A simplex lattice design for optimization of sustainable active and intelligent packaging based on gelatin films incorporated with *Hibiscus* × *archeri* wats anthocyanins and ZnO nanoparticles

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Abstract. Due to its biodegradability, edibility, transparency, film-forming properties, and excellent oxygen barrier capabilities, gelatin is a biopolymer with significant potential for use in biodegradable films. However, gelatin's high solubility in water and inadequate gas barrier properties limit its use in extensive food packaging. Incorporating anthocyanins extracted from *Hibiscus x archeri* Wats (HAE) and zinc oxide nanoparticles (ZnO-NPs) into gelatin films is expected to improve mechanical properties, the water vapor barrier, and antibacterial activity. The objective of this study was to determine the optimal formulation for biodegradable films composed of gelatin, HAE, and ZnO-NPs. An experimental approach using a Simplex Lattice Design was employed, with a total of eight trials conducted, each with two varying levels of ZnO-NPs and HAE. The experimental and dependent variables included tensile strength, elongation at break, water vapor transmission rate, and antibacterial activity. The results demonstrated good compatibility between gelatin, HAE, and ZnO-NPs. Incorporating HAE and ZnO-NPs improved the tensile strength and increased the elongation at break of the gelatin films. HAE and ZnO-NPs also influenced the WVTR and exhibited antimicrobial activity against *A. hydrophila*. The optimization results were validated, confirming that the formulation with 0.414 g HAE and 0.286 g of ZnO-NPs is optimal for producing multifunctional, eco-friendly, smart films for active food packaging.

1 Introduction

Packaging plays a crucial role in food manufacturing because it helps maintain the quality of food during storage, transportation, and use. Using packaging is essential for keeping both fresh and processed foods safe by protecting them from harmful external factors. These factors include contaminants, gas composition, the growth of spoilage bacteria, mechanical stress, and physical damage [1]. Due its synthetic and non-biodegradable polymer base, traditional food packaging poses a significant environmental risk. The development of packaging based on renewable resources and biodegradable polymers has emerged as a key area of study in the food industry [2]. Developing eco-friendly alternatives that do not only reduce their environmental impact but also provide additional benefits, such as improving food safety and prolonging shelf life, has increasingly become essential. Biopolymer-based materials, made from renewable resources such as polysaccharides, proteins, and aliphatic polyesters, are suggested as a green alternative to conventional plastics. These biodegradable materials could lessen the environmental burden caused by plastic packaging. However, they are not widely used in food preservation because they are not strong enough, do not block air and moisture well, and are very sensitive to moisture. Among various biopolymers, gelatin has attracted considerable attention due to its excellent mechanical properties, such as flexibility and strength, as well as its favorable optical characteristics, including brightness and opacity. It also exhibits good barrier properties against water vapor and gases, and its structural composition helps inhibit bacterial growth within the packaging. Owing to these attributes, gelatin is widely employed as an edible film, particularly for packaging highly perishable foods such as meat and fish [3]. Nonetheless, it is crucial to acknowledge that pure gelatin films come with certain drawbacks, such as their weak mechanical strength and inadequate water vapor barrier properties. As a result, incorporating natural bioactive compounds and functional nanomaterials into gelatin matrices has been suggested as an effective strategy to improve their performance.

The C6-C3-C6 framework is integral to anthocyanin, also referred to as the flavylium cation. This unique chemical structure of anthocyanins leads to specific structural changes across various pH levels, which are directly linked to the color variations these compounds display. In acidic environments ($pH < 3$), the red flavylium cation is most prevalent. At a neutral pH (6–7), the colorless carbinol pseudobase becomes the dominant species. Under mildly alkaline conditions (pH 8–10), the blue quinoidal base is predominant. Lastly, in strongly alkaline conditions ($pH > 10$), the yellow chalcone is the main form [4]. Owing to its high sensitivity and extensive functional pH range, anthocyanin is predominantly employed as a pH indicator to detect food spoilage. Substituting synthetic colours with anthocyanin-rich extracts enhances food safety and corresponds with the increasing demand of organic and clean-label packaging options.

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It has been demonstrated that zinc oxide nanoparticles (ZnO-NPs) exhibit antibacterial properties that work against fungi and both Gram-positive and Gram-negative bacteria. The antimicrobial effectiveness of ZnO-NPs is mainly associated with their ability to induce oxidative During this process, ions or reactive oxygen species (ROS) are released, which interact with the cell membrane and disrupt its integrity [5]. Furthermore, the United States Food and Drug Administration (FDA) has designated it as generally recognised as safe (GRAS), making it appropriate for food-contact, biomedical, and cosmetics uses [6,7]. This addition aims to provide active functions and enhance the polymer's mechanical and barrier qualities. A multipurpose platform for active and intelligent packaging systems is provided by the cooperative integration of ZnO-NPs and anthocyanins into gelatin matrices. ZnO-NPs function as antibacterial and strengthening agents, gelatin offers biodegradability, and anthocyanins are antioxidants and freshness markers.

The present research aims to develop and enhance sustainable active packaging films derived from gelatin, which are improved with *Hibiscus* × *archeri* Wats. anthocyanins and ZnO nanoparticles, using a Simplex Lattice Design. The study has two primary objectives: (i) to investigate the impact of anthocyanins and ZnO-NPs on the mechanical, antimicrobial, and barrier properties of gelatin films; and (ii) to discover the optimal formulation that improves both the performance and usefulness of the films. This research seeks to contribute to the advancement of eco-friendly, multifunctional active packaging systems, offering an alternative to conventional plastics while enhancing food security and safety.

2 Materials and methods

2.1 Plant sample preparation

Dried *Hibiscus x archeri* Wats. flower were collected from East Java, Indonesia. The species of the plant was identified by Mr. Heri Susanto from Yayasan Generasi Biologi Indonesia. The dried flowers and fruits were pulverized using the grinder. The powder was immediately packed in a plastic container (with silica gel) and stored in a freezer.

2.2 Extraction of anthocyanin

The *Hibiscus x archeri* Wats. powder (5 g) was extracted using 100 mL of ethanol 96%, and then subjected to ultrasonic-assisted extraction for 30 minutes at a frequency of 42 kHz using a Bransonic Ultrasonic Cleaner (Model 8510E MTH, USA). The obtained extract was filtered using Whatman No.1 filter paper and subsequently concentrated using Rotavapor ® R-300 rotary evaporator (Buchi, Switzerland) at a temperature of 40 °C, and flushed with nitrogen. This process was continued until the ethanol had completely evaporated. The extract was placed in an amber bottle and stored at -20 °C [8].

2.3 Film preparation

The film production process referred to F. M. Pereira et al. (2022) with slight modification [9]. 5 g of gelatin were dissolved in 100 mL of distilled water under continous stirring until complete dissolution was achieved, followed by the addition of 1 g of glycerol as a plasticizer. This was accomplished by using hot plate magnetic stirrer (Thermolyne Cimarec 3) to heat the mixture for 30 minutes at 80 °C. Next, ZnO-NPs dissolved in 1N HCl were incorporated into the gelatin solution at 70 °C for 30 minutes. The solution was then cooled, and the *HAE* was incorporated into the gelatin solution at 30 °C for an additional 30 minutes. The resulting solutions were cast onto Teflon plates (20×10×2 cm) and desiccated at 40 °C for 24 hours in a dehydrator. After drying, the films were taken out by hand and kept for a full day at 25 °C and 50% relative humidity in a humidity chamber. Following this conditioning step, the films were subjected to analysis for various responses: tensile strength, elongation at break, water vapor transmission rate (WVTR), and antibacterial activity. A Simplex Lattice Design was employed to conduct eight experimental runs, incorporating two different concentration levels of both ZnO-NPs and HAE.

2.4 Optimization of Film Properties Using Simplex Lattice Experimental Design and Statistical Analysis

A simplex lattice design (SLD) was employed to generate the experimental runs, perform statistical analysis (including ANOVA and fit statistics at a significance level of $\alpha = 0.05$), and identify and validate the ideal formulation. Two independent variables were considered, namely the concentration of HAE and ZnO-NPs, while the glycerol content was kept constant as a plasticizer. The experimental runs were arranged according to a simplex lattice design, which allowed systematic variation of the levels of the two independent variables. This method allowed for a thorough study of how the extract and ZnO-NPs concentration interacted with each other, which ultimately led to the discovery of the best formula for our purpose. The responses (dependent variables) were mechanical properties (tensile strength and elongation at break), water vapor transmission rate (WVTR), as well as antibacterial activity against *Aeromonas hydrophila* indicated by the inhibitory zone diameter.

2.5 Analysis of Films

2.5.1 Mechanical properties

The tensile strength (TS) and elongation at break (EB) of gelatin films were measured in accordance with Suyatma et al. (2023) using the texture analyzer Lloyd Ametek TA-1 [10]. The thicknesses of the film specimens were measured by a digital micrometre (Mitutoyo, Japan) prior to mechanical testing to calculate the cross-sectional area. Each film specimen, initially sized at 20 x 80 mm, was positioned within the grips of the tensile testing device and tested at a crosshead speed of 1 mm/s and an initial grip separation of 50 mm. The mechanical tests were performed in triplicate. Elongation at break (in percentage) was calculated as the rasio of the change in length at break (ΔL) to the initial gauge length, while tensile strength was determined by dividing the maximum force at break by the original cross-sectional area.

2.5.2 Water vapour transmission rate (WVTR)

The WVTR of each film was determined following the method by He et al. (2021) and Hanani et al. (2019) [11,12]. The films were placed over the dry cup containing equal amounts of silica. The cups were put in a desiccator maintained at 75% relative humidity, and their weight was recorded every hour for a total of 8 hours [13]. Water vapour transmission rate (WVTR, kg/s·m2) was calculated as follows:

$$\text{WVTR} = \frac{\Delta W}{t}\ x\ A \qquad (1)$$

where ΔW, t, and A are the weight change, time difference, and film area, respectively

2.5.3 Antibacterial activity

The antibacterial activity was assessed using the agar diffusion method, as described by Yuan et al. (2016) [14]. *Aeromonas hydrophila* was used as the test bacterium, and the films were prepared according to the procedure described previously. Tryptone Soya Agar (TSA) plates were inoculated with 0.1 mL of bacterial suspension containing 10^5-10^6 CFU/mL. The prepared films were punched into 6 mm diameter discs and placed on top surface of the microbial cultures. The plates were then incubated at 30 °C for 24 h. The diameter of the inhibition zone (mm) was measured using a caliper, and all the tests were performed in triplicate.

3 Result and Discussion

3.1 Optimization Process Utilizing SLD

3.1.1 Count of Runs and Various Responses for the Optimization

Responses were individually and jointly optimized individually using Design-Expert software. The simplex lattice design enabled simultaneous of the main and interactive of HAE and ZnO-NPs on the selected responses. Table 2 presents the eight experimental runs generated by this design. Tensile strength, elongation at break (EB), water vapour transmission rate (WVTR), and antibacterial activity were considered as response (dependent) variables, and the run order was randomized to minimize systematic bias (Table 1).

Table 1. Simple lattice design run matrix used to assess the impacts of process variables and the values of experimental responses for gelatin-based edible film

Run Number **	**ZnO-NPs (g)**	**HAE (g)**	**Responses**			
			Tensile strength (MPa)	**EB (%)**	**WVTR (g $(m^2)^{-1}$ h^{-1})**	**Antibacterial activity (mm)**
1	0.15	0.55	12.53	185.74	9.63	11.57
2	0.3	0.4	5.8	306.34	6.77	16.13
3	0.1	0.6	14.26	163.87	10.58	11.61
4	0.25	0.45	6.75	241.66	7.42	17.05
5	0.3	0.4	5.64	274.66	7.1	15.61
6	0.1	0.6	13.77	179.62	11.24	13.52
7	0.2	0.5	9.62	181.71	8.11	15.09
8	0.2	0.5	10.54	192.87	9.08	14.82

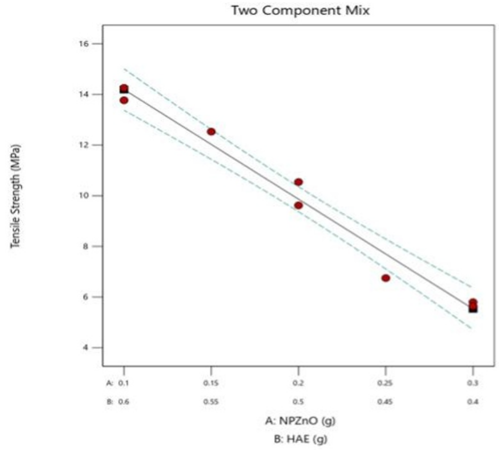
The sequence of run numbers was randomly generated using the Simplex Lattice Design.

3.1.2 Analysis of Variance (ANOVA) and Independent Variables' Effect

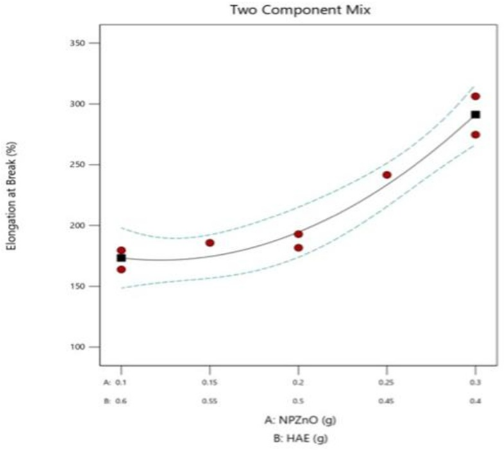
Figure 1 illustrates the interaction effects of ZnO-NPs and HAE on various response parameters, including tensile strength, elongation at break, water vapor transmission rate (WVTR), and antibacterial activity against *Aeromonas hydrophila*. Figure 1 A illustrates that an increase in HAE concentration led to a marked improvement in tensile strength, rising from 5.64 to 14.26 MPa, while elongation at break decreased from 306.34% to 163.87%. This improvement in mechanical performance is ascribedd to hydrogen bonding interactions among the hydroxyl (–OH) and carbonyl groups present in the flavonoid structure of anthocyanins and the –OH and amino (–NH) groups of gelatin. These molecule interactions improve the film matrix's mechanical integrity and functional performance by fortifying its network structure [15]. Additionally, the high specific surface area of ZnO-NPs facilitates effective interaction with the film matrix. ZnO-NPs serve as a reinforcing filler and improve the nanocomposite films' tensile strength and elastic modulus [8,16]. The decrease in elongation was mostly due to the physical segreration of polymer chains by ZnO nanoparticles, rather than the presence of strong chamical interactions. The addition of ZnO-NPs increased the intermolecular distance between gelatin chains, resulting in a loss in material cohesiveness (Figure 1B) [17].

The addition of ZnO-NPs and HAE lowered the film's water vapor transmission rate. The incorporation of ZnO-NPs into HAE dramatically decreased the water vapor permeability, likely attributable to the dimensions of the ZnO particle (Figure 1C) . The ZnO particles are affixed to the polymer matrix via hydrogen and electrostatic bonding, which diminishes the accessibility of free hydroxyl groups. Consequently, water molecules might not readily permeate the polymer matrix, thereby diminishing permeability. Consequently, when nanoparticles are present in the polymer matrix, water molecules must follow a more complex path than in pure polymer compositions, which reduces water vapour permeability. The nanoparticles block the passage of water molecules by filling up the gaps in the polymer layer [18,19]. Purghorbani et al. (2025) observed that the water vapor permeability of disposable biodegradable cups, including ZnO-NPs and red cabbage extract, diminished as the concentration of ZnO increased [18].

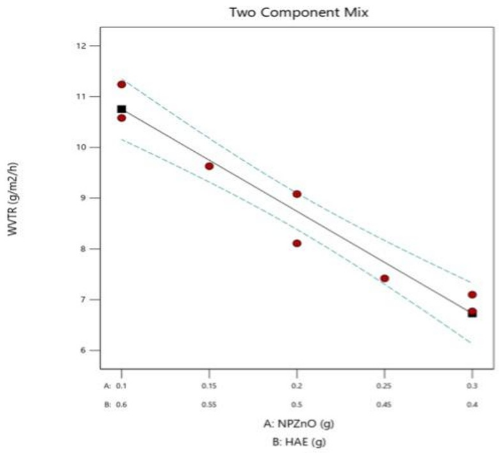
A



B



C



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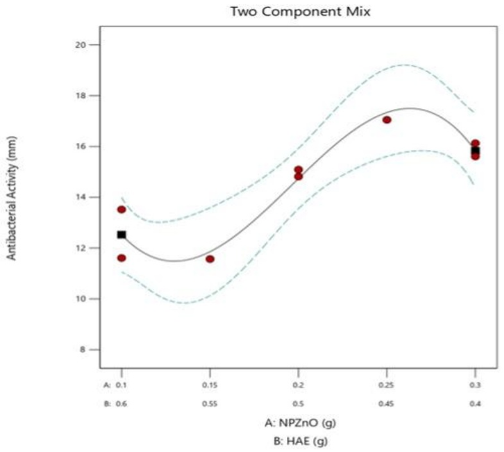

Fig. 1. Interaction impact of ZnO-NPs and HAE: (A) tensile strength; (B) elongation at break; (C) WVTR; (D) antibacterial activity

Figure 1A-C describe the mechanical and barrier properties of the films, whereas Figure 1D illustrates that the film exhibited a discernible inhibitory zone upon the addition of ZnO-NPs. The antimicrobial properties of ZnO-NPs have been attributed to two mechanisms: the generation of hydrogen peroxide on their surface and the effective inhibition of bacterial growth by Zn^{2+} released from the film matrix [20].

Table 2 indicates that the *p-values* for all response variables are below 0.05, demonstrating the viability of all models and indicating significant impacts of the components and their interactions. Table 3 additionally illustrates the primary effects of Factors A (ZnO-NPs), B (HAE), and AB (the interaction between ZnO-NPs and HAE). The high regression coefficients for TS ($R^2 = 0.99$), EB ($R^2 = 0.94$), WVTR ($R^2 = 0.95$), and antibacterial activity ($R^2 = 0.92$) indicate a strong correlation between the dependent and independent variables, suggesting a good fit. Moreover, the predicted R^2 values for all responses showed good agreement with the adjusted R^2 values, with differences below 0.2, indicating that the models and their fit statistics are appropriate for optimisation. In addition, all adequate precision values exceeded 4 (42.3431, 12.6528, 25.5774, 19.4037, and 10.3058 for TS, %EB, WVTR, and antibacterial activity, respectively), reflecting an acceptable signal-to-noise ratio. These results confirm that each response model is applicable for navigating the design space and can be reliably used in the optimisation process.

3.1.3 Optimization of the Multiple Responses

The target for the independent variables was set as "in range" during the optimisation process, and the importance level was set at 3. The research objectives determined the goals for every response (Table 3). To increase the water vapour barrier, the WVTR was reduced. Tensile strength and %EB were adjusted to "in range" because an edible film does not require rigidity. In contrast, antibacterial activity was set to maximize in order to maximize the inhibition of bacterial growth by the films.

Table 2. Outcomes of ANOVA and fitting statistics

Responses	p-value (p<0.05)	R^2	Adj. R^2	Pred. R^2	Adeq. Precision
TS (MPa)	<0.0001	0.99	0.99	0.98	42.34
EB (%)	0.0009	0.94	0.91	0.83	12.65
WVTR (g/m^2/h)	<0.0001	0.95	0.94	0.91	19.40
Antibacterial Activity (mm)	0.0113	0.92	0.86	0.66	10.30

Table 3. Parameters for the optimization procedure

Variable	Goal	Lower Limit	Upper Limit	Importance
A: ZnO-NPs	In range	0.1	0.3	+++
B: HAE	In range	0.4	0.5	+++
Tensile Strength	In range	5.64	14.25	+++
%EB	In range	163.87	306.34	+++
WVTR	Minimize	6.77	11.24	++++
Antibacterial Activity	Maximize	11.57	17.05	+++++

Using the Design Expert, two solutions were obtained, as shown in Table 4. Desirability values range between 0 and 1, with values closer to 1 indicating more optimal solutions. Among the tested formulations, solution no. 1 exhibited the highest desirability score of 0.969. Therefore, this formulation was selected as the optimal one, consisting of 0.286 g of ZnO-NPs and 0.414 g of HAE.

Table 4. Design Expert-13's solution to the optimal formula

No	ZnO-NPs (g)	HAE (g)	Desirability
1	0.286	0.414	0.969
2	0.100	0.600	0.142

3.2 Confirmation of the Optimum Formula

To confirm the Simplex Lattice Design's ideal formula, films were prepared on five replications with 0.286 g of ZnO-NPs and 0.414 g of HAE. The results of this experiment are presented in Table 5. The mean values were subsequently compared with the corresponding predictions from the Simplex Lattice Design, as presented in Table 6. All observed responses were found to lie within the 95% predicted interval (PI low to PI high), confirming good agreement between the experimental and predicted values. This finding indicates that the optimal formula was achieved through the utilisation of 0.286 g of ZnO-NPs and 0.414 g of HAE.

Table 5. Response data of confirmation formula

Run	TS (MPa)	EB (%)	WVTR (g/m^2/ h)	Antibacterial activity (mm)
1	16.21	234.16	7.31	17.74
2	11.36	255.28	7.55	17.79
3	12.72	141.89	7.39	20.43
4	11.17	147	7.08	18.62
5	15.93	130	7.15	16.61

Table 6. The result of the confirmation

Response	Prediction Value	95% PI Low	Data Mean	95% PI High
TS	13.48	12.80	13.48	14.15
%EB	171.69	143.91	181.67	199.47
WVTR	7.07	6.37	7.30	7.78
Antibacterial activity	17.05	15.39	18.24	18.71

4 Conclusion

In this research, we have successfully developed active gelatin-based films with enhanced multifunctional packaging properties. A simplex lattice design was applied to investigate the effects of Hibiscus x archeri Wats extract (HAE) and ZnO-NPs concentrations, together with key functional food-packaging properties, to identify the optimal formulation of antibacterial gelatin-based films. The optimal composition was found to be 0.286 g of ZnO-NPs and 0.414 g of HAE. Despite a reduction in tensile strength, the films maintained a safe threshold, specifically above 0.3 MPa. The inclusion of HAE and ZnO-NPs improved the elongation at break of the gelatin films. Additionally, incorporation of these components decreased the water vapor transmission rate (WVTR), indicating improved moisture barrier properties, and conferred observable antimicrobial activity against *A. hydrophila*, as shown by the formation of clear inhibition zones around the film discs.

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