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RESPONSE SURFACE METHODOLOGY (RSM): OPTIMISATION OF CASSAVA STARCH/XANTHAN GUM/ZINC OXIDE NANOPARTICLES COATING SOLUTION FOR BANANA SHELF LIFE EXTENSION

(Kaedah Rangsangan Permukaan: Pengoptimuman Larutan Salutan Tepung Ubi Kayu/Gum Xanthan/Nanopartikel Zink Oksida Untuk Meningkatkan Jangka Hayat Pisang)

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Abstract

Bananas' nutritional value contrasts with their short shelf life and requires preservation such as edible coatings. Polysaccharide-based coatings are advantageous due to their semi-permeable properties. Starch is renewable, but its retrogradation limitation can be addressed by adding gum with hydrocolloid properties. The addition of zinc oxide nanoparticles enhances antimicrobial properties and stability. However, there is limited research on the combination of starch, gum, and zinc oxide nanoparticles (ZnONPs). Thus, this research aimed to optimise edible coating solutions by varying cassava starch (CS) concentration (1.0-3.0%), xanthan gum (XG) concentration (0.5-1.5%), and ZnONPs concentration (0.5-1.5%) using response surface methodology (RSM). A central composite rotatable design (CCRD) of RSM has generated twenty (20) edible coating solution formulations. The edible coating solutions were prepared, and bananas (*Musa acuminate*) were dipped into the solutions. The responses were measured after 10 days of storage. The responses fit into a second-order polynomial model with a coefficient of determination (R^2) of 99.76% (percentage of weight loss) and 96.72% (firmness). The optimal concentration of the coating solution, which minimises weight loss and retains firmness was found to be 1.0% CS, 1.2% XG, and 0.5% ZnONPs. This research contributed valuable insights into developing an effective edible coating for extending the shelf life of perishable fruits.

Keywords: cassava starch/xanthan gum, zinc oxide nanoparticles, edible coating, banana, response surface methodology

Abstrak

Nutrisi pisang bertentangan dengan jangka hayatnya yang singkat dan memerlukan kaedah pengawetan seperti salutan yang boleh dimakan. Salutan berasaskan polisakarida menawarkan kelebihan dengan sifatnya separa telap. Kanji adalah bahan yang boleh diperbaharui, tetapi boleh berlaku proses retrogradasi dan ianya boleh diatasi dengan menambah gum yang mempunyai sifat hidrokoloid. Penambahan zink oksida nanopartikel meningkatkan sifat antimikrob dan kestabilan salutan. Walau bagaimanapun, kajian terdahulu adalah terhad mengenai gabungan kanji, gum, dan zink oksida nanopartikel (ZnONPs). Oleh itu, penyelidikan ini bertujuan untuk mengoptimumkan larutan salutan yang boleh dimakan untuk memanjangkan jangka hayat pisang dengan mengoptimumkan kepekatan tepung ubi kayu (CS) (1.0-3.0%), kepekatan gum xanthan (XG) (0.5-1.5%), dan kepekatan ZnONPs (0.5-1.5%) menggunakan Kaedah Rangsangan Permukaan (RSM). Dua puluh formulasi dihasilkan menggunakan reka bentuk komposit putaran tengah (CCRD), dan salutan diuji dengan merendam pisang (*Musa acuminate*) ke dalam salutan tersebut. Tindak balas diukur selepas 10 hari penyimpanan. Tindak balas yang sesuai dengan model polinomial kedua berkoefisien penentuan (R^2) sebanyak 99.76% (peratusan kehilangan berat) dan 96.72% (keteguhan). Kepekatan optimum salutan boleh dimakan ditentukan sebagai 1.0% CS, 1.2% XG, dan 0.5% ZnONPs. Kajian ini menyumbang dapatan yang mendalam untuk membangunkan salutan boleh dimakan yang berkesan untuk memanjangkan jangka hayat buah-buahan yang mudah rosak.

Kata kunci: tepung ubi kayu/gum xanthan, nanopartikel zink oksida, salutan boleh dimakan, pisang, kaedah rangsangan permukaan

Introduction

Bananas are elongated, curved fruits with a thick, yellow peel. The peel can be easily peeled off to reveal the soft, creamy flesh inside. The banana flesh is rich in carbohydrates, vitamins, and minerals. Bananas are composed of approximately 75% water, making them hydrating and refreshing [1]. Bananas are also low in fat and cholesterol-free, making them a healthy option for individuals looking to maintain a balanced diet. Nevertheless, bananas have a shelf life of five to seven days, depending on their ripeness when harvested. They are susceptible to rapid deterioration and spoilage [2]. The spoilage of bananas can cause the loss of important nutrients, reducing the fruit's overall nutritional value, which can lead to waste if not properly handled or stored.

To extend the shelf life and preserve the quality of bananas, edible coatings have been developed. Edible coatings are thin layers of edible materials applied to the surface of fruits and vegetables [3]. These coatings create a protective barrier that helps to reduce water loss, prevent microbial contamination, and maintain the colour and texture of the fruit [4]. The semi-permeable characteristic of the edible coating at the surface of the coated fruit helps reduce water loss [5]. The permeability of the coating regulates the exchange of gases and moisture between the fruit and its environment, creating a modified atmosphere that slows down the ripening process [6]. Microbial contamination could also be reduced as the edible coating acts as a physical barrier, preventing the entry of microorganisms onto the surface of the fruit [7].

A starch-based coating is one of the most studied edible coatings [8-10]. Starch is biodegradable, non-toxic, and readily available [5]. This starch-based edible coating can extend the shelf life of bananas by reducing water loss, slowing down the ripening process, and preventing microbial contamination [11]. However, starch has limitations as an edible fruit coating due to its moisture barrier properties, hydrophilicity, brittleness, lack of flexibility, and limited adhesion to the fruit surface [9, 12, 13]. To overcome these limitations, other materials such as chitosan [14, 15], gum [16, 17], and mineral oils [8, 18] can be incorporated into the edible coating formulation. These additional materials improve the moisture barrier properties and increase the flexibility and adhesion of the coating [16], further extending the shelf life of bananas. Microbial invasion also affects the bananas' shelf life. Therefore, the antimicrobial properties of the edible coating play a crucial role in preserving the quality and extending the shelf life of the fruit [19-21]. Incorporating antimicrobial agents can enhance the antimicrobial properties of the edible coating. Zinc Oxide Nanoparticles (ZnONPs) are one of the antimicrobial agents used to enhance the antimicrobial properties of the edible coating [22-24].

However, there is a lack of study on the optimisation of cassava starch (CS), xanthan gum (XG), and ZnONPs coating solutions for extending banana shelf life by using Response Surface Methodology (RSM), a statistical optimisation technique used to optimise factors, conditions, and processes [25]. In this study, the edible coating solutions containing CS, XG, and ZnONPs were coated to banana peels, and the optimised edible coating solution for extending the shelf life of bananas was identified. The desired outcomes of the edible coating solution in this study were determined by their minimum weight loss and maximum firmness.

Materials and Methods

Materials

Bananas (*Musa acuminate*) with a maturity index of 1 were obtained from Axes Agro Sdn. Bhd., Malaysia. The bananas were free from physical and microbiological appearance damage. They were washed with distilled water and dried at room temperature before use. CS (Cap Kapal ABC, Malaysia) and XG (Evachem, Malaysia) were obtained from the local market. The chemicals used were food-grade ZnONPs (US Research Nanomaterials Inc., USA) and glycerol (Sigma Aldrich, Germany). All substances were chemical grade and used without further purification.

Preparation of edible coating solutions

The edible coating solution was prepared based on the method described by Saekow et al. (2019) [26] with slight modifications. The edible coating solution comprised CS, XG, and ZnONPs. Firstly, the 1% (w/v) of CS was dissolved in distilled water at 90˚C and stirred for 30 minutes. Then, in another beaker, 0.5% (w/v) of XG was dissolved with 10% (v/v) glycerol at room temperature. After that, the CS solution and XG solution were mixed for 2 minutes. On the other hand, 0.5% (w/v) ZnONPs were dispersed in distilled water using a water bath sonicator (Starsonic 60, Switzerland) for 30 minutes. The ZnONPs solution was then mixed with the CS and XG mixture using a homogeniser (WiseTis, UK) for 15 minutes. The solution preparation was repeated but with the values generated by using the central composite rotatable design (CCRD) of RSM, as shown in Table 1.

Experimental design

The central composite rotatable design (CCRD) using Design Expert Version 13 RSM Software (Stat-Ease Inc., USA) was used to optimise the factors towards responses [27]. The factors selected were cassava starch (CS), xanthan gum (XG), and zinc oxide nanoparticles (ZnONPs). Meanwhile, the responses were the percentage of weight loss and firmness of the coated bananas *(Musa acuminate)* [28].

The factors and the levels of variation were determined referring to the previous and preliminary studies. The numeric factors and levels (low, high, and alpha) are shown in Table 1 [29]. The 20 edible coating solution formulations were generated with 6 central points, 8 factorial points, and 6 axial points, as shown in Table 2. The six central points show the repeatability of the method.

Table 1. The numeric factors and the four levels of each factor used in CCRD

Factor	Unit	Low	High	-α	+α
Cassava starch (CS)	%	1.00	3.00	0.32	3.68
Xanthan Gum (XG)	%	0.50	1.50	0.16	1.84
Zinc oxide nanoparticles (ZnONPs)	%	0.50	1.50	0.33	1.17

Application of edible coating solution on the peel of bananas

The bananas were coated with 20 formulations of edible coating solutions suggested by RSM software using the dipping method for 10 seconds. They were then hung at room temperature (25°C) until dry [30]. The coated bananas were then stored at temperatures of 29-33˚C with a relative humidity (ThermoPro) of 72-76%.

Evaluation of the effectiveness of the edible coating solutions

The effect (responses) of different formulations of coating solutions was evaluated on the 10th day after treatment. The responses investigated included the percentage of weight loss and firmness of the coated bananas.

Determination of percentage of weight loss

The weight loss of bananas was determined using a digital balance (Adam Equipment's PGW precision balance, UK). The bananas were weighed on day 0 (W_0). Then, the bananas were weighed again (W_1) on day 10 [31]. The weight loss (%) was calculated as follows:

$$\text{Weight loss (\%)} = \frac{W_0 - W_1}{W_0} \ x \ 100\% \qquad (1)$$

Determination of firmness

The firmness of bananas was determined using a texture analyser (Stable Micro Systems, England). The banana was penetrated to a depth of 5 mm from the peel using a 2mm cylindrical probe at a rate of 5 mmsec^{-1}. The measurement was recorded as the mean penetration force (g) [31] on day 10 for each treatment.

Table 2. Experimental design and experimental data obtained for the responses

Run	Space Type	Factor 1 A: CS %	Factor 2 B: XG %	Factor 3 C: ZnONPs %	Response 1 Weight loss (%) g	Response 2 Firmness g
1	Axial	0.32	1.00	1.00	19.03	137.11
2	Factorial	1.00	0.50	1.50	18.28	56.27
3	Factorial	3.00	1.50	1.50	20.38	20.54
4	Axial	2.00	0.16	1.00	21.27	82.72
5	Axial	2.00	1.00	0.16	17.37	53.46
6	Center	2.00	1.00	1.00	16.53	130.14
7	Factorial	1.00	1.50	0.50	21.52	71.26
8	Factorial	3.00	0.50	0.50	19.22	78.517
9	Center	2.00	1.00	1.00	16.68	156.21
10	Center	2.00	1.00	1.00	16.75	145.87
11	Factorial	1.00	1.50	1.50	18.26	124.65
12	Center	2.00	1.00	1.00	16.71	130.05
13	Axial	2.00	1.84	1.00	24.17	37.25
14	Factorial	1.00	0.50	0.50	18.91	90.41
15	Center	2.00	1.00	1.00	16.5	130.79
16	Center	2.00	1.00	1.00	16.49	143.57
17	Axial	3.68	1.00	1.00	20.57	60.02
18	Factorial	3.00	0.50	1.50	20.08	64.75
19	Factorial	3.00	1.50	0.50	22.28	10.19
20	Axial	2.00	1.00	1.84	15.75	43.25

Data analysis

The results of the responses were analysed by obtaining the coefficients of different models using a matrix approach with multiple linear regression to determine the relationship between the factors and responses from the mathematical model equation. A probability value (model significance) was used to assess the quality of the model and the calculation of the coefficient of determination (R^2) that measures the fitness of the regression model and the lack of fit. The regression analysis was used to determine the effects of variables in first-order, two-factor interaction, and second-order polynomial models. When the coefficient of determination (R^2) is close to 1 ($R^2 > 0.8\%$), the degree of correlation is high between actual and predicted values. The variability of terms in the regression equation for each response was determined using the analysis of variance (ANOVA) to analyse the interaction effects of the different factors. The significance of the model and terms was based on significant effects at the probability level of $p < 0.05$.

Optimisation and validation

The optimum values of the factors were determined using graphical and numerical optimisation. The contour plot and three-dimensional (3D) response surface plots were generated from the fitted model for each factor to better understand the interaction effects of the factors on the responses. The numerical optimisation of the responses was carried out using the desirability function approach where it targeted the desired factors. Meanwhile, the responses were set to achieve a minimum percentage of weight loss and maximum firmness. The validation of the model was performed by repeating the selected set of runs or factors at the end of CCRD. The variation between the predicted and actual responses must fall within the 95% prediction interval [32].

Results and Discussion

Weight loss (%)

The fit summary suggested that the quadratic model was the most suitable (p <0.05) to describe the effects of factors (CS, XG, and ZnONPs concentration) on the response (percentage of weight loss). The quadratic model also indicated an insignificant lack of fit (p >0.05) with a coefficient of determination (R^2) value of 0.9976. The 3D surface image below illustrates the interaction between CS and XG concentrations on the percentage weight loss of coated bananas under minimum conditions (Figure 1).

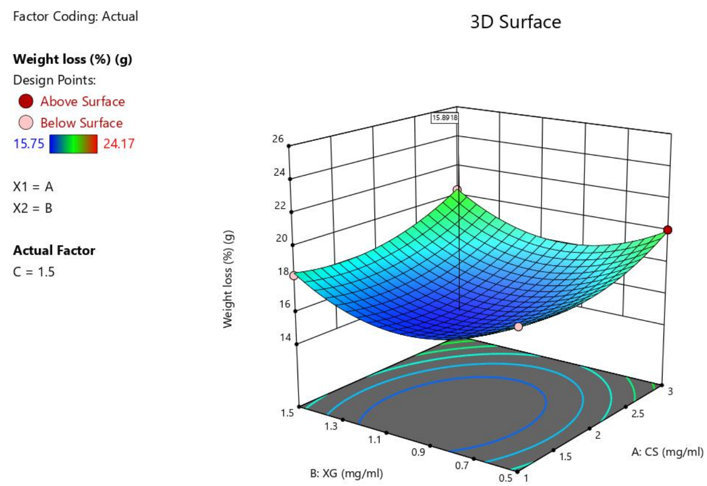

Figure 1. Response surface plot for a percentage of weight loss of coated banana under factors of CS, XG, and ZnONPs

The analysis of variance (ANOVA) is expressed in Table 3. A P-value less than 0.05 indicates the model terms are significant. In this study, the factors A, B, C, AC, BC, A^2, and B^2 are significant model terms. Meanwhile, AB and C^2 are considered as insignificant model terms. The model F-value of 424.78 implies that the model is significant. There is only a 0.01% chance that such a large F-value could occur due to noise. The lack of fit F value of 2.97 implies that the lack of fit is not significant relative to the pure error. There is a 12.84% chance that a lack of fit F-value this large could occur due to noise. A non-significant lack of fit is good as the model fits well.

In fit statistics, the R^2 value of 0.9976 agreed with the adjusted R^2 of 0.9950. Adequate Precision measures the signal-to-noise ratio and a ratio greater than 4 is desirable. In this study, the ratio of 72.891 indicates an adequate signal. This suggests that the model can capture the variation in data. The predictive model in terms of actual factors for percentage weight loss is indicated in Equation 2.

$$\text{Weight loss (\%)} = 16.6105 + 0.55503A + 0.792803B + -0.560488C + 0.35625AC + -0.67375BC + 1.12486A^2 + 2.15723B^2 \quad (2)$$

The edible coating composed of CS, XG, and ZnONPs demonstrated an effect in reducing weight loss in coated banana. The minimum weight loss percentage was achieved at 15.89% when CS, XG, and ZnONPs concentrations reached 1.77%, 1.03%, and 1.50%, respectively. It was observed that the percentage of weight loss initially increased with higher CS concentrations. However, the addition of XG concentration reduced the weight loss of the coated banana. Further increases in CS concentration led to an increase in weight loss, showing the attainment of an optimum point.

The weight loss results in textural changes and surface shrinkage that affects the shelf life of fruit [17]. In sum, the concentration of CS should be added moderately. Previous research indicates that CS functions as a semi-permeable barrier to moisture, reducing oxygen absorption, minimising carbon dioxide and ethylene emissions, preventing microbial attacks, and ultimately prolonging the shelf life of fruits [33]. Nevertheless, starch has limitations that can lead to retrogradation [9]. To overcome this, a combination of starch with other polysaccharides was employed to complement its properties. XG was introduced into the coating solution to address CS limitations and enhance the characteristics of the edible coating. With its hydrocolloid properties, XG improved the mechanical strength, flexibility, elasticity, and tear resistance of the coating, preventing retrogradation [17]. Moreover, being a colloid contributed to the coating's barrier properties against moisture, oxygen, and carbon dioxide [32], effectively hindering microbial growth.

The addition of XG concentration should also be moderate, as an increase in XG concentration can lead to higher weight loss. This result is supported by the study of Tazo et al. which observed that excessive concentration of gum Arabic in tomato coatings resulted in higher weight loss. XG and starch are hydrophilic polysaccharides with polar –OH groups and C-O covalent bonds in their structure. They interact through hydrogen and covalent bonds. This bonding plays an important role in forming a stable coating of macromolecules to improve the coating properties [29]. Therefore, the coating solution produced would slow down the weight loss of bananas.

Moreover, weight loss in bananas may result from transpiration, respiration, and ethylene release during the ripening process. The epidermis of a banana is stomatous [34]. According to Brat et al. [7], depending on the storage conditions, anywhere from 0% to 50% of the banana fruit skin's stomata remain open postharvest. Thus, transpiration remains after the banana is harvested. Bananas also undergo respiration easily, observing oxygen and releasing carbon dioxide through their peel. The ripening process naturally causes bananas to lose weight over time.

Firmness

The fit summary suggested that the quadratic model was the most suitable ($p < 0.05$) to describe the effects of factors (CS, XG, and ZnONPs concentration) on the response (firmness). Additionally, the quadratic model exhibited an insignificant lack of fit ($p > 0.05$) with a coefficient of determination (R^2) value of 0.9672. The 3D surface image below illustrates the interaction between XG and ZnONPs concentrations on the firmness of coated bananas under maximum conditions (Figure 2).

Table 3. Analysis of variance (ANOVA) for response of percentage weight loss

Source	**F-value**	**p-value**	
Model	424.78	< 0.0001	significant
A-CS	157.05	< 0.0001	
B-XG	320.43	< 0.0001	
C-ZnONP	160.15	< 0.0001	
AB	2.77	0.1272	
AC	37.90	0.0001	
BC	135.56	< 0.0001	
A^2	680.69	< 0.0001	
B^2	2503.51	< 0.0001	
C^2	0.23	0.6422	
Residual			
Lack of Fit	2.97	0.1284	not significant
Pure Error			
Cor Total			

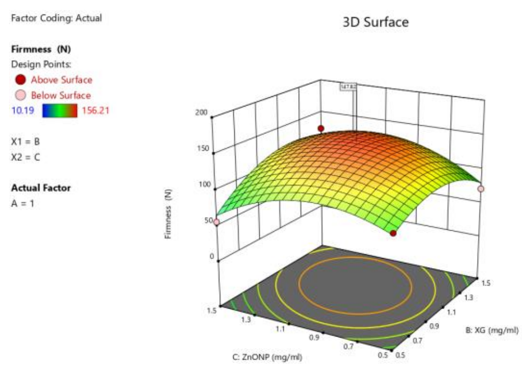

Figure 2. Response surface plot for firmness of coated banana under the factors of XG and ZnONPs

The analysis of variance (ANOVA) is expressed in Table 4. A P-value less than 0.05 indicates that model terms are significant. In this study, factors A, B, AB, BC, A^2, B^2, and C^2 are significant model terms, while C and AC are considered insignificant model terms. The model F-value of 32.75 implies that the model is significant. There is only a 0.01% chance that such an F-value could occur due to noise. The lack of fit F-value of 1.19 implies that the lack of fit is not significant relative to the pure error, with a 42.64% chance that a lack of fit F-value this large could occur due to noise. A non-significant lack of fit is good, indicating that the model fits well.

In fit statistics, the R^2 value of 0.9672 agreed with the adjusted R^2 of 0.9377. Adequate Precision measures the signal-to-noise ratio and a ratio greater than 4 is desirable. In this study, the ratio of 17.197 indicates an adequate signal. This suggests that the model can capture the variation in data. The predictive model in terms of actual factors for percentage weight loss is indicated in Equation 3.

Table 4. Analysis of variance (ANOVA) for a response of firmness

Source	F-value	p-value	
Model	32.75	< 0.0001	significant
A-CS	50.47	< 0.0001	
B-XG	11.09	0.0076	
C-ZnONP	0.00	0.9752	
AB	25.35	0.0005	
AC	0.50	0.4966	
BC	12.07	0.0060	
A^2	23.40	0.0007	
B^2	88.28	< 0.0001	
C^2	115.99	< 0.0001	
Residual			
Lack of Fit	1.19	0.4264	not significant
Pure Error			
Cor Total			

$$\text{Firmness} = 139.442 - 21.8383A - 10.235B - 20.2209AB + 13.9559BC - 14.4763A^2 - 28.1164B^2 - 32.2282C^2 \quad (3)$$

Firmness is associated with water content and the metabolic changes that occur in fruits, making it an important aspect of consumer acceptability [17]. The edible coating composed of CS, XG, and ZnONPs shows an effect on the firmness of the coated banana. The maximum firmness of the coated banana was 147.82 g when CS, XG, and ZnONPs concentrations reached 1.00%, 1.07%, and 1.02%, respectively. It was observed that the firmness increased when the XG concentration increased. The addition of ZnONP concentration also increased the firmness of the coated banana. However, further increases in XG concentration led to a decrease in firmness, showing the attainment of an optimum point.

From the results, the ZnONPs concentration must be added in moderate amounts to maximise the firmness of the coated banana. Previous research indicates that the combination of cassava starch (CS) and xanthan gum (XG) alone is insufficient as an effective coating and requires an antimicrobial agent [35]. ZnONPs serve as this agent by providing antimicrobial properties through their ability to generate reactive oxygen species (ROS) such as hydroxyl radicals, superoxide ions, and hydrogen peroxide, which are toxic to microorganisms. This interaction leads to the disruption of microbial cell walls and membranes, inhibiting their growth and proliferation [36]. Additionally, ZnONPs enhance the adhesion of the coating to fruits, ensuring a uniform and stable application. Their nano-sized structure reduces pore size in the coating, improving its barrier properties and overall stability [37]. The moderate amount of CS and XG also shows a slow decrease in firmness over time. These results were supported by Hernandez-Guerrero et al. (2020) who found that mangoes coated with 2% fruit starch exhibited greater firmness compared to untreated mangoes. By applying the coating solution, the firmness of the banana decreased slowly due to the slow degradation of the cell structure. This slow degradation of the cell structure is due to the slow hydrolysis process that occurs in the banana. The hydrolysis process hydrolyses the pectin and starch of the banana in the presence of water [29].

Optimisation and validation

The goal of formulating the edible coating solution was to minimise the percentage weight loss and maximise the firmness of the coated banana after 10 days of storage. The predicted responses were determined at 19.08% (weight loss) and 100.17 g (firmness). The predicted optimal concentrations for the factors were 1.00% CS, 1.20% XG, and 0.50% ZnONPs, with a desirability of 0.97. To validate this prediction, the experiment was run in triplicate. The measured percentage weight loss and firmness were very close to the predicted values, confirming the accuracy of the proposed quadratic model.

Conclusion

The optimisation of the CS (1.0%), XG (1.2%), and ZnONPs (0.5%) edible coating solution was successfully performed and demonstrated. The significant minimum percentage weight loss and maximum firmness were found to be 19.08% and 100.17 g, respectively, which are very close to the predicted values after validation, confirming the accuracy of the proposed quadratic model. The overall findings proposed that the formulated edible coating solution has the potential to extend the shelf life of perishable bananas.

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