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Response Surface Methodology on development and formulation optimisation of chicken skin gelatine film blended with carboxymethyl cellulose as affected by varying plasticiser concentrations

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Abstract

The present work aims to optimise chicken skin gelatine/carboxymethyl cellulose (CMC) blended film formulation at varying concentrations of CMC and plasticiser (glycerol). The influence of CMC and plasticiser concentrations on the mechanical (tensile strength, TS and elongation at break, EAB) and physical (water vapour permeability, WVP) properties of chicken skin gelatine films were studied using central composite design (CCD), a full factorial design with all combinations of the factors at two levels (high, +1, and low, −1 levels), with the centre points (coded level 0) repeated thrice. An optimised formulation obtained as a proportional mixture of CMC (3%) and glycerol (0.78%), with tensile strength of 0.08 MPa, elongation at break of 167.57 and water vapour permeability of 6.08×10^{-9} g $m^{-1}s^{-1}Pa^{-1}$. A formulation with 3% CMC and 0.78% glycerol yielded high TS and EAB, but lower WVP, which is desirable for production of food packaging. This novel research offers the packaging industry an alternative source for producing biodegradable food packaging films which are more cost-effective and at the same time reduce environmental problems.

Introduction

The stability of plastics and their potentials for various applications, including widespread use as disposable items, were anticipated early, but the problems associated with waste management and plastic rubbish were not (Thompson *et al*., 2009). Because of these problems, there is increasing interest in biodegradable polymers from renewable sources (Kolybaba *et al*., 2003). Packaging materials based on biodegradable biopolymers guarantee biodegradability and environmental compatibility (Debeaufort *et al*., 1998). Biodegradable films are usually based on polysaccharides, proteins and lipids, which are generally non-toxic, and may act as effective barriers to oxygen and carbon dioxide. Thus, they can reduce the environmental wastes and at the same time can be used as a protective coating to maintain food quality (Silva-Weiss, 2012).

Among all types of biodegradable films, protein-based films have the most attractive properties. Such films have impressive gas barrier properties as compared to those prepared from lipids and polysaccharides (Wittaya, 2012). Proteins are suitable for use as films and coating polymers, and may be derived from whey, soybeans, gluten or gelatine. Due to its abundance and biodegradability properties, gelatine has become one of protein sources that has strong potential to be used in packaging and film formation. The use of gelatine in the preparation of edible films or coating has been extensively studied (Lacey and Montero, 2010; Hanani *et al*., 2012; Fakhreddin *et al*., 2013). Several safety concerns and religious issues concerning commercial gelatine have become the main reasons for exploring different types of collagen from different animal sources such as chicken feet (Lim *et al*., 2001), chicken skin (Sarbon *et al*., 2013) and fish skin as alternative substitutes of raw materials for the production of gelatine (Cheow *et al*., 2007; Rosli and Sarbon, 2015). Because of that, there is an urgent need to find alternative source that can replace currently available gelatine as an additional option to meet consumers' needs. Waste by-products from the fisheries industry, such as fish skin, bone, fins and scales (Cheow *et al*., 2007; Jeya Shakila *et al*., 2012), and the poultry industry, such as chicken skin (Sarbon *et al*., 2013) may be potential sources to replace other mammalian sources of gelatine.

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Studies on the production and characterisation of fish gelatine films are very recent, and findings showed that fish gelatine exhibited excellent film forming properties. Studies have also shown that edible films produced by combining selected biopolymers have better properties, as compared to the films made of just one component. Due to its excellent viscosity, biocompatibility and availability, carboxymethyl cellulose (CMC) has been used for blending with gelatine. Moreover, the addition of glycerol as plasticiser agent is necessary to improve film flexibility.

Optimisation by using Response Surface Methodology (RSM) is one of the methods to find the best alternative from a specified set of alternatives (Sanaei *et al*., 2013). RSM is a technique that relates product treatment to the outputs through collection of mathematical and statistical modelling. It also establishes a regression equation to describe inter-relations between input parameters and product properties (Cho *et al*., 2004). A study by Denavi *et al*. (2009) found that the analysis performed using RSM yielded 60°C and 60% relative humidity (RH) under laboratory conditions for soy protein isolate (LSPI) and 70°C and 30% RH for commercial soy protein isolate (CSPI) as optimal drying conditions to obtain soy protein films with good mechanical properties and low solubility. Another study by Ozdemir and Floros (2008) on optimisation of film formulation using RSM proved that an optimum mixture of protein (0.53), sorbitol (0.38), beeswax (0.08) and potassium sorbate (0.01) yielded an edible film with minimum stickiness, water vapour permeability (WVP) of ≤ 9 gmm $m^2 h^{-1} kPa^{-1}$, water solubility of $\geq$ 39%, and appearance score of ≥ 80.

Extraction and characterisation of chicken skin gelatine have been successfully conducted previously by Sarbon *et al*. (2013). However, no study has been conducted on developing an edible film from chicken skin gelatine/CMC blends. Therefore, the present work aims to develop chicken skin gelatine/CMC blended film formulation which was optimised by RSM in order to obtain films with high tensile strength, high elongation at break, and low water vapour permeability. The effects of different proportions of CMC as blended material and glycerol as plasticiser on the mechanical (tensile strength and elongation at break) and physical (water vapour permeability) properties of the chicken skin gelatine film were also studied.

Materials and methods

Materials

The chicken skin used in the present work was purchased from TD Poultry Sdn. Bhd. (Terengganu, Malaysia). Glycerol (LR grade). Carboxymethyl cellulose, sodium hydroxide, sulphuric acid and citric acid were purchased from Sigma-Aldrich (UK).

Sample preparation

The visible fat on the chicken skin was removed mechanically and rinsed thoroughly in excessive water. The skins (2-3 cm in size) were freeze-dried and then grinded before being defatted using the Soxhlet method (AOAC, 2006).

Gelatine extraction

Extraction of chicken skin gelatine was conducted following the method developed by Sarbon *et al*. (2013) using acid–alkaline pre-treatment. The defatted chicken skin was grinded and soaked in sodium hydroxide (0.15%, w/v), sulphuric acid (0.15%, v/v) and citric acid (0.7%, w/v) solutions consecutively. Each soaking treatment was repeated three times. The skins were then subjected to a final wash with distilled water in order to remove any residual matter. The solution mixture was extracted in distilled water at controlled temperature within the range of 40–50°C overnight. The clear extract was filtered, concentrated by evaporation under vacuum, and freeze-dried. The dry matter obtained is referred to as ‘gelatine powder’.

Development of chicken skin gelatine films

Gelatine film was prepared using the casting technique as described by Jahit *et al*. (2016), with slight modifications. In general, the filmogenic solution was prepared according to the formulation generated by the RSM software. For film preparation, 3 g of chicken skin gelatine was dispersed in 50 mL distilled water while 0 g, 1.5 or 3g of CMC was dispersed in 50 mL distilled water separately. Both solutions were then mixed together, followed by the addition of glycerol as plasticiser with 0.5 mL, 1 mL, or 1.5 mL per formulations. The solutions were heated with continuous stirring by magnetic stirrer at 45 ± 5°C for 60 ± 5 min on a heating mantle, and then left at room temperature for another 5 min to allow the bubbles to dissipate prior to pouring. Each film forming solution was then poured onto Petri dish and oven dried (45°C) to complete dryness. Dried films were then taken out from the Petri dish for TS, EAB and WVP determinations.

Optimisation of chicken skin gelatine/CMC films with different glycerol concentrations by Response Surface Methodology

The experimental designs, statistical analysis and regression model were generated by RSM with the help of Design Expert Software Version 6 (Stat-Ease Inc., USA). In the present work, the Central Composite Design (CCD) was employed. Two independent variables, namely CMC quantity (g) and glycerol quantity (g), were chosen. The factorial portion was a full factorial design with all combinations of the factors at two levels (high, +1, and low, −1 levels) and the centre point (coded level 0), which was the midpoint between the high and low levels repeated three times. The axial or star points were for all but one factor was set at level 0, and one factor was set at the outer value corresponding to an α value of 2. The response functions measured were tensile strength (TS), water vapour permeability (WVP) and elongation at break (EAB) of the film.

Tensile strength (TS) and elongation at break (EAB)

The tensile strength (TS) and elongation at break (EAB) of the film were determined using a texture analyser (TA.TX Plus, Stable Micro System, UK) following ASTM method 0882-97 (ASTM, 1997). A 20 mm × 70 mm film strip was prepared by using a cutting blade and was placed onto grip pairs of AT/G probe attached to the texture analyser with 10 kg load cell. The initial gap between the up and down parts of the grip was set to 30 mm. The film strips were stretched by moving at a headspace of 50 mm/min until broken. The TS (MPa) was calculated using the following equation:

$$\text{Tensile strength (MPa)} = \frac{F_{max}\ (\text{N})}{\text{A}\ (\text{mm}^2)}$$

Where F_{max} was max load (N) needed to pull the sample apart, and A was the cross sectional area (mm^2) of the film sample.

Meanwhile, the EAB (%) was calculated using the following equation:

$$\text{Elongation at break (\%)} = \frac{l_{max}}{l_0} \times 100$$

Where l_{max} was the film elongation (mm) at the moment of rupture and l_0 was the initial grip length (mm) of the film sample.

Water vapour permeability (WVP)

The water vapour permeability (WVP) was determined by using a modified ASTM method following Jahit *et al*. (2016). The films were sealed onto a cup containing silica gel (0% RH) with silicone vacuum grease and a rubber band to hold the films in place. The cups with films were then weighed for an initial weight. The cups were then placed in desiccators containing distilled water at 30°C. The cups were weighed at 1 h intervals over a period of 7 h. Three films were used for the WVP determination, and measurements were conducted in triplicate. The calculation of WVP was determined according to McHugh *et al*. (1993) using the following equation:

$$\text{WVP (g m}^{-1}\text{s}^{-1}\text{Pa}^{-1}) = \text{w x A}^{-1}\text{t}^{-1}\ \Delta\ \text{Pa}^{-1}$$

Where w was the weight gain of the cup (g), x was the film thickness (m), A was the exposed area of film (m^2), t was the time of gain (s), ΔPa^{-1} was the vapour pressure difference across the film (Pa).

Statistical analysis

To optimise the best formulation for chicken skin film, the RSM design expert software (Stat-Ease Inc., USA) was used. Results were expressed as mean (± SD) for each analysis. Regression analysis was performed on the data obtained. Analysis of Variance (ANOVA), multiple comparison test, and all statistical analyses were performed using Minitab® 14 for Windows (Minitab Inc., State College, PA, USA).

Results and discussion

Optimisation of chicken skin gelatine/CMC blended film with different glycerol concentrations in terms of tensile strength (TS), elongation at break (EAB) and water vapour permeability (WVP) by response surface methodology (RSM)

Following the Central Composite Design (CCD), 13 experimental runs were performed to study the individual and interactive effects of two independent variables, namely CMC (A) and glycerol (B), on the mechanical (tensile strength, TS and elongation at break, EAB) and physical properties (water vapour permeability, WVP) of the gelatine based biodegradable films as shown in Table 1.

The TS of chicken skin gelatine blended film at different CMC and glycerol concentrations from these 13 runs ranged from 0.004 MPa to 0.148 MPa. The TS of film increased with increasing CMC but decreased with increasing glycerol. The increased amount of CMC increased the TS of gelatine based film due to the formation of intermolecular interaction between hydroxyl group of gelatine and carboxyl group of CMC. This was similar to a study reported by Tongdeesoontorn *et al*. (2011). Meanwhile, as glycerol incorporation increased, the TS were markedly reduced for blended films as glycerol enhanced molecular mobility by acting as a lubricant between the polymer chains (Chen and Zhang, 2005). Otherwise, the TS of chicken skin gelatine/CMC blended were lower in terms of comparison values, such as 1.28 – 25.03 MPa for sago starch-gelatine blended film (Al-Hassan and Norziah, 2012), 40.26 – 59.40 MPa for cuttlefish skin gelatine-chitosan blended film (Jridi *et al*., 2014) and 12.4 – 59 MPa for gelatine-gellan blended film (Yeon *et al*., 2004). The difference in TS might be due to the different types of blended material and also concentrations that would give a different result on mechanical properties. This is because different materials have different chemical structures and thus different intermolecular interactions related to the strength of film.

Table 1. Optimisation of chicken skin gelatine/CMC blended film with different glycerol concentrations in terms of tensile strength (TS), elongation at break (EAB) and water vapour permeability (WVP) by Response Surface Methodology (RSM)

Runs	CMC (g)	Glycerol (mL)	Tensile strength (MPa)	Water vapour permeability	Elongation at break (%)
1	0	0.5	0.0088	7.91119E-09	242.33
2	3	0.5	0.1480	4.74672E-09	151.33
3	0	1.5	0.0042	1.42401E-08	296.67
4	3	1.5	0.0720	9.49343E-09	172.67
5	0	1	0.0053	1.3449E-08	257.00
6	3	1	0.0542	7.12007E-09	172.33
7	1.5	0.5	0.0822	7.12007E-09	152.00
8	1.5	1.5	0.0130	1.18668E-08	209.33
9	1.5	1	0.0130	9.49343E-09	160.00
10	1.5	1	0.0293	1.02845E-08	212.67
11	1.5	1	0.0157	8.70231E-09	187.00
12	1.5	1	0.0190	1.18668E-08	187.00
13	1.5	1	0.0212	8.70231E-09	190.67

Meanwhile, the EAB of these films ranged from 151% to 296%. The incorporation of CMC markedly reduced the EAB of the films while glycerol increased the EAB of the films. The incorporation of CMC to chicken skin gelatine film would produce an intermolecular interaction between carboxyl group of CMC and hydroxyl group of gelatine (Tongdeesoontorn *et al*., 2011). This strong interaction would reduce the flexibility of chicken skin gelatine/CMC blended film. The opposite is true for glycerol, which would have enhanced molecular mobility by acting as a lubricant between the polymer chains. This plasticisation effect is useful to impart film flexibility and thus increase the elongation of films (Tong *et al*., 2008). Otherwise, the EAB values were higher when compared with values such as 5.53% - 102.1% for sago starch-gelatine blended film (Al-Hassan and Norziah, 2012), 1.26% - 4.76% for cuttlefish skin gelatine-chitosan blended film (Jridi *et al*., 2014) and 9% - 41% for gelatine-gellan blended film (Yeon *et al*., 2004). The difference in EAB might be due to the concentrations and types of plasticiser used and also the blended material added which resulted from the strength of intermolecular interaction between each material and also the effectiveness of lubricant effect.

The WVP of chicken skin gelatine film blended with CMC with different concentrations of glycerol from this 13 runs ranged from 7.911 × 10^{-9} g m^{-1} $s^{-1}Pa^{-1}$ to 1.424 × 10^{-8} g $m^{-1}s^{-1}Pa^{-1}$. The differences in WVP of blended films are also influenced by the concentrations of CMC and plasticiser incorporated into the film. The WVP values decreased with increasing amount of CMC. Increasing CMC content reduced the WVP thus resulted in an improvement of the barrier properties of these films, in terms of the hydrophilic characteristics of the matrix. The addition of CMC could introduce a twisted path for the water molecule to pass through (Kristo and Biliaderis, 2007). According to Ghanbarzadeh *et al*., (2010), at a low content of filler, CMC probably disperses well in the starch matrix and blocks water vapour transmission. Meanwhile, the WVP value increased with increasing amount of glycerol. The addition of glycerol increased film hydrophilicity and polymer chain mobility due to the plasticisation effect of polyol, which increased the diffusivity of water molecules in the film matrix (Tong *et al*., 2008).

Analysis for tensile strength (TS) of chicken skin gelatine/CMC blended film with different glycerol concentrations

Model of summary statistics of TS for chicken skin gelatine/CMC blended film with different glycerol concentrations

The multiple regression analysis technique included in the RSM was performed to determine all the coefficient of linear (A and B), quadratic (A^2 and B^2) two factor interaction (AB, A^2B and AB^2) terms to fit a full response surface model for the responses. In the present work, the model suggested for the TS was quadratic, similar to a study on soy protein based film by Nandane and Jain (2015), chitosan/cassava/gelatine blended film by Zhong and Xia (2008) and influence of glycerol and chitosan on tapioca starch based film by Chillo *et al*. (2008).

Table 2. Analysis of Variance (ANOVA) after choosing significant model for tensile strength of chicken skin/CMC/glycerol blended film

Source	Sum of Squares	DF	Mean Square	F-Value	Prob. > F	
Model	0.02083977	7	0.00297711	94.90221258	< 0.0001	significant
A	0.001193161	1	0.001193161	38.03475306	0.0016	
B	0.002395497	1	0.002395497	76.36194984	0.0003	
A^2	0.000298052	1	0.000298052	9.501107323	0.0274	
B^2	0.002197116	1	0.002197116	70.03810758	0.0004	
AB	0.001272135	1	0.001272135	40.55221904	0.0014	
A_2B	0.000278095	1	0.000278095	8.864921177	0.0309	
AB^2	0.000995541	1	0.000995541	31.73514875	0.0024	
Residual	0.000156851	5	3.13703E-05			
Lack of Fit	2.37067E-07	1	2.37067E-07	0.006054805	0.9417	not significant
Pure Error	0.000156614	4	3.91536E-05			
Cor Total	0.020996621	12				
R^2	0.992529681					
Adj R^2	0.982071235					
Pred R^2	0.987797302					
Adeq Precision	32.73602561					

Analysis of Variance (ANOVA) for tensile strength (TS) of chicken skin gelatine/CMC blended film with different glycerol concentration

Analysis of Variance (ANOVA) of the response surface quadratic model for TS is shown in Table 2. There was no model reduction as the F-value was the highest when all models included compared with reducing model. Furthermore, comparing with reducing model, the p-value of lack of fit also was the highest with all models included, confirming that with all model included more accurate to be used for prediction. The results showed that the model was significant at 95% confidence level ($p < 0.05$). This indicated that the quadratic model could explain a high percentage of variability in the observed data. Table 2 also shows that the F-value (94.9) and the “Prob. > F” value for the model were less than 0.05, which indicated that the model was significant. There was only a 0.01% chance that a "Model F-Value" this large could occur due to noise. In addition, the lack of fit test was used to predict the fitness of the model. The "Lack of Fit F-value" of 0.006 shown in Table 2 implies that the lack of fit was not significant. There was a 94.17% chance that a "Lack of Fit F-value" this large could occur due to noise. This was desirable as we would want a model that fits. Thus, the model was fitted to determine the optimum CMC and glycerol content in blended gelatine based film.

Based on the obtained results, the coefficient of determination (R^2) value was 0.9925, which was reasonably close to 1, hence acceptable. This R^2 value implied that about 99.25% of the experimental results could be explained by the fitted model over the range of factors tested for the TS of chicken skin gelatine/CMC blended film at different glycerol concentrations. The predicted R^2 was in reasonable agreement with the adjusted R^2. The adjusted R^2 value is particularly useful when comparing models with a different number of terms. Adequate precision compares the range of the predicted values at the design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination (Idris *et al*., 2006). In this case, the value of adequate precision was 32.736, which was well above 4.

The ANOVA results demonstrated that the linear model terms of CMC (A) and glycerol (B) had a significant ($p < 0.05$) effect on the TS of chicken skin gelatine/CMC blended film. Besides, the quadratic terms (A^2, B^2) and interaction terms (AB, A^2B, AB^2) also showed a significant ($p < 0.05$) effect on the TS of chicken skin gelatine/CMC/glycerol blended film model.

Response surface plots and effects of factors on tensile strength (TS) of chicken skin gelatine/CMC blended film with different glycerol concentrations

The TS’s and the response variable’s (Y) model equation of chicken skin gelatine/CMC/glycerol blended film obtained was derived using the regression coefficient on linear and interaction terms to fit a full response surface model. The best explanatory model according to the model’s regression analysis was given as follows:

$$Y = + 0.020 + 0.024A - 0.035B + 0.010A^2 + 0.028B^2 - 0.018AB + 0.014A^2B + 0.027AB^2$$

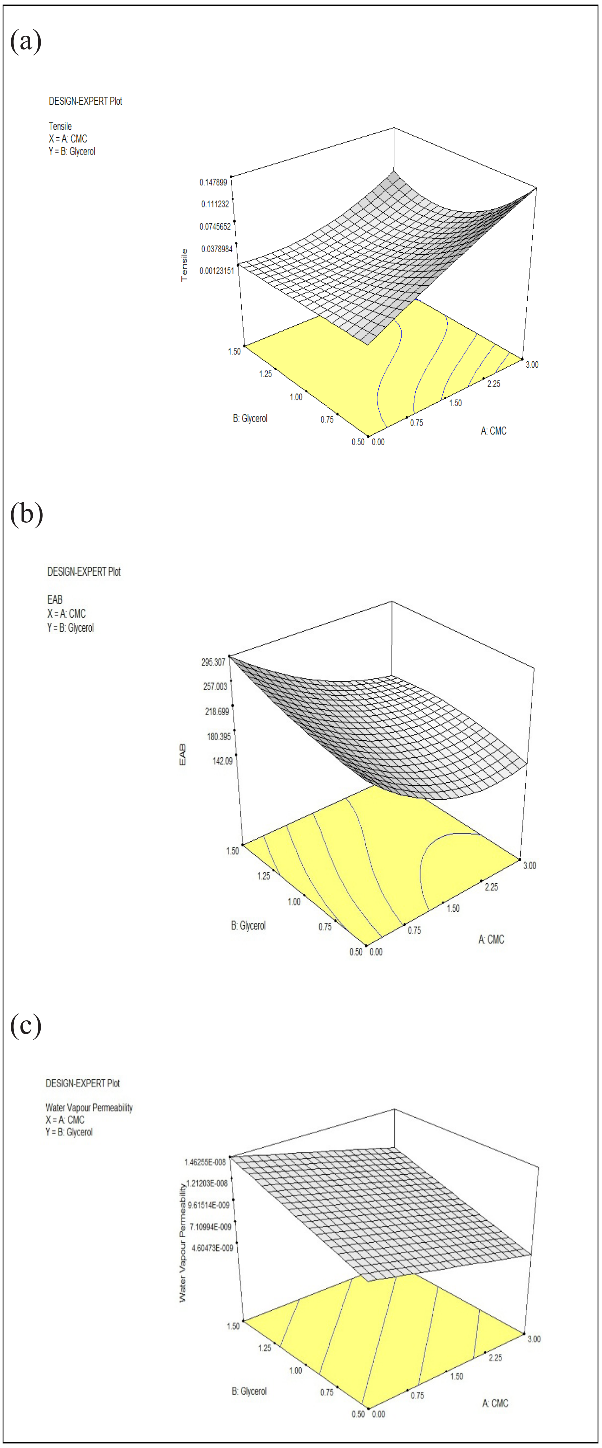

Figure 1. Response surface graph for (a) tensile strength (b) elongation at break (c) water vapour permeability of chicken skin gelatine/CMC/glycerol blended film

Three-dimensional (3D) response surface was developed to understand the interaction effect between all the factors by evaluating two variables at a time on the TS of chicken skin gelatine/CMC/glycerol blended film. Figure 1(a) shows the interactive effect of CMC and glycerol on the TS of chicken skin gelatine/CMC/glycerol blended film. Surface plot of Figure 1(a) shows that an increase in glycerol decreased the TS of film. The low molecular weight of glycerol favours the reduction of intermolecular forces along polymer chains, thus increasing film flexibility while decreasing the barrier properties of films (Pongjanyakul and Puttipipatkhachorn, 2007). It would also decrease the intramolecular hydrogen bonding in the film network (Pongjanyakul and Puttipipatkhachorn, 2007). This finding was similar with that reported by Chillo *et al*. (2008) who observed that the highest TS values of the composite films were obtained at the highest concentrations of chitosan (1%) and at the lowest concentrations of glycerol (0.5%). CMC content also had an impact on the TS of blended film. Surface plot of Figure 1(a) shows that increasing the CMC would increase the TS of film. This is in agreement with Ghanbarzadeh *et al*., (2010) who found an increase in the TS with increasing CMC content in edible modified starch/CMC film. This was probably due to the interfacial interaction between the matrix and filler (Ghanbarzadeh *et al*., 2010).

Analysis for elongation at break (EAB) of chicken skin gelatine/CMC blended film with different glycerol concentrations

Model of summary statistics for EAB of chicken skin gelatine/CMC blended film with different glycerol concentrations

In the present work, the model suggested for the EAB response was quadratic. This was similar to studies on EAB of soy protein based film by Nandane and Jain (2015) and chitosan/cassava/gelatine blended film by Zhong and Xia (2008).

Analysis of variance (ANOVA) for elongation at break (EAB) of chicken skin gelatine/CMC blended film with different glycerol concentrations

The Analysis of Variance (ANOVA) of the response surface quadratic model for EAB is shown in Table 3. There was no model reduction as the *F*-value was the highest when all models included. The *p*-value of lack of fit was also the highest, confirming that all models were accurate for use in the prediction. The results showed that the model was significant ($p < 0.05$) with the quadratic model, indicating that quadratic model could explain a high percentage of the variability in the observed data. Table 3 shows that the *F*-value (10.59) and the "Prob. > *F*" value were less than 0.05 which indicated that the model was significant and desirable, and that the terms in the model had a significant effect on the response. There was only a 0.97% chance that a "Model F-Value" this large could occur due to noise. In addition, the lack of fit test was used to predict the fitness of the model. The "Lack of Fit *F*-value"

Table 3. Analysis of Variance (ANOVA) after choosing significant model for elongation at break of chicken skin/CMC/glycerol blended film

Source	Sum of Squares	DF	Mean Square	*F*-Value	Prob. > *F*	
Model	21369.20	6	3561.53	14.56	0.0024	significant
A	3584.23	1	3584.23	14.65	0.0087	
B	1643.55	1	1643.55	6.72	0.0411	
A^2	2881.60	1	2881.60	11.78	0.0139	
AB	272.25	1	272.25	1.11	0.3321	
A2B	126.74	1	126.74	0.52	0.4987	
AB^2	173.78	1	173.78	0.71	0.4316	
Residual	1467.76	6	244.63			
Lack of Fit	67.62	2	33.81	0.097	0.9100	not significant
Pure Error	1400.14	4	350.03			
Cor Total	22836.96	12				
R^2	0.9357					
Adj R^2	0.87151					
Pred R^2	0.8955					
Adeq Precision	12.663					

of 0.12 shown in Table 3 implies that the lack of fit was not significant. There was a 74.40% chance that a "Lack of Fit *F*-value" this large could occur due to noise. Thus, the model was fitted to determine the optimum CMC and glycerol content in blended gelatine based film.

In addition, the coefficient of determination (R^2) value was 0.9368 and reasonably close to 1, hence acceptable. This R^2 value implied that about 93.68% of the experimental results could be explained by the fitted model over the range of factors tested for the EAB of chicken skin gelatine/CMC/glycerol film blended. The predicted R^2 was in reasonable agreement with the adjusted R^2. The value of ratio adequate precision was 10.905 which indicated an adequate signal; thus, this model could be used to navigate the design space.

The ANOVA results demonstrated that the linear model terms of A (CMC) and B (glycerol) had a significant ($p < 0.05$) effect on the EAB of chicken skin gelatine/CMC/glycerol blended film. Besides, the quadratic terms (A^2, B^2) and interaction terms (AB, A^2B, AB^2) also showed significant ($p < 0.05$) effect on the EAB of chicken skin gelatine/CMC/glycerol blended film model.

Response surface plots and effects of factors on elongation at break (EAB) of chicken skin gelatine/CMC blended film with different glycerol concentrations

The model equation for EAB and the response variable (Y) of chicken skin gelatine/CMC/glycerol blended film obtained was derived using the regression coefficient on linear and interaction terms to fit a full response surface model. The best explanatory model according to model's regression analysis was given as follows:

$$Y = +186.38 - 42.33A + 28.67B + 31.01A^2 - 2.99B^2 - 8.25AB - 9.75A^2B - 11.42AB^2$$

Three-dimensional (3D) response surface was developed to understand the interaction effect between all the factors by evaluating two variables at a time on the EAB of chicken skin gelatine/CMC/glycerol blended film. Figure 1(b) shows the interactive effect of CMC and glycerol on the EAB of chicken skin gelatine/CMC/glycerol blended film. Surface plot of figure 1(b) shows that an increase in glycerol increased the EAB of the film while an increase in CMC decreased the EAB of the film. This result is in agreement with that of Jost *et al*. (2014) who found that a significant increase in EAB was correlated with an increasing glycerol concentration. Glycerol as plasticiser will reduce the intermolecular forces and intramolecular hydrogen bonding along polymer chains, thus increasing the film flexibility (Pongjanyakul and Puttipipatkhachorn, 2007).

Analysis for water vapour permeability (WVP) of chicken skin gelatine/CMC blended film with different glycerol concentrations

Model of summary statistics for WVP of chicken skin gelatine/CMC blended film with different glycerol concentrations

In the present work, the model suggested for the WVP response was linear, which was dissimilar to the findings of a study by Wang *et al*. (2008) in which WVP values for WPI films had a significant quadratic relationship with both corn oil levels and pH.

Table 4. Analysis of Variance (ANOVA) after choosing significant model for water vapour permeability of chicken skin/CMC/glycerol blended film

Source	Sum of Squares	DF	Mean Square	F-Value	Prob. > F	
Model	7.55211E-17	2	3.77605E-17	31.7113313	< 0.0001	significant
A	3.37966E-17	1	3.37966E-17	28.38241841	0.0003	
B	4.17245E-17	1	4.17245E-17	35.04024418	0.0001	
Residual	1.19076E-17	10	1.19076E-18			
Lack of Fit	4.89779E-18	6	8.16298E-19	0.465804723	0.8075	not significant
Pure Error	7.00979E-18	4	1.75245E-18			
Cor Total	8.74286E-17	12				
R^2	0.86380227					
Adj R^2	0.836562724					
Pred R^2	0.778396386					
Adeq Precision	19.11621613					

Analysis of variance (ANOVA) for water vapour permeability (WVP) of chicken skin gelatine/CMC blended film with different concentrations of glycerol

Analysis of Variance (ANOVA) of the response surface linear model for the WVP is shown in Table 4. A model reduction was done to reduce the insignificant terms of model. The *F*-value of the model reductions was higher after backward elimination of non-significant terms, as compared to the unreduced model. The p-value of lack of fit was also the highest, confirming that the reduced model was more accurate for prediction. The result showed that the model was significant ($p < 0.05$) with linear model, indicating that linear model could explain a high percentage of the variability in the observed data. Table 4 shows the *F*-value (37.71) and the "Prob. > *F*" value for the model which were less than 0.05. This was desirable as it indicated that the terms in the model had a significant effect on the response. There was only a 0.01% chance that a "Model *F*-Value" this large could occur due to noise. In addition, the lack of fit test was used to predict the fitness of the model. The "Lack of Fit *F*-value" of 0.47 shown in Table 4 implied that the lack of fit was not significant. There was a 80.75% chance that a "Lack of Fit *F*-value" this large could occur due to noise. Thus, the model was fitted to determine the optimum CMC and glycerol content in blended gelatine based film.

Based on the results presented in Table 4, the coefficient of determination (R^2) value was 0.8638, which was reasonably close to 1, hence acceptable. This R^2 value implied that about 86.38% of the experimental results could be explained by the fitted model over the range of factors tested for the WVP of chicken skin gelatine/CMC/glycerol film blended. The predicted R^2 was in reasonable agreement with the adjusted R^2. The value of ratio adequate precision was 19.116, indicating an adequate signal. Therefore, this model could be used to navigate the design space. The ANOVA results demonstrated that the linear model terms of A (CMC) and B (glycerol) had a significant ($p < 0.05$) effect on WVP of chicken skin gelatine/CMC/glycerol blended film.

Response surface plots and effects of factors on water vapour permeability (WVP) of chicken skin gelatine/CMC blended film with different concentrations of glycerol

The model equation for the WVP and the response variable (Y) of chicken skin gelatine/CMC/glycerol blended film obtained was derived using the regression coefficient on linear and interaction terms to fit a full response surface model. The best explanatory model according to model's regression analysis was given as follows:

$$Y = +9.615E\text{-}009 - 2.373E\text{-}009A + 2.637E\text{-}009B$$

A three-dimensional (3D) response surface was developed to understand the interaction effect between all the factors by evaluating two variables at a time on the WVP of chicken skin gelatine/CMC/glycerol blended film. shows the interactive effect of CMC and glycerol on the WVP of chicken skin gelatine/CMC/glycerol blended film. Surface plot of Figure 1(c) shows that the increase in glycerol increased the WVP of film while the increase in CMC decreased the WVP of the film. This behaviour could be explained by the size of the plasticiser. Glycerol with a molecular weight of 92.1 Da is a smaller molecule, with only three hydroxyl groups. Therefore, glycerol can effectively hinder intermolecular and intramolecular bonding in the network, thereby increasing the free volume and the permeability of oxygen and water vapour. This finding is similar to a study by Jost *et al*. (2014) who stated that glycerol significantly influenced the WVP of alginate films,

Table 5. Recommended solution for optimal formulation of chicken skin gelatine film blended with carboxymethyl cellulose (CMC) and plasticised with glycerol

No	CMC (%)	Glycerol (%)	Tensile (MPa)	Elongation at break (%)	Water Vapour Permeability (g $m^{-1}s^{-1}Pa^{-1}$)	Desirability	
1	0.73	0.50	6.81	190.85	8.20×10^{-5}	0.37	
2	3.00	0.77	12.43	165.903	6.05×10^{-5}	0.36	Selected
3	3.00	1.45	9.736	190.84	8.25×10^{-5}	0.32	

as increasing glycerol concentration led to a higher transmission rate of alginate films. From this linear equation, there was no interaction found between the effect of glycerol on water vapour and effect of CMC on water vapour.

Optimisation of chicken skin gelatine film's tensile strength (TS), elongation at break (EAB), and water vapour permeability (WVP)

Optimal response conditions

The desirability profiles for the optimum conditions suggested by the RSM are shown in Table 3.4. The selected optimisation formulation was depending on the desirability value from each solution suggested. The desirability values showed that the selected conditions was suitable for optimum responses (TS, EAB, WVP) of chicken skin gelatine/CMC blended film. Therefore, the suggested film formulation for chicken skin gelatine film blended with CMC was 3.00 g CMC and 0.77 mL glycerol. However, the desirability of all solution recommended actually was not really strong as the value was below 0.8. The optimal formulation should be chosen with result of desirability of above 0.8. The desirability of suggested optimised formulation may depend on the data obtained from 13 experimental design and/or from analysis of each response.

Validation test

To confirm the validity of the model, an experiment was conducted using the optimal conditions suggested with three replicates for each response. The TS obtained was 13.66 MPa, which was slightly higher and significantly different ($p < 0.05$) from the predicted value (12.43 MPa). However, the EAB obtained (146.67%) was significantly lower than the predicted value (167.57%) ($p < 0.05$). Meanwhile, the WVP obtained was 1.84×10^{-4} $gm^{-1}s^{-1}Pa^{-1}$, which was significantly higher than the predicted value (6.08×10^{-5} $gm^{-1}s^{-1}Pa^{-1}$). The predicted values were shown to disagree with the experimental response values. This might result from the selected solution which had a low desirability value thus making the experimental response value significantly different from the predicted response value.

Conclusion

In conclusion, the TS, EAB and WVP of chicken skin gelatine/CMC composite film with different glycerol concentrations were significantly affected by different concentrations of CMC and glycerol. Based on the model, the optimum conditions selected were CMC of 3.00 g and glycerol of 0.77 mL. However, desirability value of all suggested solution by RSM and selected solution were not convincing as the value was below 0.8. This resulted in significantly different result in the predicted and experimental response values. The predicted values should be in agreement with the experimental response values. Therefore, the optimisation of film formulation was a very crucial part in order to optimise the formulation that would yield the best film properties desired by the food packaging industry. The analysis of each response needs to be thoroughly analysed to get the optimised formulation with high desirability value. The RSM was successfully used to investigate the effects of CMC and glycerol and to optimise the formulation of a chicken skin gelatine/CMC blended film of different glycerol concentrations for the production of biodegradable food packaging.

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