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Optimization of Processing Parameters of Nanocomposite Film for Fresh Sliced Okra Packaging

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ABSTRACT

Nanocomposite film can be regarded as an active packaging material which is capable of curtailing microbial growth and keeping food for an extended life. In this research, the optimum processing parameters of cassava starch-zinc nanocomposite film was determined for packaging fresh-sliced okra. Samples of the films, with thickness ranging between 15–17 μm , were developed from the blends of 24g of cassava starch, 0–2 % zinc nanoparticles (NP) and 45–55 % glycerol in 600 ml distilled water. The ideal film was determined by optimizing the film processing parameters using Box-Behnken Design in Surface Response Methodology. It was subsequently used to package fresh-sliced okra at 10–27°C temperature and 3–9 days storage period; and compared with a low density polyethylene film (LDPE, 10 μm). The results showed that the 17 μm thick film, whose desirability function is closer to the optimal goal, gave values of 7.4×10^4 CFU/g, 21.62 mg/100g, 0.44 mg/l and 10.46 IU for bacteria count, ascorbic acid, titratable acidity and β -carotene contents, respectively. Also, there was a progressive decrease in the quality of the packaged products with an increase in the temperature and the storage period ($p^\circ\text{C}$ was not significant). Thus, the nanocomposite film can essentially be used for the packaging of fresh-sliced okra.

KEY WORDS

Fresh-sliced okra, Quality, Optimization, Modelling, Nanocomposites

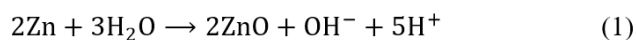
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INTRODUCTION

Nanocomposite films are special kind of packaging materials that can be used to extend the shelf life of perishable agricultural produce[1],[2]. Unlike polymers and nylons which are nondegradable, nanocomposite films can be degraded by the action of living organisms such as bacteria, yeast and fungi[1]. According to Rouilly and Rigal[3], the presence of the nanoparticles in the film, usually introduced in controlled amounts, may be directly responsible for keeping packaged foods beyond their normal storage period. There is, however, a growing concern among consumers of foods packaged with the nanocomposite film because of the likely toxins the nanoparticles added in the film might introduce. In most cases, they are not sure if this would not potentially cause any health issue as a result of accumulation of toxic particles in the blood cells. For this reason, food engineers are researching into different types of organic and inorganic nanoparticles to ensure the safety and create a healthy food culture. They are additionally confident of upgrading the nutritional quality of food through selected additives, thus changing the way the body processes and retains sustenance[3].

Potential application of inorganic nanoparticles, like zinc as an antimicrobial agent for inhibiting growths of microbes on the surfaces of foods has attracted attention in recent time[4]. For instance, ceramic powder of zinc has been reported by Yamamoto[5] to show appreciable activity against the growth of spoilage bacteria and fungi on the surface of food. Sawai and Yoshikawa[6] also reported that zinc nanoparticles can help in bone development and can inhibit the growth of microorganisms even when minimally administered. Jin et al.[7] reported that zinc nanoparticles inhibit the survival rates of *L. monocytogenes* and *S. enteritidis* in egg white and culture media, respectively. Additionally, the mechanism of antimicrobial activity of the zinc nanoparticles could be explained by the d-orbital

reaction of the metal with water molecule ligands to generate reactive hydrogen peroxide as shown in Eq. (1) to (4). Padmavathy and Vijayaraghavan[8] reported that the generated hydrogen peroxide can penetrate the cell membrane and kill the bacteria or any pathogenic microorganism. The concentration of the hydrogen peroxide generally increased with decreasing particle size, since the number of zinc particles per unit volume of the slurry increased with decreasing particle size[1],[9].



Following this established fact, an increase in antibacterial activity of the zinc nanoparticles may be associated with an increase in the peroxide concentration. Thus, the greater the concentration of the zinc nanoparticles the better is the activity of the metal particles against the influence of spoilage microorganisms. The effect of zinc nanoparticles on the survival rate of *S. aureus* and *E. coli* has been reported by Yamamoto[5]. The author revealed that the antimicrobial activity of the zinc nanoparticle is different for two microorganisms because of the difference in the sensitivity to hydrogen peroxide in the resulting complex[5].

Based on the foregoing, it can be stated that zinc nanoparticles can be blended with biodegradable film to control the spread of microbial loads on the surface of vegetables. The use of biodegradable nanocomposite films for microbial load inhibition and quality retention has been reported for many vegetables. For instance, Ayaala-zavala et al.[10] and Azarakhsh et al.[11] reported significant reductions in the growth of microorganisms on packaged fresh-cut peach and packaged fresh-cut

pineapple using pectin-cinnamon leaf oil and alginate-lemongrass oil nanocomposite films, respectively. Fadeyibi et al.[1] reported that zinc nanoparticles are essential in curtailing the growth of deterioration microorganisms on tomatoes. However, the application of biodegradable nanocomposite film for fresh sliced okra packaging has not been reported. Also, there is no reported research on the optimization of the film parameters for the quality retention of the packaged fresh-sliced okra. It is therefore necessary to study the response of starch-zinc nanocomposite film on the keeping quality of fresh-sliced okra. The objective of this research was to investigate the optimum film processing parameters for microbial growth inhibition of fresh-sliced okra using cassava starch-zinc nanocomposite film.

MATERIALS AND METHODS

Sample preparation

Freshly harvested okra (500 g) were bought from Maizube Farms, Minna, Niger State and transported to the Crop Processing and Storage Laboratory of the Federal University of Technology, Minna. The vegetables were sliced into thin layers of an approximate size of 10 mm and immediately sealed in polyethylene carrier bags.

Nanocomposite films were first prepared from a blend of cassava starch, zinc nanoparticles, glycerol and distilled water. The zinc nanoparticles were prepared by Sol-gel method and characterized using the procedure reported by Fadeyibi et al.[1]. The method involves mixing two homogeneous solutions. The first solution was prepared by adding 30 ml of distil water to 20 ml of triethanolamine with constant stirring while adding 2 ml (100 drops) of ethanol in drop wise. A 5.39 gm of zinc acetate di-hydrate was added to 50 ml of distil water with continuous stirring, to obtain a 0.5 M solution, as the second homogeneous solution. The two solutions were mixed together in

500 ml beaker and a solution ammonium hydroxide and 10 ml distil water was added, in drop wise, with continuous heating and stirring for 20 minutes. A white bulky solution was formed when the resulting solution is left for 30 minutes. It was then washed 8-10 times with distil water and filtered in a filter paper. The resulting residue was dried in the oven at 95°C for 8hrs[1]. The particle size distribution of the nanoparticles was determined using zetasizer equipment (ver. 7.01), and found to be in the range of 4–6 nm. Analytical grade glycerol with 98% purity was obtained from Science Equipment and Development Institute (SEDI), Minna, Niger State, Nigeria. The starch was prepared by peeling 250 kg of cassava root followed by soaking in a clean bowl containing water for 24 hours; and thereafter ground into pastes. The ground pastes were then sieved using a muslin cloth and the resulting filtrates was left undisturbed for 24 hours to allow the starch to settle at the bottom of the bowls. The prepared starch of the cassava was dried under the sun for one week and a moisture meter was used to monitor its moisture contents up to 2% (wb).

The nanocomposite film was developed by mixing 24 g of cassava starch, 0% of zinc nanoparticles and 45% of glycerol in 600 ml of distilled water. This was followed by heating to 100°C and constant stirring until gelatinization occurs. The product was poured in three plastic moulds of 360× 370 mm size and varying depths (8 mm, 10 mm and 12 mm). This was followed by drying in an air circulated oven dryer with a drying condition of 95°C temperature and 60% relative humidity. The products obtained after drying were carefully removed from the moulds, and this gives corresponding films with thickness 15 µm, 16 µm and 17 µm, with respect to the depth of the mould used. This procedure was repeated for a blend of 24 g cassava starch, 1% zinc nanoparticles and 50% glycerol; and for a blend of 24 g cassava starch, 2% zinc nanoparticles and 55% glycerol. A total of film 27 samples, obtained from each blend category, was used to package the fresh-sliced okra.

Packaging and quality parameters determination

The fresh-sliced okra was divided into 28 portions of equal weight and packaged in the 27 samples of the nanocomposite films plus a low density polyethylene (10 μm size). The packaged samples were each sealed using the sealing machine, and kept in the incubator for storage. The incubation temperature was varied in the range of 10–27°C; and the quality parameters were determined for 0–9 days storage periods.

The total coliform and quality parameters of the fresh sliced okra were evaluated at day 0, and then re-evaluated at an interval of 3 days for a total storage period of 9 days. The microbial loads, which are the bacteria and fungi counts, of the packaged fresh-sliced okra samples were determined according to the standard guidelines for the determination of microbial loads as reported by AOAC [12] and Guilbert et al.[13]. Also, the methods of quality analyses, as described by Onwuka [14] were used

for the analyses of the total soluble solids, moisture content, vitamin C, β -carotene and titratable acidity of the packaged fresh-sliced okra samples.

Determination of optimum film processing parameter

Optimization framework: Response surface methodology (RSM) was applied to determine the optimum conditions of process variables for the physical properties of the films. Data from a previous report and preliminary trials were taken into account to select the number and range of process variables in the experimental design. The process variables considered in this studies were the thickness of the film ($X_1 = t_f$, 15, 16, 17 μm), concentration of glycerol ($X_2 = C_g$, 45, 50, 55 % w/w) and the concentration of zinc nanoparticles ($X_3 = C_{np}$, 0, 1, 2 % w/w), while the dependent response variables chosen were the quality attributes of the films. A Box-Behnken Design (BBD), which includes 17 experiments formed by 5 central points,

Table 1: Combination of process variables of the films in BBD protocol

Sdt	Run	Factor			Response					
		t_f	C_g	C_{np}	AA	βC	TA	TSS	BC	FC
15	1	16	50	1	++	++	++	++	++	++
3	2	15	55	1	++	++	++	++	++	++
5	3	15	50	0	++	++	++	++	++	++
7	4	15	50	2	++	++	++	++	++	++
13	5	16	50	1	++	++	++	++	++	++
2	6	17	45	1	++	++	++	++	++	++
11	7	16	45	2	++	++	++	++	++	++
1	8	15	45	1	++	++	++	++	++	++
17	9	16	50	1	++	++	++	++	++	++
9	10	16	45	0	++	++	++	++	++	++
14	11	16	50	1	++	++	++	++	++	++
16	12	16	50	1	++	++	++	++	++	++
4	13	17	55	1	++	++	++	++	++	++
6	14	17	50	0	++	++	++	++	++	++
10	15	16	55	0	++	++	++	++	++	++
12	16	16	55	2	++	++	++	++	++	++
8	17	17	50	2	++	++	++	++	++	++

AA = Ascorbic acid, mg/100g; βC = β -carotene, IU; TA = Titratable acidity, mg/l; TSS = Total soluble solids, %; BC = bacteria count, cfu/g; FC = fungi count, cfu/g; ++ = response data; t_f , c_g and C_{np} retain their usual meaning

was employed to minimize the cost of running a 3³ full factorial experiment. The layout of the combinations of the process variables and the quality attributes of the cassava starch nanocomposite films in the BBD, with individual experiments carried out in random order, are presented in Table 1.

Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The characteristics of a goal may be altered by adjusting the weight or importance. For several responses and factors, all goals can be combined into one desirability function[15]. In this investigation, the objective function is to ‘maximize’ all responses, including the ascorbic acid, β-carotene, moisture content, total soluble solids and titratable acidity of the packaged product. All the independent variables are kept ‘in range’ based on the specified design protocol of the experiment. The reason for the choice of a particular objective function is based on past experience and preliminary studies on the quality indexes of okra.

Modelling development: The quadratic polynomial regression model was assumed for predicting (Y) response variable. Other forms of the polynomial regression models were tested against the chosen quadratic model before it was accepted for use. Model of the form expressed in Eq. (5) was developed to describe the response (Y) surface:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (5)$$

where, Y is the value of the considered experimental predicted response variable (Characterized attributes of the films), X_1 , X_2 and X_3 are the values of t_p , Cg and Cnp, respectively, β_0 is the constant value, β_1 , β_2 and β_3 are linear coefficients, β_{12} , β_{13} and β_{23} are the interaction coefficients, β_{11} , β_{22} and β_{33} are quadratic coefficients. Contour plots for each of the response variables were used by applying superimposed surfaces methodologies, to obtain a contour plot for observation and selection of the zone of Optimization of the thickness of the films,

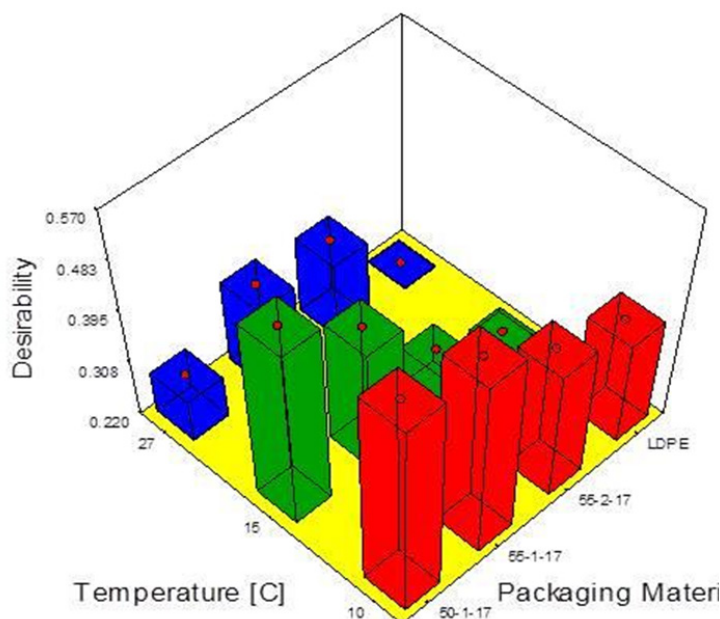


Fig. 1. Desirability of Optimizing the Quality Parameters of Okra at different Temperatures and Materials of Packaging

the concentrations of glycerol and the zinc nanoparticles. All statistical analyses were performed using Design Expert Package (ver. 9.0.3.1).

Fitting of Data to the Model and Validation:

The experimental data were fitted to the regression models generated using the Design Expert Package (ver. 9.0.3.1). The degree of fitness of the models was determined by both the R^2 and R_{adj}^2 . The models were validated by comparing the experimental and the predicted data and computing their mean square error (mse) at $p \leq 0.05$ significant levels.

Analysis of Variance of the Model: The analyses of variance of the developed models for predicting the Characterized attributes of the cassava starch nanocomposite films was carried out to investigate the significance of the model terms at $p \leq 0.05$. In the event that the model terms are not significant, the model was improved by stepwise regression or backward elimination regression procedures, as the case may be, to remove the insignificant terms of the models[15], [16]. This was also achieved using the Design Expert Software Package (ver. 9.0.3.1).

RESULTS AND DISCUSSION

Effects of temperature and packaging material on quality of packaged products

The effects of temperature and packaging material on quality of the packaged fresh-sliced okra are shown in Fig. 1. The nanocomposite films and the 10 μm LDPE film, which were used for the packaging of the okra, were subjected to optimization to determine the most desirable film for optimizing the quality parameters at varying temperatures for a packaging period of 9 days. The influence of temperature and material of the packaging on the desirability function of maximizing the total soluble solids, moisture contents, ascorbic acid and β -carotene contents; and minimizing the titratable acidity of okra is shown in Fig. 1. The optimum film, whose desirability function is closer to the optimal goal, gave values of 21.57 %, 78.43 % (wb), 21.62 mg/ 100g, 0.44 mg/l and 10.46 IU for total soluble solids, moisture content, ascorbic acid, titratable acidity and β -carotene contents, respectively (Table

Table 2: Quality parameters optimization of packaged okra

PM	T (°C)	P (days)	TSS (%)	MC (% , wb)	VC (mg/100g)	TA (mg/l)	β -C (IU)	D
55-2-17	27	3	21.573	78.427	21.622	0.468	10.461	0.594
55-1-17	27	3	19.680	80.320	22.424	0.381	9.297	0.587
50-1-17	10	9	16.213	83.787	10.568	0.243	12.526	0.556
50-1-17	27	3	19.970	80.030	17.283	0.357	9.381	0.553
50-1-17	15	9	19.468	80.532	14.113	0.434	9.920	0.525
55-1-17	10	9	15.258	84.742	10.162	0.323	12.124	0.524
10 μm LDPE	27	3	18.492	81.508	19.331	0.593	9.218	0.517
50-1-17	15	3	19.994	80.006	8.959	0.284	12.290	0.511
55-1-17	10	6	11.183	88.817	11.337	0.340	12.485	0.507
50-1-17	15	6	21.344	78.656	10.325	0.309	10.530	0.499
55-2-17	10	6	13.108	86.892	11.737	0.419	10.833	0.495
55-1-17	15	3	22.225	77.775	8.082	0.173	11.962	0.481
55-1-17	15	6	21.957	78.043	8.656	0.248	10.172	0.459

PM = packaging material, T = packaging temperature, P = Packaging period, TSS = total soluble solids, MC = moisture content, VC = ascorbic acid, β -C = β -carotene, D = desirability

Table 3: Optimization Solution of the Microbial Loads of Packaged Okra

Packaging Material	T (°C)	P (days)	BC (CFU/ g)	FC (CFU/ g)	Desirability
50-1-17	10	3	7.40×10^4	1.26×10^6	0.703
50-1-17	10	6	1.80×10^4	7.61×10^6	0.695
55-2-17	10	3	9.44×10^4	9.09×10^6	0.600
50-1-17	10	9	1.27×10^4	7.51×10^6	0.592
55-1-17	27	3	6.80×10^4	6.34×10^5	0.570
55-1-17	10	6	9.47×10^4	1.76×10^7	0.567
55-2-17	10	6	5.89×10^3	3.04×10^7	0.562
55-1-17	10	3	8.24×10^3	2.51×10^7	0.556
55-2-17	27	3	6.82×10^4	1.69×10^6	0.538
55-1-17	15	3	8.62×10^4	1.18×10^6	0.531
55-1-17	10	9	4.02×10^4	1.11×10^7	0.505
55-1-17	15	9	2.35×10^5	2.63×10^5	0.477
10 μ m LDPE	10	6	4.27×10^4	3.08×10^7	0.457
55-2-17	15	3	7.30×10^4	1.56×10^7	0.453
10 μ m LDPE	15	9	1.76×10^4	2.18×10^6	0.450
55-1-17	27	6	2.82×10^5	4.14×10^5	0.446
55-1-17	15	6	1.89×10^5	2.25×10^6	0.443
55-2-17	27	6	1.53×10^5	5.25×10^6	0.435
50-1-17	15	3	1.92×10^5	4.10×10^6	0.423
50-1-17	27	3	1.92×10^5	5.31×10^6	0.415

T = Packaging temperature, P = Packaging period, BC = Bacterial Counts, FC = Fungi Counts

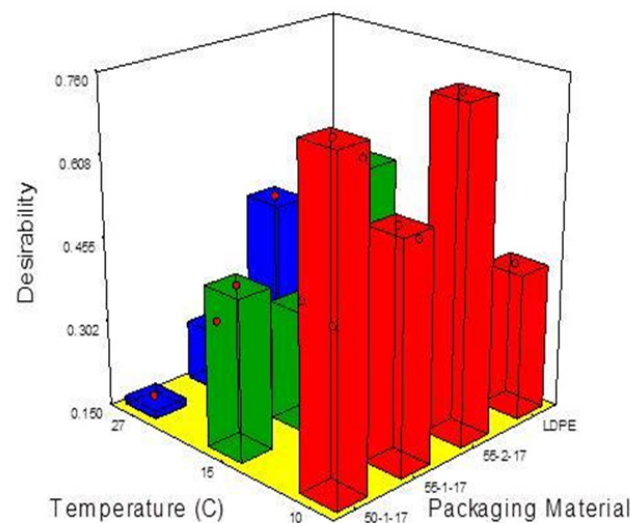


Fig 2: Desirability of Minimizing the Microbial Loads of Okra at different Temperatures and Materials of Packaging

2). Similar results were reported by Babarinde and Fabunmi [17] in their work on effect of packaging materials and storage temperature on quality of okra.

Effects of temperature and packaging material on shelf-life of packaged products

The effects of temperature and packaging material on the microbial counts of the packaged fresh-sliced okra are shown in Fig. 2. The cassava starch-zinc nanocomposite films and the 10µm LDPE film, which were used for the packaging of the okra, were subjected to optimization to determine the most desirable film for minimizing the microbial loads of the packaged okra at varying temperatures for a packaging period of 9 days. The influence of temperature and material of packaging on the desirability function of minimizing the microbial loads of okra is shown in Fig. 2. The optimum film, whose desirability function is closer to the optimization goal, gave values of 7.4×10^4 and 1.26×10^6 CFU/g for bacteria and fungi counts, respectively (Table 3). Consequently, the cassava starch-zinc nanocomposite films can be used for extending the shelf-life of okra at 27°C.

Prediction of quality and microbial indices of the packaged products

Moisture content of the packaged fresh-sliced okra

The empirical equations for the prediction of the moisture content of packaged sliced okra for the optimally selected cassava starch nanocomposite films and a 10 µm LDPE were developed and fitted in Eq. (6) to Eq. (9).

where, T = packaging temperature (°C), p = packaging period (days), MC_{50117} , MC_{55117} , MC_{55217} and MC_{LDPE} = moisture content of packaged okra using the cassava starch-zinc nanocomposite films (50117, 55117 and 55217) and a low density polyethylene (LDPE), respectively.

It can be seen that Eq. (8) has R^2 closer to that of the Eq. (9). This suggests the ability of the nanocomposite film containing 2% of the zinc nanoparticle and 55% of the glycerol to retain moisture of the fresh-sliced okra than the one containing 1% of the nanoparticles. Similar results were reported by Fadeyibi et al.[18] in their work on prediction of some physical attributes of zinc-nanocomposite films for food packaging applications. Also, Rajesh et al.[19] developed biodegradable film based on banana flour-plasticizer for quality packaging of food carrot, banana and cashew nuts, and reported that

$$\begin{aligned}
 \text{(a) } MC_{50117} &= 122.97 - 3.76T - 1.67p + 0.087T^2 + 0.092p^2 & \begin{cases} R^2 = 0.819 \\ R^2_{Adj} = 0.638 \\ mse = 9.6902 \end{cases} & \text{(6)} \\
 \text{(b) } MC_{55117} &= 127.04 - 4.89T - 0.29p + 0.118T^2 + 0.001p^2 & \begin{cases} R^2 = 0.7601 \\ R^2_{Adj} = 0.5216 \\ mse = 13.593 \end{cases} & \text{(7)} \\
 \text{(c) } MC_{55217} &= 129.6 - 3.30T - 5.22p + 0.07T^2 + 0.391p^2 & \begin{cases} R^2 = 0.8467 \\ R^2_{Adj} = 0.6934 \\ mse = 13.015 \end{cases} & \text{(8)} \\
 \text{(d) } MC_{LDPE} &= 118.9 - 3.72T + 0.116p + 0.04Tp + 0.08T^2 - 0.101p^2 & \begin{cases} R^2 = 0.8475 \\ R^2_{Adj} = 0.59325 \\ mse = 6.6585 \end{cases} & \text{(9)}
 \end{aligned}$$

moisture gain was more in composite films compared to poly propylene (PP) film. The stored products in poly propylene packages attributed to greatest protection against water vapour than biodegradable films.

Total soluble solid of the packaged fresh-sliced okra

The empirical equations for the prediction of the total soluble solids of packaged sliced okra for the optimally selected cassava starch nanocomposite films and a 10 µm LDPE were developed and fitted in Eq. (10) to Eq. (13).

where, T = packaging temperature (°C), p = packaging period (days), TSS₅₀₁₁₇, TSS₅₅₁₁₇, TSS₅₅₂₁₇ and TSS_{LDPE} = total soluble solids of packaged okra using the cassava starch-zinc nanocomposite films (50117, 55117 and 55217) and a low density polyethylene (LDPE), respectively.

It can be seen that Eq. (10) has R² closer to that of the Eq. (13). This suggests the ability of the nanocomposite film containing 1% of the zinc nanoparticle and

50% of the glycerol to retain TSS of the fresh-sliced okra than the one containing 2% of the nanoparticles and 55% nanoparticles. Similar results were reported by Fadeyibi et al.[18] in their work on prediction of some physical attributes of zinc-nanocomposite films for food packaging applications.

Ascorbic acid content of the packaged fresh-sliced okra

The empirical equations for the prediction of the ascorbic acid content of packaged sliced okra for the optimally selected cassava starch nanocomposite films and a 10 µm LDPE were developed and fitted in Eq. (14) to Eq. (17).

where, T = packaging temperature (°C), p = packaging period (days), Vc₅₀₁₁₇, Vc₅₅₁₁₇, Vc₅₅₂₁₇ and Vc_{LDPE} = moisture content of packaged sliced okra using the cassava starch-zinc nanocomposite films (50117, 55117 and 55217) and a low density polyethylene (LDPE), respectively.

$$\begin{aligned}
 \text{(a) } TSS_{50117} &= -22.97 + 3.76T + 1.67p - 0.087T^2 - 0.092p^2 & \begin{cases} R^2 = 0.819 \\ R^2_{Adj} = 0.638 \\ mse = 9.6902 \end{cases} & \text{(10)} \\
 \text{(b) } TSS_{55117} &= -27.04 + 4.89T + 0.29p - 0.118T^2 - 0.001p^2 & \begin{cases} R^2 = 0.7601 \\ R^2_{Adj} = 0.5216 \\ mse = 13.593 \end{cases} & \text{(11)} \\
 \text{(c) } TSS_{55217} &= -29.6 + 3.30T + 5.22p + 0.07T^2 - 0.391p^2 & \begin{cases} R^2 = 0.8467 \\ R^2_{Adj} = 0.6934 \\ mse = 13.015 \end{cases} & \text{(12)} \\
 \text{(d) } TSS_{LDPE} &= -18.87 + 3.72T - 0.116p - 0.035Tp - 0.084T^2 + 0.101p^2 & \begin{cases} R^2 = 0.8475 \\ R^2_{Adj} = 0.59325 \\ mse = 6.6585 \end{cases} & \text{(13)} \\
 \text{(a) } Vc_{50117} &= 22.22 - 1.76T + 0.619p - 0.146Tp + 0.077T^2 + 0.083p^2 & \begin{cases} R^2 = 0.7492 \\ R^2_{Adj} = 0.3311 \\ mse = 29.298 \end{cases} & \text{(14)} \\
 \text{(b) } Vc_{55117} &= 24.19 - 1.523T - 0.995p - 0.141Tp + 0.07T^2 + 0.23p^2 & \begin{cases} R^2 = 0.7576 \\ R^2_{Adj} = 0.3536 \\ mse = 17.165 \end{cases} & \text{(15)} \\
 \text{(c) } Vc_{55217} &= 10.02 + 1.31T - 5.11p - 0.029T^2 + 0.446p^2 & \begin{cases} R^2 = 0.659 \\ R^2_{Adj} = 0.318 \\ mse = 7.7046 \end{cases} & \text{(16)} \\
 \text{(d) } Vc_{LDPE} &= 50.04 - 3.71T - 2.455p + 0.081Tp + 0.083T^2 + 0.026p^2 & \begin{cases} R^2 = 0.65234 \\ R^2_{Adj} = 0.07289 \\ mse = 17.4227 \end{cases} & \text{(17)}
 \end{aligned}$$

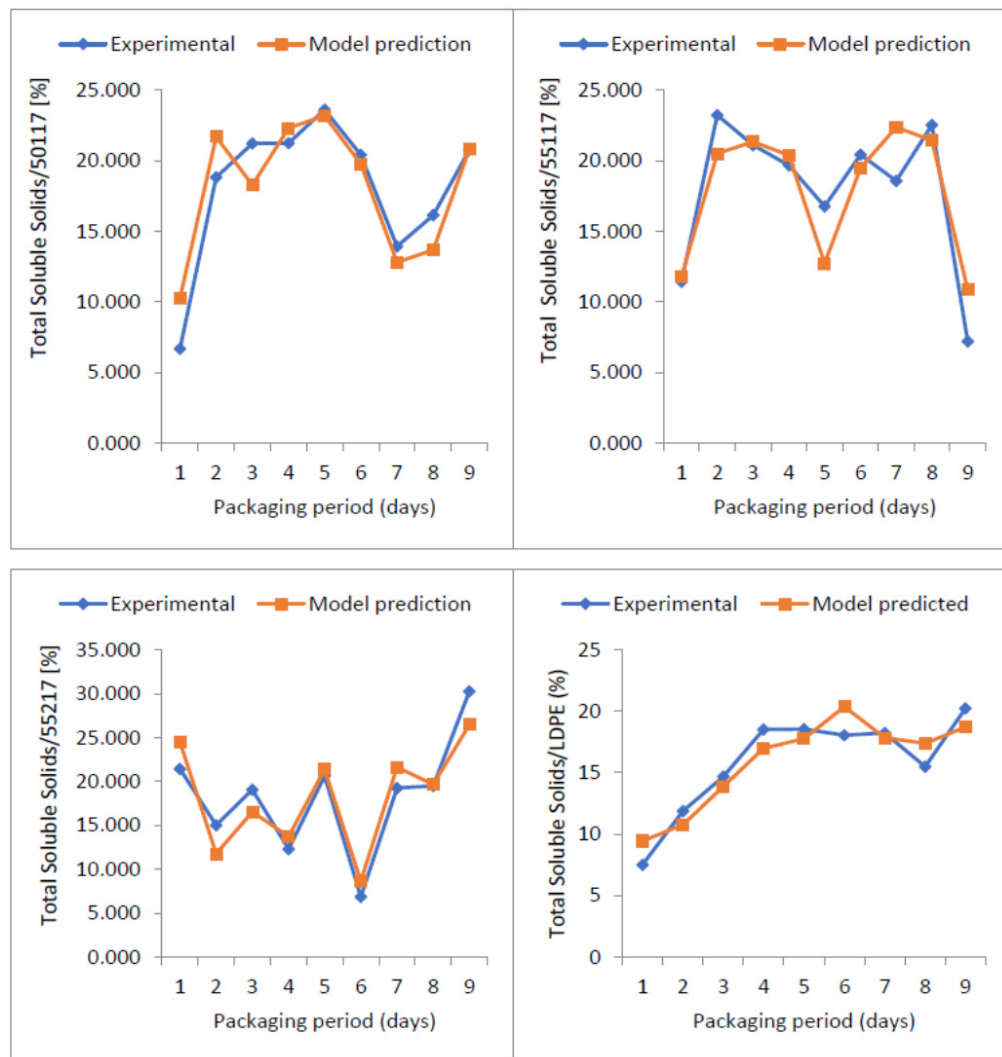


Fig 3a. Effect of packaging period on total soluble solids and titratable acidity of packaged fresh-sliced okra

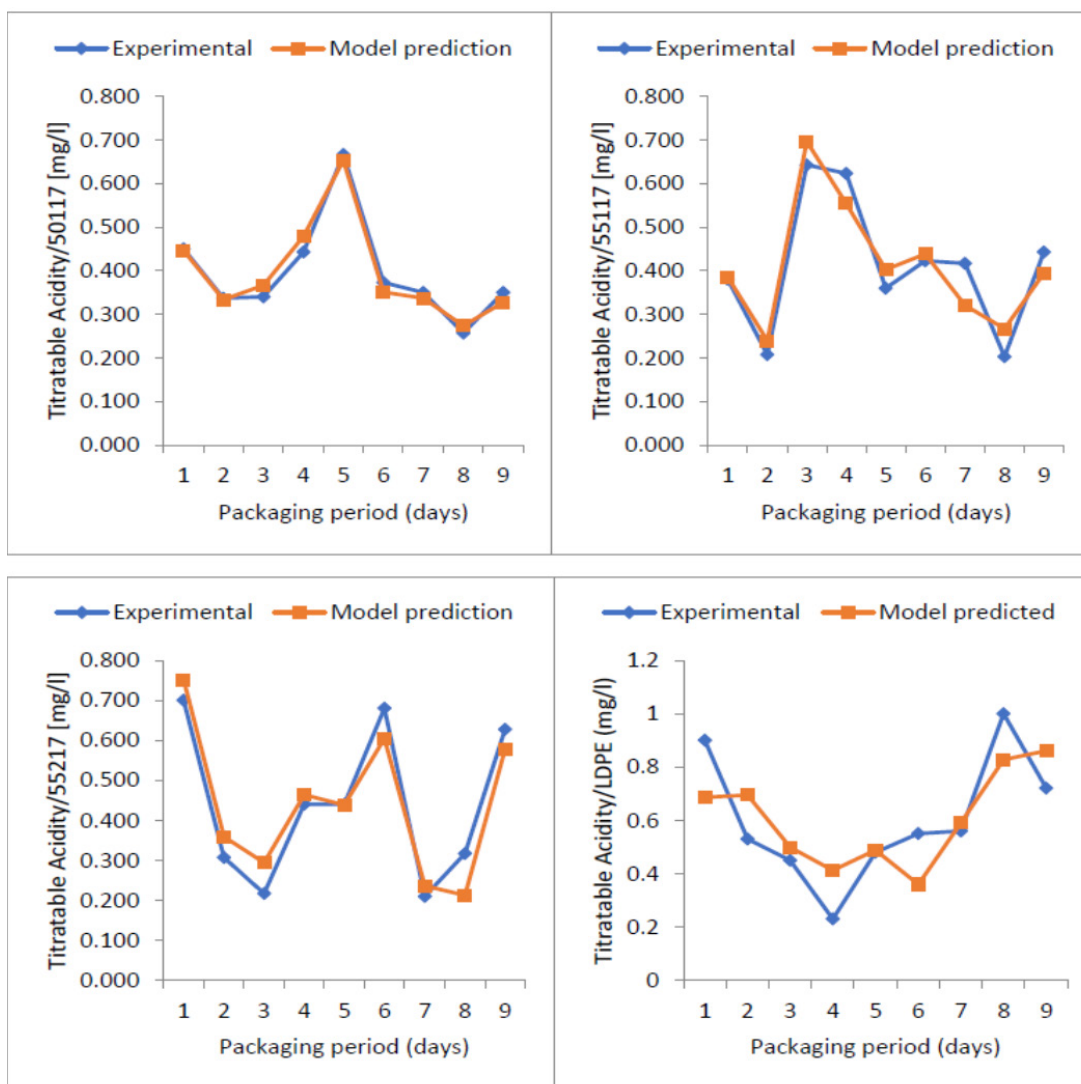


Fig 3b. Effect of packaging period on total soluble solids and titratable acidity of packaged fresh-sliced okra

It can be seen that Eq. (15) has the highest R^2 than the other equations. This suggests the ability of the nanocomposite film containing 1% of the zinc nanoparticle and 55% of the glycerol to retain vitamin C content of the fresh-sliced okra than the one containing 2% of the nanoparticles and 50% glycerol. Nkechi et al.[20] studied the effect of packaging materials, storage conditions on vitamin C content of cashew apple and observed a progressive decrease in the values of Vitamin C as time of storage progressed. Therefore, the fresh sliced okra can conveniently be stored in nanocomposite film for up to 9 days at temperature of 5, 15 and 27°C to retain its vitamin C content. Beltrán-González et al.[21] studied the effect of packaging materials on colour, vitamin C and sensory quality of refrigerated mandarin juice, and reported that type of container played a predominant role in determining the juice quality.

β-carotene content of the packaged fresh-sliced okra

The empirical equations for the prediction of the β-carotene content of packaged sliced okra for the optimally selected cassava starch nanocomposite films and a 10 μm LDPE were developed and fitted in Eq. (18) to Eq. (21).

where, T = packaging temperature (°C), p = packaging period (days), βc_{50117} , βc_{55117} , βc_{55217} and βc_{LDPE} = β- Carotene content of packaged okra using the cassava starch-zinc nanocomposite films (50117, 55117 and 55217) and a low density polyethylene (LDPE), respectively.

It can be seen that Eq. (19) has R^2 closer to that of the Eq. (21). This suggests the ability of the nanocomposite film containing 1% of the zinc nanoparticle and 55% of the glycerol to retain β-carotene of the fresh-sliced okra than the one containing 2% of the nanoparticles and 50% glycerol. Similar results were reported by Fadeyibi et al. [18] in their work on prediction of some physical attributes of zinc-nanocomposite films for food packaging applications.

Titrateable acidity of the packaged fresh-sliced okra

The empirical equations for the prediction of the titrateable acidity of packaged sliced okra for the optimally selected cassava starch nanocomposite films and a 10 μm low density polyethylene were developed and fitted in Eq. (22) to Eq. (25).

where, T = packaging temperature (°C), p = packaging period (days), Tt A₅₀₁₁₇, Tt A₅₅₁₁₇, Tt A₅₅₂₁₇ and

$$(a) \beta c_{50117} = 14.63 - 0.276T - 0.615p - 0.023Tp - 0.007T^2 + 0.058p^2 \begin{cases} R^2 = 0.9334 \\ R^2_{Adj} = 0.8224 \\ mse = 0.39244 \end{cases} \quad (18)$$

$$(b) \beta c_{55117} = 12.11 - 0.045T - 0.42p + 0.034Tp \begin{cases} R^2 = 0.9864 \\ R^2_{Adj} = 0.9639 \\ mse = 3.2847 \end{cases} \quad (19)$$

$$(c) \beta c_{55217} = 13.59 + 0.04T - 0.931p - 0.013Tp - 0.002T^2 + 0.073p^2 \begin{cases} R^2 = 0.883 \\ R^2_{Adj} = 0.688 \\ mse = 0.45291 \end{cases} \quad (20)$$

$$(d) \beta c_{LDPE} = 22.43 - 0.80T - 0.50p + 0.002Tp + 0.012T^2 + 0.011p^2 \begin{cases} R^2 = 0.9864 \\ R^2_{Adj} = 0.9639 \\ mse = 0.24066 \end{cases} \quad (21)$$

$$(a) Tt A_{50117} = 1.03 - 0.052T - 0.106p + 0.005Tp + 8.6 \times 10^{-4}T^2 + 0.003p^2 \begin{cases} R^2 = 0.966 \\ R^2_{Adj} = 0.9102 \\ mse = 0.00123 \end{cases} \quad (22)$$

$$(b) Tt A_{55117} = 1.24 - 0.11T - 0.04p + 0.003Tp + 0.003T^2 + 0.002p^2 \begin{cases} R^2 = 0.860 \\ R^2_{Adj} = 0.6267 \\ mse = 0.008793 \end{cases} \quad (23)$$

$$(c) Tt A_{55217} = 2.22 - 0.187T - 0.119p + 0.006Tp + 0.0043T^2 + 0.002p^2 \begin{cases} R^2 = 0.893 \\ R^2_{Adj} = 0.715 \\ mse = 0.010573 \end{cases} \quad (24)$$

$$(d) Tt A_{LDPE} = 1.7713 - 0.17T + 0.058p + 0.005Tp + 0.004T^2 - 0.011p^2 \begin{cases} R^2 = 0.56349 \\ R^2_{Adj} = -0.1640 \\ mse = 0.065063 \end{cases} \quad (25)$$

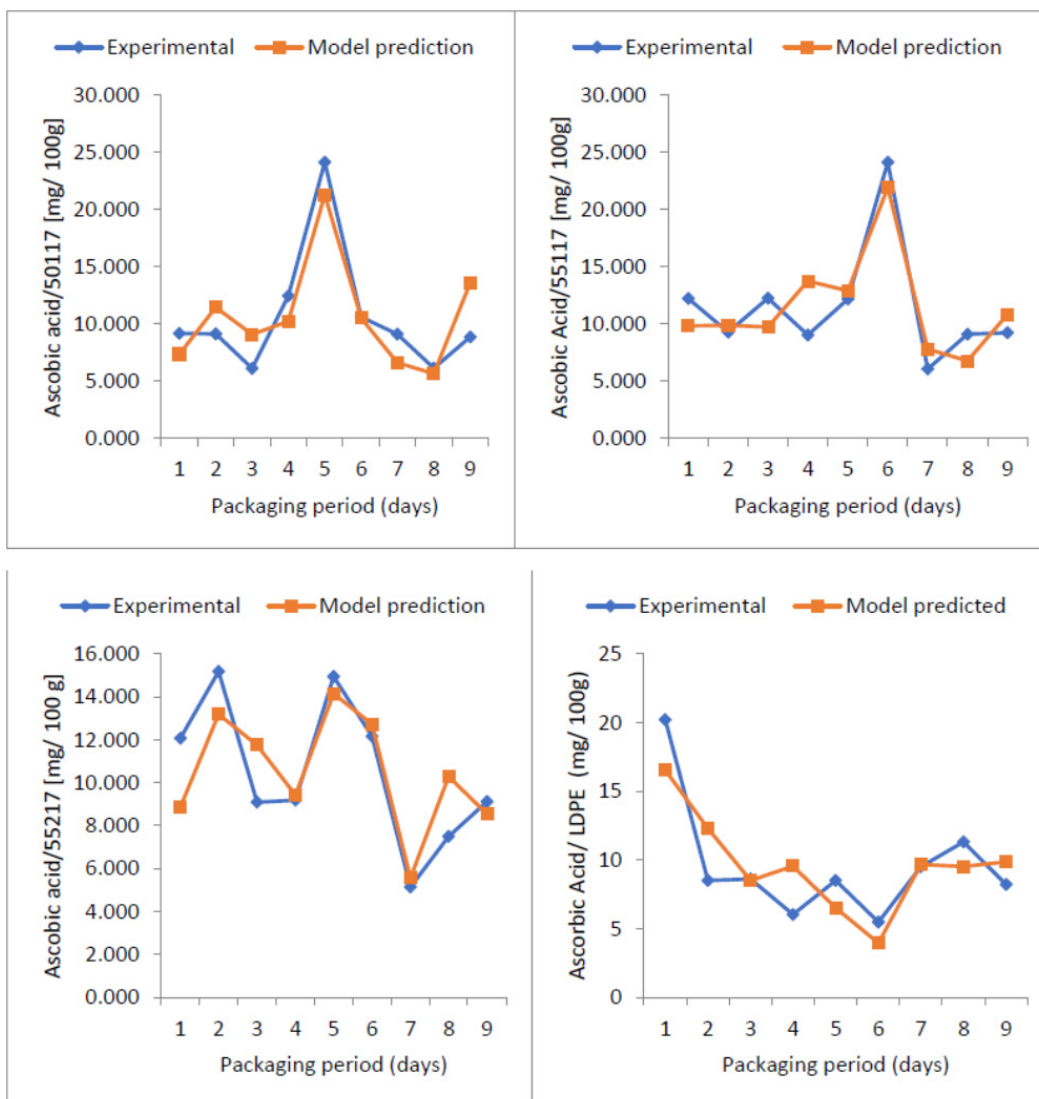


Fig 4a. Effect of packaging period on β -carotene and ascorbic acid of packaged fresh-sliced okra

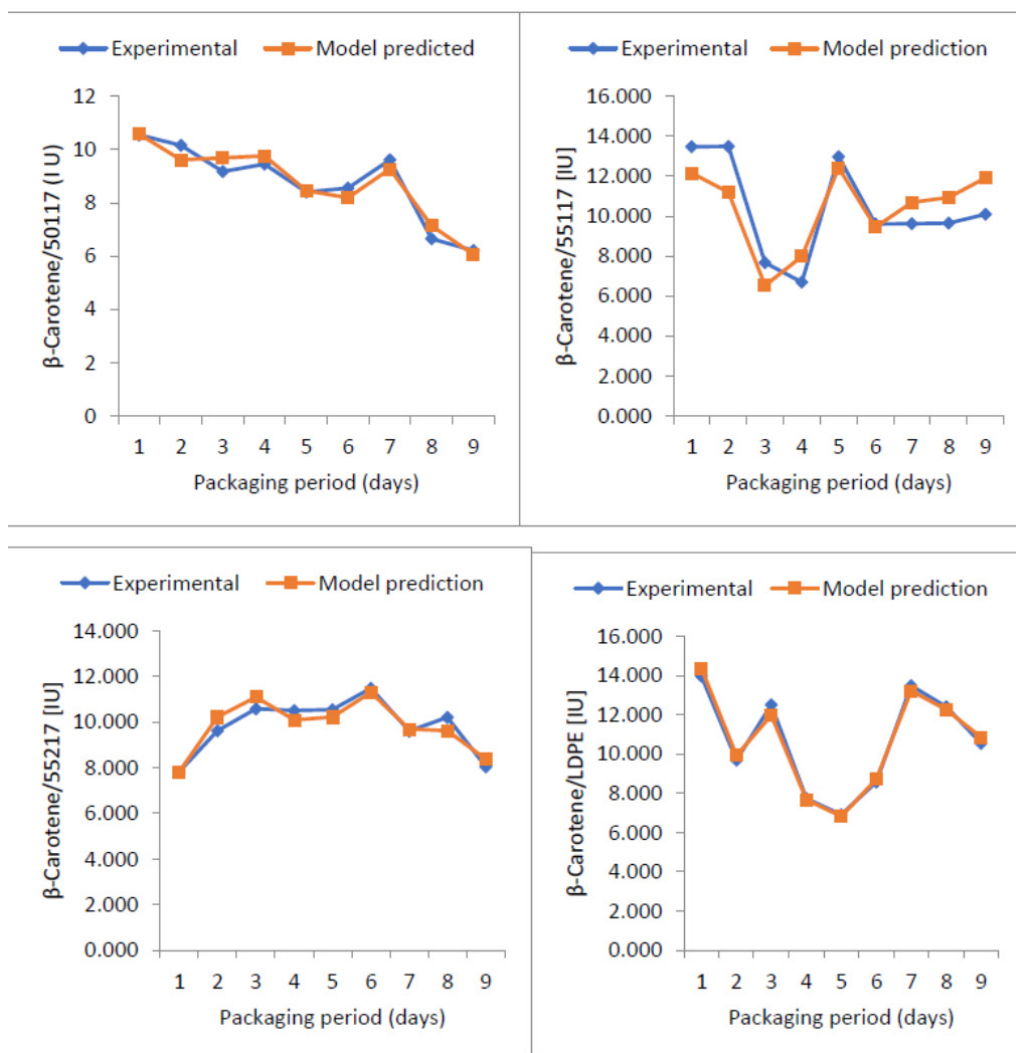


Fig 4b. Effect of packaging period on β -carotene and ascorbic acid of packaged fresh-sliced okra

Tt A_{LDPE} = titratable acidity of packaged okra using the cassava starch-zinc nanocomposite films (50117, 55117 and 55217) and a low density polyethylene (LDPE), respectively.

It can be seen that Eq. (22) has the highest value of R^2 than the other predictive equations. This suggests the ability of the nanocomposite film containing 1% of the zinc nanoparticle and 50% of the glycerol to retain titratable acidity of the fresh-sliced okra than the one containing 2% of the nanoparticles and 55% glycerol. Similar results were reported by Fadeyibi et al.[18] in their work on prediction of some physical attributes of zinc-nanocomposite films for food packaging applications.

Bacteria count of the packaged fresh-sliced okra

The empirical equations for the prediction of the bacteria load of packaged sliced okra for the optimally selected nanocomposite films and a 10 μ m LDPE were developed and fitted in Eq. (26) to Eq. (29).

where, T = packaging temperature ($^{\circ}$ C), p = packaging period (days), BL_{50117} , BL_{55117} , BL_{55217} and BL_{LDPE} = bacteria loads of Fresh-sliced okra packaged in the cassava starch-zinc nanocomposite films (50117, 55117 and 55217) and a low density polyethylene (LDPE), respectively.

It can be seen that all the predictive equations have satisfactory R^2 values. This suggests the ability of the nanocomposite film containing 2% of the zinc nanoparticle and 55% of the glycerol to inhibit bacterial growth on the fresh-sliced okra than the one containing 1% of the nanoparticles. Babarinde and Fabunmi[17] corroborate this findings in their work on the effects of packaging materials and storage temperature on quality of fresh okra. Rajesh et al.[19] developed biodegradable film based on banana flour-plasticizer for quality packaging of food carrot, banana and cashew nuts, and reported that the bacterial load of the products stored in biodegradable packaging was less compared to polypropylene package.

Fungi load of the packaged fresh-sliced okra

The empirical equations for the prediction of the fungi load of packaged sliced okra for the optimally selected nanocomposite films and a 10 μ m LDPE were developed and fitted in Eq. (30) to Eq. (34).

where, T = packaging temperature ($^{\circ}$ C), p = packaging period (days), FL_{50117} , FL_{55117} , FL_{55217} and FL_{LDPE} = fungi loads of Fresh-sliced okra packaged in the cassava starch-zinc nanocomposite films (50117, 55117 and 55217) and a low density polyethylene (LDPE), respectively.

It can be seen that Eq. (30) has R^2 closer to that

$$(a) \quad BL_{50117}(o) = 1.52 \times 10^6 - 2.51 \times 10^5 T + 7.31 p + 2.03 \times 10^4 T + 5.56 \times 10^3 T^2 - 2.65 \times 10^4 p^2 \quad \begin{cases} R^2 = 0.91378 \\ R^2_{Adj} = 0.7701 \\ mse = 25.81394 \end{cases} \quad (26)$$

$$(b) \quad BL_{55117}(o) = -2.59 \times 10^5 + 2.59 \times 10^4 T + 1.84 \times 10^4 p - 279.3 T p - 279.3 T^2 - 1.34 \times 10^3 p^2 \quad \begin{cases} R^2 = 0.96032 \\ R^2_{Adj} = 0.89418 \\ mse = 18.13449 \end{cases} \quad (27)$$

$$(c) \quad BL_{55217}(o) = -2.83 \times 10^5 + 4.02 \times 10^4 T - 1.68 \times 10^4 p - 695.1 T p - 664.8 T^2 + 3.53 \times 10^3 p^2 \quad \begin{cases} R^2 = 0.9352 \\ R^2_{Adj} = 0.8272 \\ mse = 21.20402 \end{cases} \quad (28)$$

$$(d) \quad BL_{LDPE} = 8.01 \times 10^5 - 2.15 \times 10^4 T - 2.88 \times 10^5 p + 8.95 \times 10^3 T p - 178.43 T^2 + 1.50 \times 10^5 p^2 \quad \begin{cases} R^2 = 0.8865 \\ R^2_{Adj} = 0.6974 \\ mse = 24.08364 \end{cases} \quad (29)$$

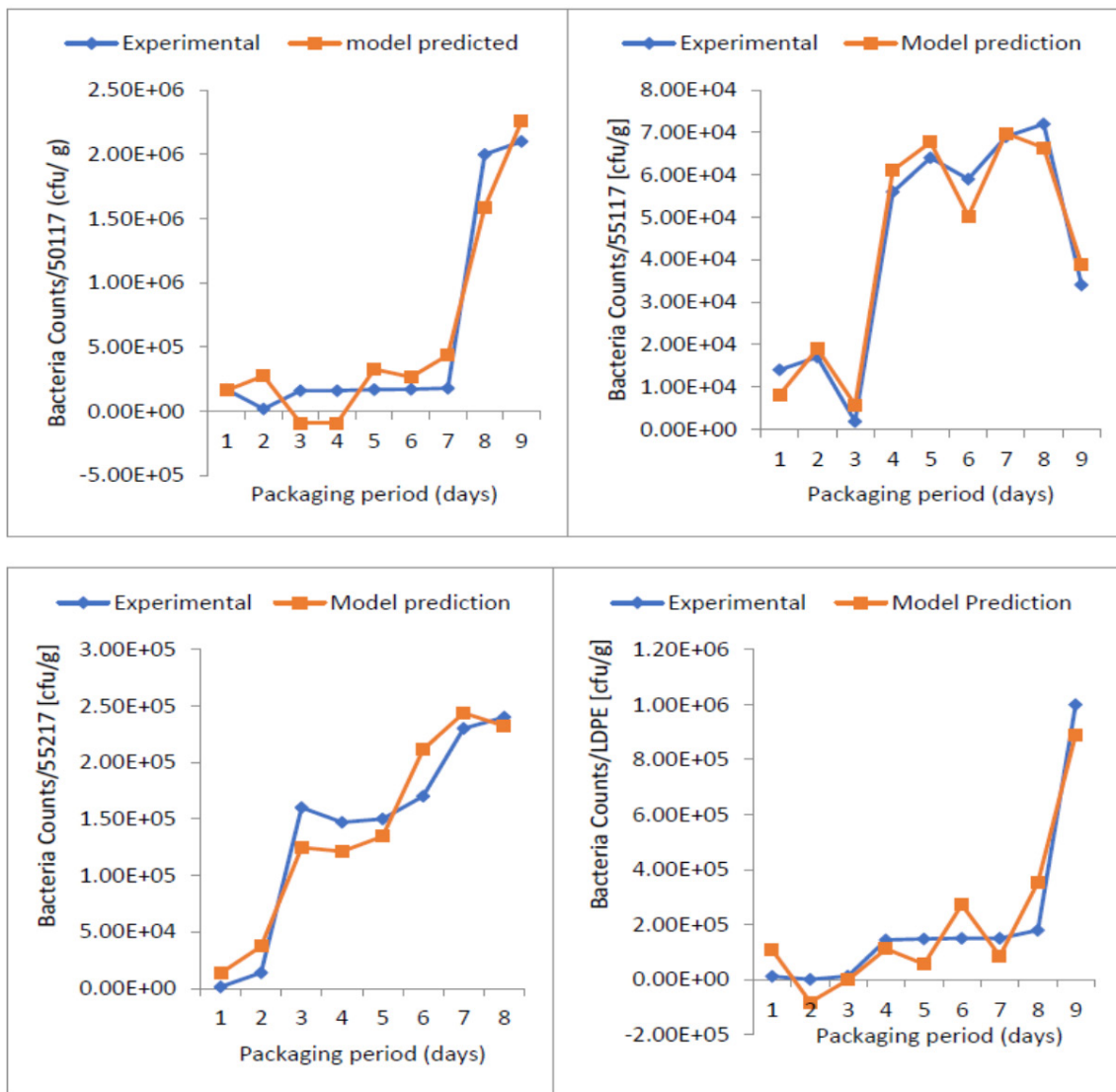


Fig 5a. Effect of packaging period on microbial loads of packaged fresh-sliced okra

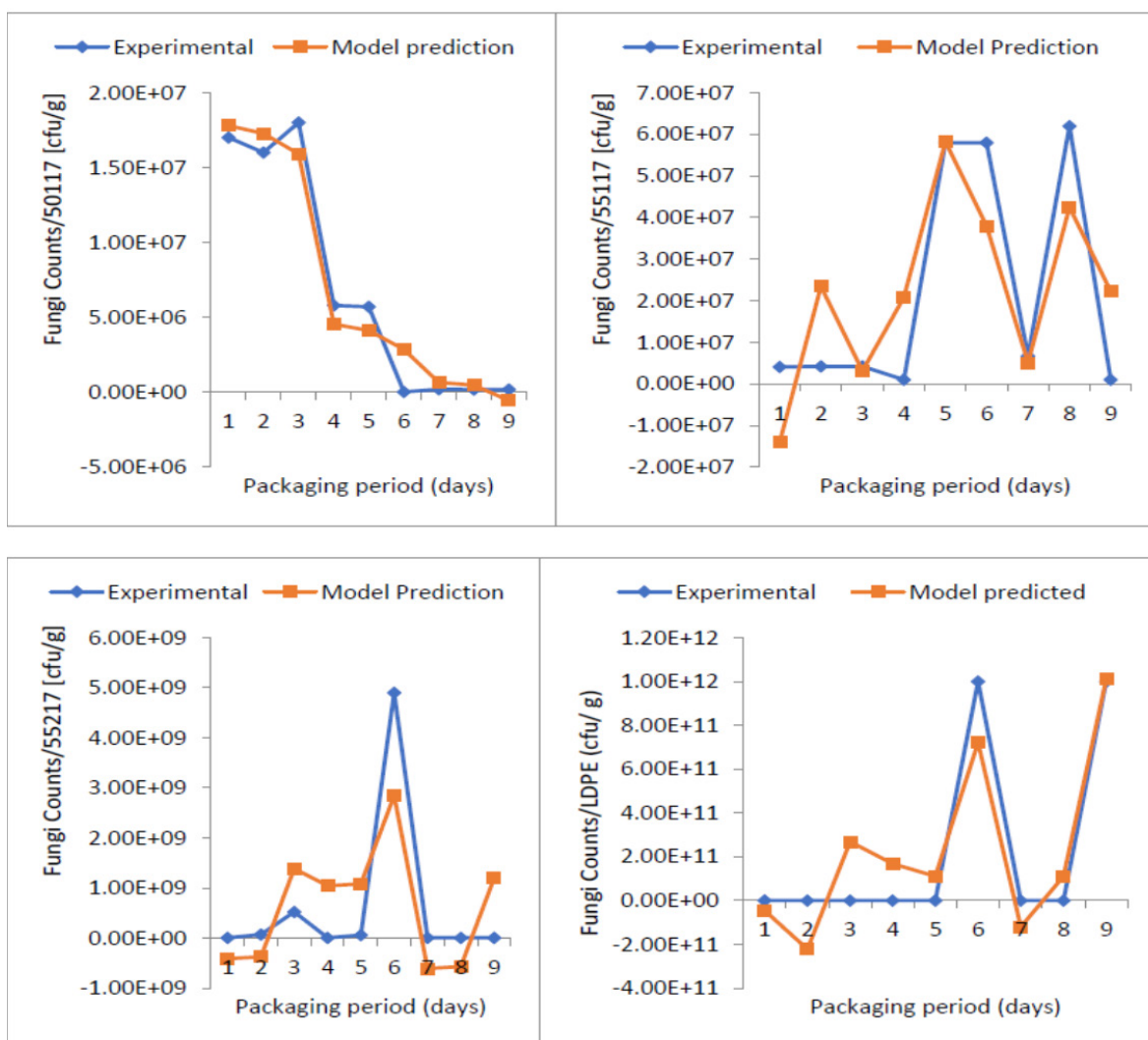


Fig 5b. Effect of packaging period on microbial loads of packaged fresh-sliced okra

$$\begin{aligned}
\text{(a) } Fl_{50117}(o) &= 2.21 \times 10^{10} + 1.17 \times 10^{11}T - 4.72 \times 10^{11}p + 8.01 \times 10^9Tp \\
&\quad - 3.92 \times 10^9T^2 + 3.70 \times 10^9p^2 \quad \begin{cases} R^2 = 0.82751 \\ R^2_{Adj} = 0.54003 \\ mse = 52.84779 \end{cases} \quad (30) \\
\text{(b) } Fl_{55117}(o) &= -5.42 \times 10^9 + 9.21 \times 10^8T - 8.49 \times 10^8p + 9.58 \times 10^7p^2 \\
&\quad - 2.52 \times 10^7T^2 \quad \begin{cases} R^2 = 0.5390 \\ R^2_{Adj} = 0.08 \\ mse = 42.3214 \end{cases} \quad (32) \\
\text{(c) } Fl_{55217}(o) &= -2.52 \times 10^8 + 1.90 \times 10^7T + 4.14 \times 10^7p - 4.87 \times 10^5T^2 \\
&\quad - 3.21 \times 10^6p^2 \quad \begin{cases} R^2 = 0.62859 \\ R^2_{Adj} = 0.2572 \\ mse = 33.9958 \end{cases} \quad (33) \\
\text{(d) } Fl_{LDPE} &= 6.47 \times 10^7 - 6.09 \times 10^6T + 1.90 \times 10^5p + 6.61 \times 10^3Tp \\
&\quad + 1.37 \times 10^5T^2 - 4.76 \times 10^4p^2 \quad \begin{cases} R^2 = 0.96031 \\ R^2_{Adj} = 0.8942 \\ mse = 29.5068 \end{cases} \quad (34)
\end{aligned}$$

of the Eq. (34). This suggests the ability of the nanocomposite film containing 1% of the zinc nanoparticle and 50% of the glycerol to inhibit fungi growth on the fresh-sliced okra than the one containing 1% of the nanoparticles. Babarinde and Fabunmi [17] corroborate this findings in their work on the effects of packaging materials and storage temperature on quality of fresh okra. Also, Rajesh et al.[19] developed biodegradable film based on banana flour-plasticizer for quality packaging of food carrot, banana and cashew nuts, and reported that the fungi load of the products stored in biodegradable packaging was less compared to polypropylene package.

CONCLUSIONS

This research reports the optimum processing parameters for cassava starch-zinc nanocomposite film for use in the packaging of fresh-sliced okra products. The results indicate that the 17 μ m thick film, which was processed with 50% glycerol and 1% zinc nanocomposite performs higher than the other counterpart films at 10°C and 15°C with respect to the quality retention of the packaged products. There was a progressive decrease in the quality of the packaged okra products with an increase in the temperature and the storage period ($p < 0.05$). The quality difference between the packaged products and that of the fresh okra at 27°C was not significant. Thus, the nanocomposite film can essentially be used for the packaging of the fresh-sliced okra.

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