding capacity within chitosan inter-chain also increased. Also, it was observed from the figure the higher the glycerol inclusion, the higher the moisture content of the polymer matrix. Glycerol is hydrophilic, so it has very strong affinity for water. Hydroxyl group in glycerol easily bind with the water molecules causing films with high glycerol to retain moisture. Conversely, the higher the inclusion rate of sorbitol the lower the moisture content of the matrix film. With sorbitol inclusion, the film plasticity increased. Films having sorbitol in their compositions are more hydrophobic and better packaging materials most especially perishable food products. This result is in-line with that of Sanyang et al. (2015) and Utami et al. (2020).
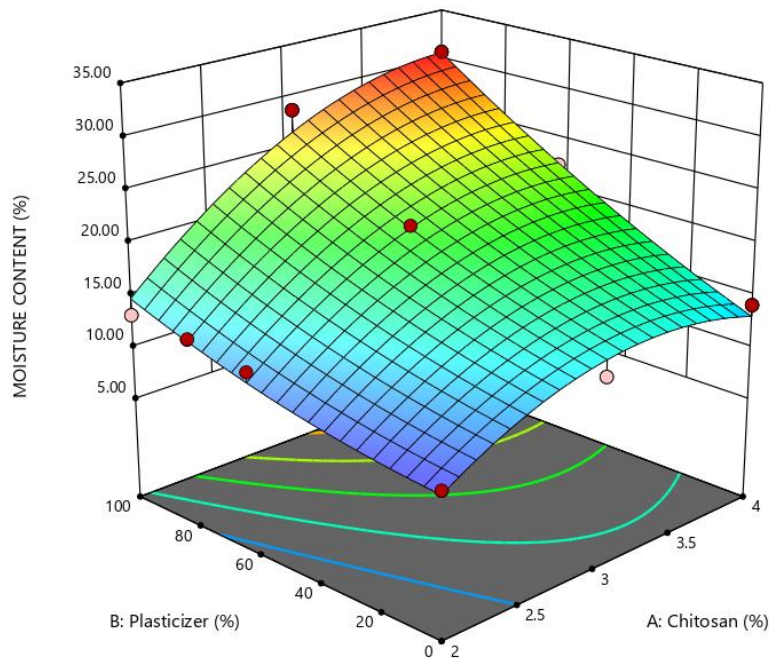
Figure 6. Effect of Plasticized Chitosan-Based Polymer Film Moisture Content

3.1.7 Film Water Solubility.

The ability of edible film to dissolve in water is a measure of its easy digestibility in man and animal systems when consumed with the coated product and it also determines its biodegradability when thrown away as waste without causing any environmental hazard (Hafnimardiyanti et al., 2016). Membrane film solubility in water in Table 4 was of a linear model with p-value 0.0001 (less than 0.05). This is an indication that the probability of the model equation failing was less than 5 %. The final mathematical expression based on the coded factors was as given in equation (14):

Y = 3.82B − 1.26A + 28.40   (14)

Where Y = Membrane Solubility in Water

      A = Chitosan composition

      B = Plasticizer composition (glycerol: sorbitol)

Table 4 showed that only chitosan (A) and plasticizer (B) were significant to film solubility at (p<0.05). It can be observed from Table 4 that glycerol at 100% for all samples had highest solubility. The high water-solubility of glycerol films for different polymer concentrations may be ascribed to the strong affinity of glycerol to water molecules, as well as, its lower molecular weight which easily facilitates its reaction between the polymer chains to increase the void volume between the chains. As the inclusion rate of sorbitol was increasing for all samples of different chitosan concentrations, solubility values were significantly reducing. This reduction can be as a result of heavy molecular weight and hydrophobic properties of sorbitol. The model graph presentation of statistical analysis was as presented in Figure 7.
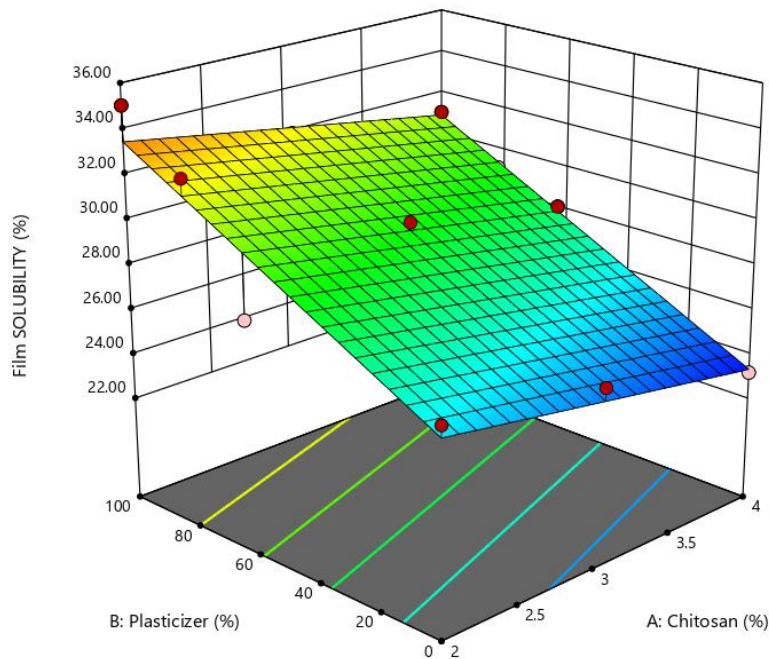
Figure 7. Effect of Plasticized Chitosan-Based Polymer on Film Water Solubility

3.1.8 The Degree of Swelling.
        The film membrane is the ability of biofilm to absorb moisture and increase in size. This property will help to determine the type of food product a particular biofilm could be applied to depending on its swelling rate. There's always an interaction between product and its polymer packaging material. Hence, the integrity of the product can easily be compromised by the degree of swelling of its packaging materials. Table 4 gave the degree of swelling for polymer films as a quadratic model with p-value 0.0001 (less than 0.05). This implies that the probability of the equation having error is less than 5 %. Based on the statistical coded factors, the final equation

$$Y = 101.77B^2 + 58.43A - 55.73AB - 100.13B + 130.87 \tag{15}$$

Where: -
Y = Swelling degree
A = Chitosan concentration
B = Plasticizer (glycerol: sorbitol)
Table 4 indicated that the square of plasticizer (B^2), chitosan concentration (A), interaction between chitosan and plasticizer (AB) and plasticizer have significant effect (p-value <0.05) on the degree of swelling whereas the square of chitosan (A^2) had no significant effect.
  It was observed that regardless of chitosan and plasticizer concentrations, all samples absorb enough water to swell. For samples with zero plasticizers P_0(s) as chitosan concentration increases from 2-4 %, (Figure 8) swelling values was significantly rising. The higher the chitosan concentration in a polymer matrix, the higher the percentage of swelling. This can be attributed to the fact that more solid matter was available within the film to absorb moisture hence, swells accordingly (Kozlowska et. al. 2018). Similar to solubility, films with 100 % glycerol plasticizer for the various chitosan compositions were highest in degree of swelling. Higher film swelling property could make the coated product more vulnerable to moisture and can compromise its integrity. On the other hand, as the inclusion rate of sorbitol increased in the glycerol: sorbitol plasticizing blend, the swelling was reducing. The free hydrogen ion in sorbitol had stronger linkage with the chitosan interchain molecules resulting into less film swelling compared with glycerol. With more sorbitol inclusion as plasticizer in polymer matrix, the integrity of food product is more preserved. This result is in-line with that of Kozlowska et al. (2018) during their determination of the effect of sorbitol on swelling property of carrageenan- based hydrogels edible film.
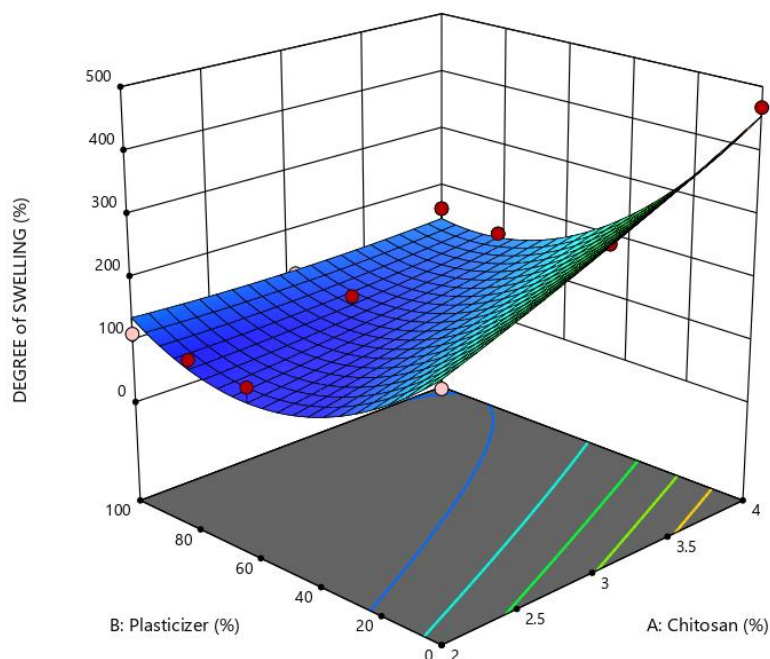
Figure 8. Effect of Plasticized Chitosan-Based Polymer on Degree of Swelling of Film Membrane.

3.2 Optimization of Membrane Film

An optimization exercise was carried out using Design-Expert version-13 software programming to determine the optimum formulation for the production of polymer membranes of the edible films. The goal of chitosan and plasticizer were set based on their level of importance, while that of tensile strength, puncture strength, Young's Modulus, elongation at break, and solubility were maximized whereas water vapor permeability, swelling and moisture content were minimized. The result of optimum membrane formulation was chitosan 2% and plasticizer (70 % glycerol: 30 % sorbitol) with a desirability value of 0.510 or 51.0 %. For this simulation, the surface responses were determined as tensile strength 12.32MPa, puncture strength 11.67N, elongation at break 4.7%, Young's Modulus 2.63MPa, water vapour permeability 10.38 $gm^{-1}s^{-1}KPa^{-1}$, moisture content 9.11 %, film solubility 27.86 % and degree of swelling 124.70 %.

IV. CONCLUSION

The formulation, production and characterization of a chitosan-based bio-polymer plasticized with glycerol and sorbitol was successfully carried out. Its quality parameters were also modelled and optimized. Quadratic equation model was best fitted for puncture strength, tensile strength, elongation at break, Young's Modulus, water vapour permeability, moisture content, and degree of swelling. Only film solubility was best fitted with linear model. Optimum membrane formulation carried out gave factors of chitosan as 2 % and plasticizer (glycerol 70 %, sorbitol 30 %). Response surface results at this optimized formulation was tensile strength 12.32MPa, puncture strength 11.67 N, elongation at break 4.7 %, Young's Modulus 2.63 MPa, water vapour permeability 10.38 $gm^{-1}s^{-1}KPa^{-1}$, moisture content 9.11 %, film solubility 27.86 % and degree of swelling 124.70 %. From the findings chitosan decreased the barrier properties but it increased the mechanical properties of the film membrane. Glycerol increased the moisture permeability and improved the flexibility while increase in sorbitol concentration increased the plasticity nature of the films. This study has been able to shed a significant light on the use of biodegradable films as a primary packaging material for the shelf-life elongation of food products.

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