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Optimization of agar and glycerol concentration in the manufacture of edible film

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Abstract

Optimization of the formulation in the manufacture of edible film is necessary to obtain the optimum concentration of base materials and plasticizer. The research was aimed to determine the optimum concentration of agar and glycerol in the manufacture of agar-based edible film. The experiment was conducted using central composite design with three levels and five center points. The middle value for each treatment was 3% for agar and 10% for glycerol. The lower and upper limits for each treatment were 2% and 4% for agar, and 6% and 14% for glycerol. The response values of the edible film were thickness, moisture, solubility and mechanical properties. The verification of the optimum formulation is repeated three times. The optimum formulation verification data were analyzed using T test. The results showed that the optimum formulation to manufacture edible film was 2.75% agar and 6.80% glycerol with 0.98 of the desirability values. The verification of the optimum formulation on laboratory scale produced edible film was better than the results of the simulation program in terms of thickness, moisture content, solubility, and Young modulus.

Introduction

Development of food product packaging is always performed to acquire the environmentally friendly packaging and is not harmful to human health. Edible film is one of the appropriate packaging materials for food products because it has some properties that can overcome some of the problems experienced by the food product such as oxidation, migration of moisture, reduction of aroma and flavor, and microbial contamination (Bourtoom, 2008a; Pavlath and Orts, 2009; Avena-Bustillos and McHugh, 2012). Edible film is environmentally friendly and safe for human health since it is made from natural ingredients. Besides edible film also has some advantages compared to packaging made of polymers with basic ingredients of petroleum, the material is available, biodegradable, and can be made from a single polymer or a combination of polymeric materials (Espitia *et al.*, 2014).

The characteristics of edible film were determined by the type of base material and plasticizer. Edible films with base materials such as proteins and polysaccharide have good protective properties against oxygen, carbon dioxide and lipid, but it is not effective to hold moisture (Wang *et al.,* 2007; Bourtoom, 2008a). One of the marine polysaccharide used as base material for edible film preparation is agar. The advantage of being the base material for edible film production is its ability to form a strong gel with a melting point well above the initial gelation temperature (Lacroix and Tien, 2005). Agar-based edible film has protective properties against moisture similar to other hydrocolloid edible film, and it has good mechanical properties, transparent, clean, homogeneous and easily handled (Phan *et al.*, 2005).

Glycerol is the most widely used plasticizer in edible film making, because it has the stability and compatibility with the hydrophilic biopolymer chain (Cevera *et al*., 2004). Since glycerol is hydrophilic, its use as plasticizer may increase the water vapor permeability of the edible film (Gontard *et al.*, 1993). Glycerol as a plasticizer is an important variable affecting the mechanical properties of the film due to the effect of plasticizers on the formation of a polymer matrix (Mali *et al*., 2005; Maran *et al*., 2013a). The use of glycerol as a plasticizer in the manufacture of edible film is better than sorbitol, because the glycerol-pasticized edible film is more flexible and not brittle. Similarly, the mechanical properties and appearance of edible film with glycerol plasticizer do not change during storage (Oses *et al*., 2009). However, mechanical resistance of edible film with plasticizer of sorbitol is better than plasticizer of

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glycerol and polyethylene glycol (Bourtoom, 2008b).

In addition, the concentration of base materials and plasticizers also determines the characteristics of edible film. Film thickness, oxygen and water vapor permeability of the edible film are influenced by the concentration of the base materials and plasticizers (Gounga *et al*., 2007; Arham *et al.*, 2016). Increasing concentration of plasticizer in the manufacture of edible film will decrease the tensile strength, but increase the elongation, water vapor permeability and solubility (Bourtoom, 2008b).

Optimization of the formulation in the manufacture of edible film is necessary to obtain the optimum concentration of base materials and plasticizers. Optimization of the formulation using response surface methodology has been carried out on edible film with a basis of whey protein, tapioca starch, corn starch, chitosan, tapioca starch and chitosan composite, xanthan gum and tapioca starch composite (Ozdemir and Floros, 2008; Chillo *et al.*, 2008; Arismendi *et al.*, 2013; Maran *et al.*, 2013a; Maran *et al*., 2013b; Azevedo *et al*., 2014).

This research investigated agar-based edible film formulated with the glycerol plasticizer using response surface methodology. The research was aimed to determine the optimum concentration of agar and glycerol in the manufacture of agar-based edible film.

Materials and Methods

Materials

Commercial agar powder (gel strength 900±40 g/cm^2, particle size 80 mesh, moisture 20%, and yellowish color) was purchased from Golden Agar Sentosa (Surabaya, Indonesia). Other reagents for film formulations i.e. glycerol (food grade) and distilled water were obtained from a local chemist store.

Film preparation

Films were prepared according to the method of Sousa *et al.* (2010) with a slight modification. Agar powder was dissolved in distilled water at 95ºC for at least 30 mins under stirring. The concentration of an agar solution was 1, 2, and 3% (w/v). Glycerol was then added into the agar solution at a concentration of 5, 10, and 15% (w/w). The solution was kept at 95ºC and stirred for 10 mins, after which the solution was cooled down to 75ºC prior to cast in glass petri dishes (10 cm diameters). The solution provided 0.20 ml/cm^2 and was evenly spread over the dish. The dish was placed on a leveled surface to obtain film of homogeneous thickness, and subsequently dried in an oven at 50ºC for 24 hr. Dried film was peeled off from the dishes and stored in desiccators at room temperature.

Thickness

The thickness of the films was measured using a digital micrometer with an accuracy of ±1 µm (Krisbow KW06-85). Nine thickness measurements were randomly taken on each film sample, and a mean value was used in the calculation.

Solubility

The solubility of film samples was determined according to the method of Ahmad *et al.* (2012). Solubility test was done using film samples of 3 x 2 cm in size. Samples were dried at 105ºC for 24 hr and weighed (W1). Each sample was then inserted into a 50 ml centrifuge tube containing 10 ml of distilled water. Samples were stored for 24 hr at room temperature and stirred slowly on a periodic basis using a shaker. The solution was filtered, and the residues remained on the filter paper were dried in an oven at 105ºC for 24 hr, after which the samples were weighed to determine the dry matter soluble in water (W2). Solubility was calculated using the formula:

$$\text{Solubility (\%)} = (W1 - W2)/ W1 \times 100 \quad (1)$$

The mechanical properties of the film

Tensile strength, elongation, and Young's modulus were measured using a Testometric (BS EN ISO 527-3, 1996). Film sample was cut into square pieces with a width of 25 mm and a length of 50 mm. The film pieces fitted to handle the testometric with the distance between the handle of 50 mm. The handle top actuated with a speed of 5 mm/min. Measurements were made at room temperature. The result of the measurement of tensile strength, elongation, and Young's modulus is shown in the form of numbers and charts. Tensile strength and Young's modulus are expressed in MPa, and elongation is expressed in percentage.

Experimental design and statistical analysis

The experiment was conducted using response surface methodology to central composite design (CCD). The treatment has been applied consisting of two factors, three levels and five replications of the center point. The applied factors were the base material and plasticizer concentration. Middle value for base material and plasticizer in the manufacture of edible film was 3% for agar and 10% for glycerol. The lower and upper limits for each treatment were 2% and 4% for agar, and 6% and 14% for glycerol.

The response values of the edible film were thickness, moisture, solubility and mechanical properties. Data analysis was performed using Design Expert 9 software (trial version). The verification of the optimum formulation suggested by the program simulation is repeated three times. The optimum formulation verification data were analyzed using T test.

Results and Discussion

Film thickness

The film thickness depends on the base material and its composition (Abugoch *et al*., 2011). The result of a statistical analysis showed that the fit model to describe the treatment effect of agar and glycerol concentration for the film thickness is a quadratic model with p value 0.04%, showing that the chance of a model error is less than 5%. The lack of fit value of the quadratic model of film thickness response was not significantly different (0.11), which indicates that the model is suitable to describe the treatment effect of agar and glycerol concentration to the film thickness. Bas and Boyaci (2007) reported that the model will be considered appropriate if lack of fit value model is not significantly different at the level of specificity α. The actual quadratic equation of the film thickness response with $R^2 = 0.94$ is as follows.

$$Y = 166.64 + 4.29X_1 \;\; 18.45X_2 + 0.07X_1^2 + 1.00X_2^2 + 0.81X_1X_2 \quad (2)$$

Description: Y = response
X_1 = agar concentration (%)
X_2 = glycerol concentration (%)

Statistical analysis showed that factors that significantly affect the film thickness response were the agar concentration (X_1), the glycerol concentration (X_2) and the square of the glycerol concentration (X_2^2). While the square of the agar concentration (x_1^2) and the interaction between treatments (X_1X_2) did not significantly affect the film thickness response. Positive values of coefficient X_1 in the equation 2 showed that increasing the concentration of the agar linearly increase the film thickness. The addition of agar concentration will increase the amount of dissolved solids resulting in the increase of the thickness of the edible film. Increasing the thickness due to the effect of an increase base material concentration has previously been reported by Bhuvaneshwari *et al.* (2011) in the manufacture of chitosan film. The response surface plot of the agar and glycerol concentration effect

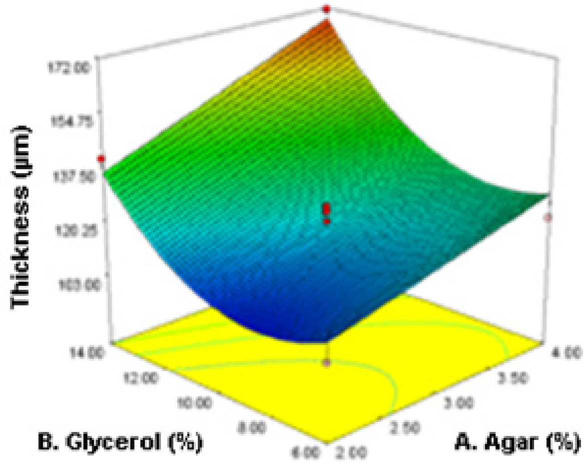

Figure 1. Response surface plot of the film thickness

against the film thickness is presented in Figure 1.

The film thickness tends to decrease up to the middle value of glycerol concentration, but increase sharply above the middle value (Figure 1). High concentration of glycerol that causes a strong bond between the agar and glycerol compound has an impact on the increase in the film thickness. The increasing concentrations of glycerol will also increase the viscosity of which increased the film thickness (Bertuzzi *et al.*, 2007). Similarly, Vieira *et al.* (2011) have reported that the addition of high concentrations of plasticizers in the manufacture of edible film will lead to an increase in water diffusion in the polymer. Increased water diffusion in the polymer will have an impact on increasing the film thickness.

Moisture content

Moisture content is one of the important properties of edible film because the moisture content influences the mechanical properties of the film. The fit model analysis of the response of the moisture content of edible film gives a quadratic model with a coefficient of determination (R^2) 0.92 and a lack of fit of 0.11. Mathematical response model of moisture content of the edible film is presented by equation 3.

$$Y = 30.44 \;\; 5.04X_1 \;\; 0.26X_2 + 0.73X_1^2 + 0.08X_2^2 \;\; 0.17X_1X2 \quad (3)$$

The statistical analysis showed that the concentration of base material and plasticizer had a significant effect on the moisture content of edible film ($p<0.05$). Polynomial equation of the moisture content response presented in equation 3 showed a negative correlation for all treatment variables and their interactions, but the treatment quadratic was positively correlated. The negative correlation shown by the interaction between treatments indicated that

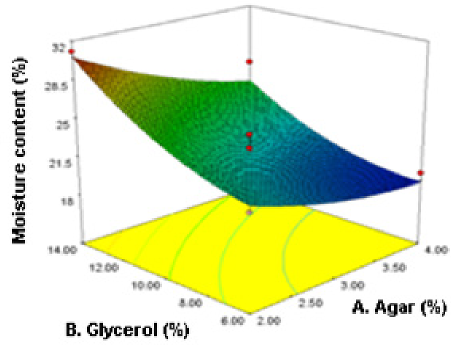

Figure 2. Response surface plot of the moisture content

the interaction between agar and glycerol produces an antagonistic effect on the moisture content. The response surface graph plot of the moisture content is presented in Figure 2.

Increasing the agar concentration tends to lower the moisture content of edible film, whereas the increase in glycerol concentration is directly proportional to the increase in the moisture content of edible film (Figure 2). This is consistent with the results of Ghasemlou (2011) that the moisture content of the hydrocolloid film increases with increasing concentrations of plasticizers. Similarly, Huri and Nisa (2014) reported that the increasing glycerol concentration in the manufacture of edible film lead to an increase in the -OH groups derived from glycerol, so that the amount of water that is bound to increase. In addition, glycerol is also a humectant as its hydroxyl groups can form hydrogen bonds in water. Humectants added onto a product to serve as a water binder are able to increase the compactness of bonding matrix (hydrogen bonds) which will increase the moisture content of the product.

Solubility

Measurement of solubility aimed to determine the ability of edible film to dissolve and the ability of edible film to retain water when used as food packaging. The use of edible film as a food packaging that has high water activity and acts as a food product protector; the low of solubility is one of the most important requirements (Atef *et al*., 2015).

The analysis of the edible film solubility based on the agar and glycerol concentration produced a linear model with $R^2 = 0.84$ and a lack of fit of 0.18. The mathematical model of solubility response is presented in equation 4.

$$Y = 42.23\ \ 4.93X_1 + 0.63X_2 \quad (4)$$

The statistical analysis showed that the concentration of base material and plasticizer had a significant effect on the solubility of edible film ($p<0.05$). The linear regression model of the solubility response indicates that the solubility of the edible film tends to decrease with increasing the agar concentration, while the solubility increases with increasing the concentration of the glycerol.

Reduction in solubility of edible film with increasing of agar concentrations is caused by water-insoluble particles that are contained in the agar namely agaropectin containing sulphate and pyruvate. Thus, if the agar concentration is increased then the impact of the insoluble particles on the solubility of the edible film is also increasing. On the contrary, increasing the concentration of glycerol tends to increase the solubility of edible film as glycerol is hydrophilic. This is consistent with the report Nugroho *et al.* (2013), that the addition of the hydrophilic component in the edible film will lead to an increase in solubility. Besides, the addition of glycerol can lower intermolecular forces and internal hydrogen bonds in molecular bonding of edible film, thus improving the solubility (Zulferiyenni *et al.,* 2014).

Mechanical properties

The mechanical properties of edible film such as tensile strength, elongation, and Young's modulus are very important, because the packaging materials must have good mechanical strength to maintain its integrity during handling and storage. The mechanical properties of the film depend on the material compositions and environmental conditions (Wihodo and Moraru, 2013).

Glycerol as a plasticizer in the manufacture of edible film is an important variable affecting the mechanical properties of the film, due to the effect of plasticizer in the polymer matrix (Maran *et al*., 2013a). The mechanical properties responses analysis showed that the agar and glycerol concentrations significantly affected the tensile strength and Young's modulus, but did not significantly affect the elongation. The quadratic model of the tensile strength response is presented in Equation 5, with the R^2 and lack of fit value of 0.88 and 0.84, respectively. The high value of the lack of fit showed that the model is good to describe the effect of independence variables on the response.

$$Y = (-109.82) + 40.66X_1 + 14.11X_2\ \ 5.22X_1^2\ \ 0.62X_2^2\ \ 0.35\ X_1X_2 \quad (5)$$

The statistical analysis showed that the concentration of base material had a significant effect on the tensile strength of edible film ($p <0.05$), but the plasticizer concentration had no significant effect (p>

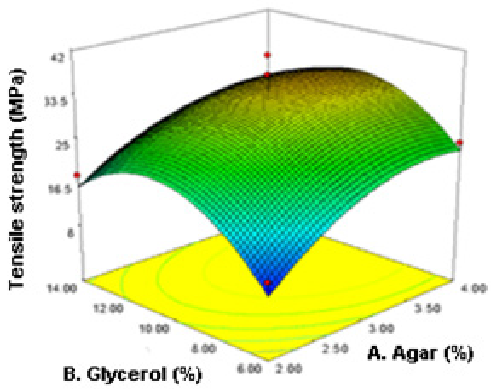

Figure 3. Response surface plot of tensile strength

0.05). The quadratic model of equation showed that the tensile strength response is positively correlated with increased concentrations of agar and glycerol, but negatively correlated with treatment squared and interaction between treatments. The response surface plots of the tensile strength are presented in Figure 3.

Figure 3 showed that increasing the agar concentration tends to increase the tensile strength of edible film. The influence of base material concentrations for tensile strength of edible film has been reported by Handito (2011). This author argues that increasing the concentration of carrageenan in the manufacture of edible film will increase the molecular interaction of carrageenan with glycerol in the matrix film, which causes the matrix film that formed become more solid and compact, so that larger force is required to break the film.

The increase of glycerol concentrations above the middle value tends to lower the tensile strength of edible film (Figure 3). This is consistent with the report of Mali *et al*. (2005) who found a decrease in the tensile strength of the edible film with glycerol as a plasticizer due to the hygroscopic characteristic of glycerol, which tends to provide additional water into the matrix of the film.

Young's modulus or modulus of elasticity is a basic rule of the film stiffness. The high value of Young's modulus indicates a high degree of film stiffness (Mali *et al*., 2005). The analysis of the Young's modulus response generated a quadratic model with R^2 and lack of fit of 0.86 and 0.71, respectively. It showed that 86% of the Young's modulus value is determined by the agar and glycerol concentration factors, and 14% is determined by other factors. The statistical analysis showed that the concentration of base material had a significant effect on the Young's modulus of edible film ($p<0.05$), but the plasticizer concentration had no significant effect ($p>0.05$). Quadratic model equations of Young's modulus response are as follows.

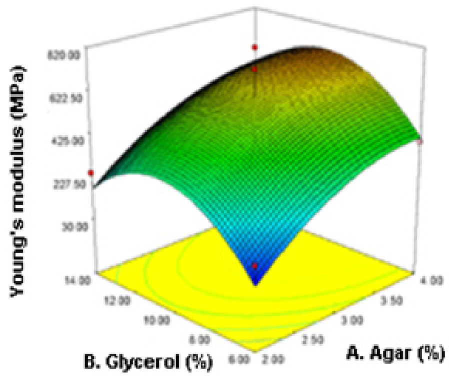

Figure 4. Response surface plot of Young's modulus

$$Y = (-2373.15) + 749.37X_1 + 305.51X_2 - 93.89X_1^2 - 14.42X_2^2 - 0.81X_1X_2 \quad (6)$$

Equation 6 showed that the response of Young's modulus value is positively correlated with the increase concentrations of agar and glycerol at below the middle concentration value, and negatively correlated with the squared of agar and glycerol concentration and interaction between the treatments. This indicates that the use of agar and glycerol in high concentrations tends to decrease the modulus of elasticity value of the edible film.

Mali *et al*. (2005) have reported that increase concentrations of plasticizers resulted in a decline in value of the modulus of elasticity which produces more flexible edible films. The graph plots of the response surface of the Young's modulus values indicate that the optimum value of the Young's modulus of the edible film is around the upper limit of the agar concentration and at approximately the middle value for the glycerol concentration (Figure 4).

Optimization of formulation

Optimization of the edible film formulation was based on the criteria of thickness, moisture content, solubility and tensile strength with target values were 115μm, 20%, 33%, and 23 MPa, respectively, while other responses were set in the range of the lower and upper limit. Each criterion was given a weighting of 5 (highest) for the thickness, moisture content, solubility and a tensile strength response, while other responses were given a weighting of 3 (moderate). This weighting was done based on the importance of each characteristic of edible film for the needs as food packaging, where the thickness, moisture content, solubility and tensile strength greatly determine the ability of the edible film to protect the overlaid material and stability of the edible film during storage. Simulation program Design Expert generates an optimum solution formulation for the manufacture

Table 1. Values of prediction and verification of the agar based edible film characteristics

Characteristics	Prediction	Verification
Thickness (μm)	115.00[b]	98.48±8.43[a]
Moisture content (%)	20.92[a]	19.74±0.39[a]
Solubility (%)	32.96[b]	31.60±1.42[a]
Tensile strength (MPa)	23.00[b]	21.36±2.78[a]
Elongation (%)	18.77[b]	16.03±5.44[a]
Young's Modulus (MPa)	373.34[a]	284.14±62.88[b]

Values were mean ± standard deviation. Values with different superscript letters on the same rows were significantly on confidence level 95%.

of the agar-based edible film that is 2.75% agar and 6.80% glycerol concentrations with the desirability value of 0.98. The optimum formulation provides responses or characteristics of edible film to be as presented in Table 1.

Verification of optimum formulation

Verification or validation was aimed to prove that the optimum formulation solution suggested by the program provides characteristics of edible film in accordance with the predicted values. Verification of the optimum formulation is done by producing edible films on a laboratory scale following the formulation suggested by the program. The verification of the optimum formulation produces edible films with characteristics as shown in Table 1.

Statistical analysis using T test showed that significant difference between the experimental and predicted value. Nevertheless, the edible film produced in the verification process showed that the values of the thickness, moisture content, solubility and the Young's modulus was lower than predicted values. These results suggest that the edible film produced from the verification process has better characteristics as compared to the characteristics of the edible film generated from the simulation program. Thus the optimum formulation suggested by the program can be applied to manufacture agar-based edible film.

Conclusion

The concentration of agar and glycerol determines the characteristics of the agar-based edible film. The use of agar and glycerol in the production of edible film has an optimum concentration 2.75% and 6.80%, respectively, with a desirability value of 0.98. The verification of the optimum formulation a laboratory scale produced edible film having better characteristics than those of the edible film produced from the simulation program. The thickness, moisture content, solubility, and Young's modulus values of the agar-based edible film produced in the verification process were lower than the values resulted from the simulation program.

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