Research Article

ANN and RSM Modeling for the Synthesis of Avocado Seed Starch Combined Orange Peel Extract Antimicrobial Packaging Film

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Cooperation of essential oil into film formation results in active packaging materials, which can improve the quality and freshness of the foods and extend the shelf life. In the present study, extraction of starch and essential oil was performed. The active films were developed through the solvent casting method. The influence of avocado seed starch and orange peel essential oil was investigated and optimized using response surface methodology and an artificial neural network on the tensile strength, water vapor permeability, and antimicrobial properties of active films. The results showed that both models performed reasonably well, but trained artificial neural networks have more modeling capability rather than the response surface method. The optimum conditions were found to be orange peel oil of 0.57 g and 30% w/w of avocado seed starch with the values of the corresponding responses of 3.94 MPa, $3.098 \times 10^{-10}$ g/ms Pa, and 17.273 mm for tensile strength, water vapor permeability, and inhibition zone, respectively. Generally, the orange peel extract had an effective and promising alternative for the commercial production of antimicrobial packaging films.

1. Introduction

Nowadays, replacing non-renewable sources of packaging materials emerged due to environmental pollution and sustainability issues [1, 2]. From natural polymers, starch has been regarded as the most promising to this goal for the reason that this material has being biodegradable, abundantly available, and renewable [3]. Besides, there are many researchers who reported the film-forming ability of (amylose) starches extracted from different sources [4–11]. Starch-based film is recognized as odorless, transparent, biodegradable, colorless, and non-toxic [6, 7]. Avocado seed starch has high values of gelatinization temperatures differential scanning calorimetry and good gel stability [12].

Types of starch, types of plasticizer used, processing conditions, and the effect of co-biopolymers and other additives on film have been stated as factors affecting the physical and mechanical properties of the starch films [6, 13, 14]. Furthermore, the functionality of the films has improved by the inclusion of active substances like essential oils (antioxidant and/or antimicrobial properties), which intern result in consumer health and reduce the amount of additives to preserve quality food products [3, 13, 15–24]. In up-to-date, antimicrobial-containing films have great importance in the food industries [6, 17]. Due to the health concern of synthetic additives, natural additives are favored [25–31].

Fruit peels are waste products that have antimicrobial and antioxidant properties [32, 33]. It has been stated that extracts from *Citrus sinensis* (sweet orange) peels have great antibacterial activity against different bacterial and fungal strains [34–39]. Several authors have studied the effect of incorporation of orange peel extract and in its powder form on antimicrobial packaging film [40–46]. However, to the best of our knowledge, there was no report on avocado seed starch films containing orange peel extracts by combination response surface methodology (RSM) and artificial neural network (ANN).

RSM includes a set of statistical methods for designing experiments, model building, and assessment of the impact of processing parameters on response and process optimization [47–49]. In the present study, the effects of process
variables on responses using RSM with a face-centered central composite design (CCD) were employed in the formulation of antimicrobial films. ANN is the complex mathematical modeling commonly used to mimic the biological neural networks and processes information. ANN provides a complex relationship between input and output variables and can be used in the replacement of polynomial regression modeling tools [50]. ANN is more reliable in capturing the nonlinear relationship between the dependent variable and independent process variables compared with the RSM focusing on the statistical importance of the linear process parameters and their interactions via analysis of variance (ANOVA) [51]. The mathematical models developed by RSM and ANN were compared using error functions.

Thus, the objective of the present study was to examine the effect of orange peel extracts and avocado seed starch on the physic mechanical and antimicrobial properties of avocado seed starch films and optimize these process variables by using RSM and ANN. The collaboration effect of the process variables with the responses using the response surface plots was demonstrated. Additionally, the feedforward with the backward propagation ANN model was also developed, and the predictive abilities and modeling competencies of the two models are differentiated. For the development of ANN model based on Feed Forward Backpropagation Network Mat lab, 2014b was used.

2. Materials and Methods

2.1. Raw Material. The raw materials used for the experimental work were avocado seed (*Persea americana*), orange peels were obtained from Jimma Zone, and *Escherichia coli* was obtained from Jimma University College of Agricultural and Veterinary Medicine, Ethiopia. The microorganism was cultured in a Mueller–Hinton agar at 37°C for 12–24 hours.

2.2. Starch Extraction. The starch isolation techniques were done following [52], the avocado seeds were stripped and washed with tap water, and done following [52], the avocado seeds were stripped and water at 15°C for three times. The remaining starch was washed with distilled water at 15°C for 20 minutes, with continuous stirring at 500 rpm; different concentrations of orange peel oil (0.1, 0.5, and 0.9 g) were added up into the mixed gelatin solutions with stirred continuously for 35 minutes. The blend was cast on the Petri dish and allowed to dry at 45°C temperature in an oven till completely dry. A prepared film, after being removed from the Petri dish, was kept for further analysis. All variables and their respective levels were selected based on earlier studies [9], as illustrated in Table 1.

2.3. Orange Peel Preparation and Oil Extraction. Fresh orange peel was collected from Jimma, Oromia regional state, Ethiopia. The essential oil was extracted from fresh orange peel using the hydrodistillation method, following [53]. Before extraction, the peel was washed, air-dried for 7 days, and then sized into powder using mortar and pestle. A total of 200g of sized orange peels were mixed with 500 mL of distilled water using a 2000 mL round-bottomed flask and was heated to 135°C until boiling and then it was heated more for the next 2 hours. Afterward, the condensed vapor was separated throughout the distillatory process, during which oil accumulated in the upper layer while water settled in the bottom layer. The water was then recycled into flasks again. This process was repeated till the required amount of oil was extracted, and the oil sample was collected in a dark bottle.

2.4. Preparation of Antimicrobial Films. Avocado seed starch films containing orange peel oil were prepared using solvent casting by the procedure adapted from [53]. Film-forming solutions were prepared through gelatinization of avocado seed starch (15, 20, and 25% w/w) at a temperature of 75°C±5°C for 20 minutes, with continuous stirring at 500 rpm; different concentrations of orange peel oil (0.1, 0.5, and 0.9 g) were added up into the mixed gelatin solutions with stirred continuously for 35 minutes. The blend was cast on the Petri dish and allowed to dry at 45°C temperature in an oven till completely dry. A prepared film, after being removed from the Petri dish, was kept for further analysis. All variables and their respective levels were selected based on earlier studies [9], as illustrated in Table 1.

2.5. Film Characterization

2.5.1. Tensile Strength and Water Vapor Permeability. Tensile strength (TS, MPa) was measured using a texture operating following ASTM D882-09 (ASTM, 2009). Water vapor permeability (WVP) was determined according to ASTM E 96 (ASTM, 2001) in a desiccator (75% relative humidity).

2.5.2. Antimicrobial Properties of Films. The antibacterial activity of the produced films was determined by the agar disc diffusion method, as described by Radfar et al. [22] with modification. The produced films were cut into 6 mm discs and then placed on the prepared culture of *E. coli* containing Mueller–Hinton agar in Petri dishes. After incubation of cultures at 37°C for 24 hours, the diameters of inhibition zones were examined.

2.6. RSM Modeling and Statistical Analysis. In the present study, RSM was employed to determine the optimum process variables (orange peel oil (A) and avocado seed starch (B)) for the mechanical, physical, and antimicrobial film formulation. A CCD with two factors and three levels for each variable and independent process variables compared with the dependent variable in an oven till completely dry. A prepared film, after being removed from the Petri dish, was kept for further analysis. All variables and their respective levels were selected based on earlier studies [9], as illustrated in Table 1.

where $Y$ is the predicted response, $x_i$ and $x_j$ are the variables (for which $i$ and $j$ range from 1 to $n$), $n$ is the number of independent parameters (two in this study), $b_0$ is the constant coefficient, $b_i$ is the linear coefficient, $b_{ij}$ is the second-order interaction coefficient, $b_{ij}$ is the interaction coefficient, and $e_i$ is the error. The significant terms in the model were determined by ANOVA for each response, and
the coefficient of determination $R^2$, adjusted $R^2$, and predicted coefficient $R^2$, lack of fit were used to resolve the adequacy of the developed model. In addition, predicted versus actual responses were plotted to identify whether the developed models were the experimental (actual) values or not.

2.7. ANN-Based Modeling. The feed-forward architecture of ANN, also known as multilayer perceptron (MLP), was used for two inputs neurons (orange peel oil and avocado seed starch) and an output layer of three neurons (TS, WVP, and inhibition zone), and a hidden layer was employed to construct the predictive for the same set of experimental data used for RSM. 60% of the data points were selected for training to develop a virtual network, 20% of the data sets that have been validated, and 20% of the data sets that have been tested. In order to minimize both the error of prediction and the number of neurons required, it is necessary to determine how many neurons are needed in this hidden layer. The number of neurons in the hidden layer has been set at six after a series of tests. In order to achieve a rapid convergence, the input and target values for each ANN node have been normalized within a range of 0–1 so as to obtain minimal RMSE values. ANN modeling using toolbox of MATLAB version 8.1 (R2014a) was applied to evaluate the goodness of fitting model using RSM data output.

2.8. Comparison of RSM and ANN Models. The goodness of fit and prediction abilities of computed models were evaluated by root-mean-squared error (RMSE), mean absolute error (MAE), standard error of prediction (SEP%), absolute average deviation (AAD%), and correlation coefficients ($R^2$). The formulas used for these error functions are adopted from Haefez et al. [50] and presented in equations (2) and (3), respectively.

\[
RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (Y_{ie} - Y_{ip})^2}
\]

\[
R^2 = 1 - \frac{\sum_{i=1}^{n} (Y_{ie} - Y_{ip})^2}{\sum_{i=1}^{n} (Y_{ie} - \bar{Y}_e)^2}
\]

\[
MAE = \frac{1}{n} \sum_{i=1}^{n} |Y_{ie} - Y_{ip}|
\]

\[
SEP = \frac{\text{RMSE}}{Y_e} \times 100,
\]

where $n$ is the number of experiments; $Y_{ie}$ is the experimental value of the $i^{th}$ experiment; $Y_{ip}$ is the predicted value of the $i^{th}$ experiment by model; and $Y_e$ is the average value of experimentally determined values. The models to be higher acceptable, the $R^2$ was closer to 1, and RMSE was as small as possible.

3. Results and Discussion

3.1. Modeling and Prediction Using RSM. As observed from the experimental results in Table 2, a total of 13 experiments were performed to analyze the effects of orange peel oil and avocado seed starch on TS, WVP, and inhibition zone. The statistical significance of the model was analyzed using ANOVA, as shown in Table 3.

3.2. Analysis of ANOVA and Model Adequacy. In this study, TS, WVP, and inhibition zone were determined, and ANOVA was used to obtain the interaction between the process variables and the responses. Based on the ANOVA, all responses, namely TS and EA, were significantly affected by quadratic process variables (Table 3). The significance of the factors was checked using P-value and F-value with 95% confidence level generating regression coefficients and ANOVA for the quadratic model of the response surfaces. A P-value less than 0.05 indicates the significance of the model terms, while greater than 0.05 indicates the model terms are not significant. The ANOVA results showed a perfect fit of the quadratic regression model for TS and WVP, while two-factor interaction (2FI) for inhibition zone with their F-value of 175.05, 28.50, and 30.78, indicating that the model is highly significant.

The significance of each parameter coefficient was determined by p-values, the smaller the p-values the more significance of the coefficient. In this case, A, B, A^2, B^2, and AB were found to have a significant effect on the TS, A, B, A^2, and B^2 for WVP and A, B, and AB for inhibition zone of 0.9921, 0.9532, and 0.9112.

The goodness of fit of the model was confirmed by the coefficient of determination $R^2$ and adjusted $R^2$ (Table 4). In the current study, the $R^2$ value of 0.9921, 0.9532, and 0.9112 for TS, WVP, and inhibition zone, while the Adj-$R^2$ values were 0.9864, 0.9197, and 0.8816, respectively. A high $R^2$ value indicates that the model fitted the experimental data [15]. Thus, all responses have reflected that the model is suitable to predict the film’s TS, WVP, and antimicrobial properties. The percentage variation of actual values based on independent variables is measured by the adjusted $R^2$ [9]. Therefore, the quadratic models were significant ($P < 0.05$) for TS and WVP, while 2FI significant ($P < 0.05$) for the inhibition zone.

The predicted $R^2$ value for TS, WVP, and inhibition zone was found at 0.9364, 0.8628, and 0.7229, respectively, as shown in Table 4. The predicted $R^2$ is in reasonable agreement with the adjusted $R^2$; i.e., the difference should be less than 0.2 (20%), as recommended by Alam et al. [54]. The coefficient of variation (CV%) and standard deviation for

<table>
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<th>Factors</th>
<th>Unit</th>
<th>Coded levels</th>
<th>Coded variables</th>
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<td>Orange peel oil</td>
<td>g</td>
<td>A 0.1 0.5 0.9</td>
<td></td>
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<tr>
<td>Avocado seed starch</td>
<td>% (w/w)</td>
<td>B 15 20 25</td>
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the response in this study were reasonably low and acceptable, which indicates that the deviations between experimental and predicted values are low (Table 4). Thus, the model is adequate for predictions in the range of the experimental variables [15].

3.3. Development of Regression Model Equation. The mathematical models were expressed in terms of coded variables. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The final equations obtained in terms of coded factors after excluding the insignificant terms were depicted in equations (7), (8), and (9), and the responses in terms of coded were expressed with independent factors of orange peel oil (A) and avocado seed starch (B). Equations (7), (8), and (9) showed that TS, WVP, and inhibition zone were positively affected by orange peel oil. Additionally, the inhibition zone was affected by avocado seed starch.

\[ \text{TS} (Y_1) = +4.77 + 0.9388A - 3.13B - 0.3760AB - 1.20A^2 + 2.37B^2, \]  \hspace{1cm} (7)

\[ \text{WVP} (Y_2) = +4.19 + 0.9388A - 3.13B - 0.3760AB - 1.40A^2 + 2.17B^2, \]  \hspace{1cm} (8)

\[ \text{Inhibition zone} (Y_3) = +11.93 + 1A + 3.61B + 2.01AB. \]  \hspace{1cm} (9)

Besides, the adequacy of the model was checked through the predicted versus experimental values plot (Figures 1(a), 1(b), and 1(c)). As depicted in Figures 1(a), 1(b), and 1(c), the points of all predicted and experimental responses nearly in 45° lines, indicating that the developed models are appropriate to predict TS, WVP, and inhibition zone. From the graph, it is clear that the values derived experimentally match closely with the developed model. The actual data points in the graph closely resemble those predicted, suggesting that the quadratic model and 2FI are efficient models for estimating the responses of independent variables [54].

3.4. Response Surface Analysis. From the model equation, the coefficients of the independent variables show the effect of each independent variable on TS, WVP, and inhibition zone. To visualize and study the individual and interactive relationship between the response and process parameters, three-dimensional (3D) surface plots were generated from the fitted polynomial equation and presented in Figure 2.

3.4.1. Effects of Process Variables on the TS. TS is the maximum amount of force or stress required to pull material to the point where it breaks or before distortion occurred [23]. In the present study, the TS of the produced films was varied between 2.133 and 10.606 Pa, as shown in Table 2. Linear and quadratic avocado seed starch and orange peel oil amounts and their interaction were found to have a significant effect on the TS value (p < 0.05), as described in Table 3.

As increase in orange peel oil up to intermediate value, result in increasing the TS of the films. Increasing orange oil content beyond optimum (0.5 g) resulting in a reduction of TS. The results were in correspondence with other works [3, 22, 55]. The hydrophobic nature of orange peel oil results in TS reduction. Compounds present in the orange peel can disrupt the interaction with proteins in the membrane network, as gelatin composed of hydrophilic structures can become incompatible with orange peel and reduce the binding of the structural integrity of the film [9]. This can cause discontinuities in the film matrix and reduce the mechanical strength of the film [55]. As can be seen in Figure 2(a), TS was increased as decreasing amount of starch.

3.4.2. Effects of Process Variables on the WVP. Films with minimal WVP reduce the water transport and help to extend the shelf life of food products. The results reported that WVP values were found between 1.223 × 10^{-10} and 9.696 × 10^{-10} g/ms Pa. Meanwhile, the ANOVA results (Table 3) showed the linear and interaction effect of orange peel oil and avocado seed starch and their quadratic term were found to have a significant effect on the WVP values (p < 0.05). Figure 2(b) showed that a higher WVP value was observed at optimum value of extract (0.5 g), while further increasing the oil contents results in a reduction of WVP. The present results are in correspondence with previous reports [6, 9, 16, 56–58]. This activity may be due to the internal cross-linking process; consequence in the formation of the porous microstructure by the addition of essential oil to the films [56] and this outcome increasing hydrophobic nature of the films.

The addition of avocado seed starch was found to have a negative effect on WVP value (equation (8)). The WVP was observed to rapidly decrease with an increased avocado seed starch compared to that of orange peel oil. The decreases in the WVP of films in the presence of starch were reported by many scholars [9, 59, 60]. This may be the reason that starch-based films are hydrophilic and have the tendency...
3.4.3. Effects of Process Variables on the Inhibition Zone. Antimicrobial properties confirm the potency of films in preventing microorganism attacks on food products, which can cause the food to become rotten, therefore shortening the shelf life [62]. The linear effects of orange peel oil and avocado seed and their interaction effects on the inhibition zone value of the films were significant (P < 0.05). However, the quadratic effects were non-significant (P > 0.05) (equation (6)). The antimicrobial activity of films integrated with orange peel oil at various contents against E. coli is presented in Table 3. The results revealed that the films incorporated with 0.1% (w/v) orange peel oil showed the minimum inhibitory effect against E. coli. Further increasing the oil concentration increased the inhibitory effect (Figure 2(c)). Such a trend was examined by Chaivarit et al. [43], who have reported the use of orange oil-loaded pectin films as antibacterial material for food packaging against Staphylococcus aureus. Azarifar et al. [15] similarly reported that gelatin-carboxymethyl cellulose-based active films containing Tracheliopsernum ammi essential oil have an inhibitory effect against S. aureus and E. coli. Furthermore, this similar result can be observed in other studies of biocomposite films integrated with different essentials oil [22–24, 55].

Oikeh et al. [38] reported the powerful inhibitory effect of orange peel extract against S. aureus, Enterococcus faecalis, Pseudomonas aeruginosa, E. coli and Salmonella typhi- murium bacterial strains, and Candida albicans, Aspergillus niger, and Penicillium notatum fungal strains. The antibacterial efficiency of orange peel oil is due to the concentration and amount of phenolic compounds [44].

3.5. ANN-Based Modeling. The predicted values for TS, WVP, and inhibition zone models by ANN are tabulated in Table 5. TS, WVP, and inhibition zone predicted by the network were between in the range of 2.10 to 10.54 Pa, 1.20 × 10⁻¹⁰ to 9.6 × 10⁻¹⁰ g/ms Pa, and 7.03 to 17.69 mm. This demonstrated that the best grouping of the ANN parameters to predict the responses satisfactorily.

3.6. Performance Assessment of the Predictive Capability of the Developed Models. The effectiveness of the developed RSM and ANN models to predict the TS, WVP, and inhibition zone of the films were statistically measured, in terms of the RMSE, MAE, SEP%, AAD%, and R². The results indicated that both models performed realistically well, but ANN models have better modeling competency compared to the RSM model for all responses. As can be seen, the ANN predicted value is much nearer to that of the actual data, signifying that the ANN model has higher prediction ability than the RSM model (Table 6). Ajesh Kumar et al. [63] reported that the experimental data were better predicted by ANN models compared to RSM with higher R², lower RMSE, and MAE in the soybean aqueous extract-based composite film production. Furthermore, Hafeez et al. [50] observed that RMSE obtained from RSM (5.0737) is approximately four times higher as compared to ANN (1.1779) for cleaner intensification of ozone production.

3.7. Numerical Optimization and Validation Test of the Model by Using Response Surface Modeling. The reason for optimization is to find the best set of independent variables with minimization of TS and inhibition zone, and minimization of WVP through numerical optimization. The optimum condition was obtained at 0.57 g orange peel extract and 30%
w/w avocado seed starch. Under these conditions, the model predicted the corresponding TS, WVP, and inhibition zone values of 3.94 MPa, 3.098 × 10^-10 g/ms Pa, and 17.273 mm, respectively. To validate the optimum conditions predicted by the model using desirability ramp, triplicate experiments were performed under the predicted process conditions, and mean TS of 4 MPa, WVP of 3.097 × 10^-10 g/ms Pa, and inhibition zone of 17.287 mm were obtained. Saberi et al. [8] identified that starch-based film as odorless physical, and optical properties of biodegradable edible films based on pea starch and guar gum. At the optimum condition of pea starch of 2.5 g, glycerol of 25%, and guar of 0.1 g, the model predicate solubility (%) of 28.771 ± 2.234, and the correspondence experimental value of 27.673 ± 2.724 was attained at the given optimum values. Similarly, Said and Sarbon [9] performed validation (experimental) test in order to determine the actual TS, EAB (elongation at the break), WVP, and DPPH antioxidant activity value under optimized condition (20% rice starch and 0.03 g curcumin). The validation results demonstrated that TS, EAB, and WVP values were found to be higher than predicted values. The validity of the estimation models built through the statistical experimental

Figure 1: Correlation between the experimental and predicted value for (a) TS, (b) WVP, and (c) inhibition zone.
Design-Expert® Software
Factor Coding: Actual

**TS (MPa)**
- Design points above predicted value: 2.133
- Design points below predicted value: 10.606

X₁ = A: Orange peel oil
X₂ = B: Avocado seed starch

**Figure 2: Continued.**
design was verified by the small differences (<4%) between the experimental and the predicted responses. The result indicated that face-centered CCD is effectively used to optimize the process parameters that affect the TS, WVP, and inhibition zone of the films.

4. Conclusions

The effect of TS, WVP, and inhibition zone of starch-based films having orange peel extract was analyzed using RSM. The outcomes verified that the investigated responses for TS and WVP were influenced by both individual variables, while inhibition zone was much significantly by orange peel oil than avocado seed starch. In this work, the RSM and ANN were employed and compared in terms of their predictive modeling capabilities. The neural network approach showed superior predictive capabilities by its better $R^2$ and error function (RMSE). The finding of the study indicated that 0.57 g% orange peel extract and 30% w/w avocado seed starch are the best optimized process parameters that result to the corresponded to the actual value of TS, WVP, and inhibition zone of 3.94 MPa, $3.097 \times 10^{-10}$ g/ms Pa, and 17.27 mm, respectively. Validation of these conditions gave TS of 4 MPa, WVP of $3.097 \times 10^{-10}$ g/ms Pa, and inhibition

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<th>TS (%w/w) ANN</th>
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zone of 17.287 mm. The films containing orange peel extract demonstrated commendable antimicrobial, against *E. coli*.

**Data Availability**

All data are included within the article.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

All authors conceived, designed, and performed the experiments and wrote this paper, and they also participated in experiment design and research supervision. The authors read and approved the final manuscript.

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