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Development of model for barrier and optical properties of tapioca starch based edible films

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ABSTRACT

The film forming solutions composed of tapioca (cassava) starch (1–3 g), glycerol (0.5–1.0 ml), agar (0.5–1.0 g) and span 80 (0.1–0.5 ml) were prepared according to a three-level, four-factor Box-Behnken response surface experimental design. The films were obtained by casting method and they are homogenous and transparent. The influence of film composition (tapioca starch, glycerol, agar and span80) on the barrier and optical properties of the tapioca starch based edible films was evaluated. The results showed that, hydrophilic nature and plasticizing effect of glycerol increases the water vapor permeability, oxygen permeability, moisture content, solubility and swelling capacity of the films. But surfactant (span80) incorporation reduces the mobility of the polysaccharide matrix and decreases the barrier properties of the films. Transparency of the films was influenced by plasticizer and surfactant concentration due to the dilution effect of glycerol and span80. The results were analyzed by Pareto analysis of variance (ANOVA) and second-order polynomial models were developed using multiple regression analysis. The models developed from the experimental design were predictive and good fit with the experimental data with high coefficient of determination (R^2) values (more than 0.95). The optimized conditions were obtained were tapioca starch of 1.95 g, glycerol of 0.8 ml, agar of 0.7 g and span 80 of 0.3 ml, respectively.

1. Introduction

Recent environmental regulations, societal concerns and growing environmental awareness throughout the world have triggered the efforts in plastic industry to develop new products and processes that cause less or no harm to the environment. Development and characterization of environmentally friendly polymeric materials have attracted extensive interest due to the concerns on environmental impact (Siracusa, Rocculi, Romani, & Rossa, 2008; Weber, Haugaard, Festersen, & Bertelsen, 2002). A significant proportion of research on biodegradable films has been made using biopolymers derived from renewable sources. Biopolymers are usually derived from annually renewable natural resources including carbohydrates and proteins of plant or animal origin (Boredes, Pollet, & Avérous, 2009). Starch has been considered one of the most promising natural renewable resources because of its lower cost, biodegradability, thermoplastic behavior (Mali, Grossmann, García, Martino, & Zaritzky, 2005a) and availability in abundance than other natural resources. The use of starch material for preparation of edible or biodegradable films has been widely recognized (Krochta & De Mulder-Johnston, 1997). Films developed from tapioca starch are described as isotropic, odorless, tasteless, colorless, non-toxic and biologically degradable (Flores, Famá, Rojas, Goyanes, & Gerschenson, 2007). From the literature, it is found that most of the researchers studied the development of biodegradable films using pure tapioca starch that are very brittle in nature as a result of the strong cohesive bond between the polymer molecules (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007; Chang, Abd Karim, & Seow, 2006; Flores et al., 2007; Lu, Xiao, & Xu, 2009; Zhang & Han, 2006).

The addition of an appropriate plasticizing agent to starch based edible films reduces their brittleness and improves the flexibility and extensibility by acting as spacers between polymer chains and decreasing the intermolecular forces between adjacent polymeric chains (García, Martino, & Zaritzky, 2000b; Koskinen, Suortti, Autio, Myllärinen, & Poutanen, 1996; Romero-Bastida et al., 2005). Glycerol is the most widely used plasticizer for improving the mechanical properties and transparency of the edible films (Mali, Sakanaka, Yamashita, & Grossmann, 2005b; Myllarinen, Partanen, Seppala, & Forssell, 2002; Yang & Paulson, 2000). The

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hydroxyl groups present in glycerol are responsible for inter and intramolecular interactions (hydrogen bonds) in polymeric chains, providing films with a more flexible structure (Souza et al., 2012). Surfactants are amphibious substances (possessing hydrophilicity and hydrophobicity simultaneously) that are conventionally added to enhance the stability of the emulsified films (Chen, Kuo, & Lai, 2009). The addition of surfactant into film formulation to reduces the surface tension of the film-forming solution and decreases the water permeability of the film due to the hydrophobic nature (Ziani, Osés, Coma, & Maté, 2008). Rodriguez, Oses, Ziani, and Mate (2006) observed that water vapor permeability (WVP) and wettability of potato starch films were affected by the use of span 80 as the surfactant. Agar is a fibrous carbohydrate extracted from a number of marine algae of the class *Rhodophyceae* (called 'red seaweeds') such as *Gelidium* sp. and *Gracilaria* sp. (Rhim, 2011). Agar has been used to prepare environmental friendly packaging materials such as foams, films and coatings (Freile-Pelegrín et al., 2007; Lee, Lee, & Song, 1997; Phan, Debeaufort, Luu, & Voilley, 2005; Phan, Debaufort, Voilley, & Luu, 2009). Wu, Geng, Chang, Yu, and Ma (2009) reported that the addition of agar to starch improved the barrier properties of the film.

Response surface methodology (RSM) is a collection of statistical techniques for designing experiments, building models and evaluating the effect of process parameters. Box-Behnken is a spherical, revolving response surface methodology design that provides efficient solutions compared with a three-level full-factorial design and reducing the number of required experiments which becomes more significant as the number of factors increases (Borkowski, 1995; White, Willis, Keshav, & Dutton, 2001). The present work is focused on the development of model and investigating the individual and interactive effects of process parameters on the barrier and optical properties of tapioca starch-based edible films using Box-Behnken response surface design.

2. Materials and methods

2.1. Raw materials

Tapioca starch was obtained from Local market, Erode, India. Landers, Gbur, and Sharp (1991) method was used for determining the amylose and amylopectin content of tapioca starch. Ash, moisture and starch contents were determined according to the AOAC (1995) techniques. Glycerol (98% purity, Food grade) and span 80 (99% purity, Food grade) were purchased from Merk chemicals, Mumbai, whereas Agar was purchased from Hi-media chemicals, Mumbai.

2.2. Preparation of edible films

Solutions with various proportions of starch, glycerol, agar and span 80 were processed to form films by casting method (Prakash Maran, Sivakumar, Sridhar, & Prince Immanuel, 2013). The homogenous and clear film forming solution was prepared by dispersing starch and additives in distilled water and gradually heating the contents up to 70 ± 5 °C with stirring (100 rpm) and then kept for 30 min at 70 ± 5 °C. By degassing under vacuum, the dissolved air from the solution was removed and then 20 ml of the solution was transferred to 9 cm internal diameter Petri dishes resting on a leveled surface for casting and then the films were dried in a controlled temperature chamber at 25 °C at 60% RH for 48 h. From Petri dishes, the films were carefully separated and equilibrated at 25 °C, 58% relative humidity for 72 h.

2.3. Thickness (THI)

The thicknesses of the dried films were determined using a digital micrometer (Mitutoyo Co., Japan) with an accuracy of ±1 μm. The electronic digital micrometer was calibrated using standards prior to film thickness measurements. Fifty thickness measurements were taken at different random locations of each film and the mean values of each film were calculated and reported in millimeter (mm).

2.4. Water vapor permeability

Water vapor permeability of film was determined according to the methodology described by Mali, Grossmann, García, Martino, and Zaritzky (2004). Anhydrous calcium chloride (0% RH) was taken in a permeation cell and the circular mouth (area = 0.00181 m^2) of the cell was sealed with the film to be tested. The permeation cell was placed in the desiccator that was maintained at 75% RH using saturated sodium chloride solution in order to maintain RH gradient of 75% across the film. The RH inside the cell was always lower than that in the desiccator, and the water vapor transport was determined from the weight gain of the permeation cell after the steady state condition was reached (about 3 h). Weight measurements were made at regular time intervals (about 1 h) and the changes in the weight were plotted as a function of time. The slope was calculated by linear regression method and the water vapor transmission rate (WVTR) was calculated from the slope of the straight line (g/s) divided by the transfer area (m^2). After the permeation tests, film thickness was measured and WVP ($g\,m^{-1}\,s^{-1}\,Pa^{-1}$) was calculated using the following equation (Mali et al., 2004):

$$\mathrm{WVP} = \frac{\mathrm{WVTR}}{S(R_1 - R_2)d} \quad (1)$$

where S is the saturation vapor pressure of water (Pa) at the test temperature (25 °C), R_1 is the RH in the desiccator, R_2 is the RH in the permeation cell and d is the film thickness (m). All tests were conducted in triplicates and average values were recorded.

2.5. Gas permeability

Oxygen (O_2) permeability (OP) of the films was evaluated by the accumulation method described by Mali et al. (2004). O_2 concentration was measured in a gas chromatograph (Omega GC, Sc No-303). All the experiments were conducted in triplicates and O_2 was stabilized passing through a NaCl saturated solution. O_2 permeability of films was calculated and expressed in $cm^3/m\,s\,Pa$ at standard temperature (20 °C) and RH (75%).

2.6. Moisture content (MC)

Film moisture content was determined gravimetrically in a hot air oven at 105 °C overnight. Determination of moisture content was performed for five film specimens of each formulation and the average value was recorded. The moisture content was calculated using the following equation (Zhang & Li, 2011):

$$\mathrm{MC}\ (\%) = \left(\frac{M_1 - M_0}{M_1}\right) \times 100 \quad (2)$$

where M_1 is the initial weight of the film and M_0 is the final weight of the film.

2.7. Solubility (SOL)

Solubility of the film was measured according to the method described by Romero-Bastida et al. (2005). Discs of film (2 cm diameter) were cut, weighed, immersed in 50 ml of distilled water. The

beaker was sealed to prevent the evaporation of water, and stored at 25 °C for 24 h, and was periodically wobbled slightly. Then the samples were dried in a vacuum oven at 40 °C until the weight of the samples became constant. The total soluble matter of the sample was calculated as follows:

$$\text{SOL } (\%) = \left(\frac{M_0 - M_1}{M_1}\right) \times 100 \quad (3)$$

where M_0 and M_1 were the dry sample weight before and after the test, respectively. For each film, tests were performed in triplicate, and average value was used for calculation.

2.8. Swelling capacity (SWE)

Swelling capacity of starch film in distilled water was measured according to Hu, Chen, and Gao (2009). The initial weight (M_0) of dry sample with a size of 40 mm × 20 mm was determined after drying to a constant weight in a vacuum oven at 40 °C. The weighed samples were immersed in beaker filled with 50 ml distilled water. The beaker was sealed to prevent from dust and the evaporation of the contents, and kept at 25 °C for 24 h. The samples were taken out and dried to a constant weight at 40 °C in a vacuum oven, and then weighed (M). Tests were performed in triplicate and each value of swelling capacity was calculated as follows:

$$\text{SWE } (\%) = \left(\frac{M_1 - M_0}{M_0}\right) \times 100 \quad (4)$$

where M_0 and M_1 were the initial weight and the weight of swelled sample, respectively.

2.9. Transparency (TR)

Film transparency was determined according to method described by Ozdemir and Floros (2008). The films were cut into rectangular shapes (15 mm × 50 mm) and placed inside a spectrophotometer cell. Transparency of films was measured using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 560 nm. Three replicates of each film were tested. All tests were conducted at 23 ± 2 °C and 50 ± 5% RH. The percent transparency was calculated as follows:

$$\text{TR } (\%) = \left(\frac{I_r}{I_0}\right) \times 100 \quad (5)$$

where I_r is the light intensity with the specimen in the beam and I_0 is the light intensity with no specimen in the beam.

2.10. Experimental design

Box-Behnken statistical screening design was used to statistically develop model and to study and evaluate main effects, interaction effects and quadratic effects of the process parameters (tapioca starch, glycerol, agar and span 80) on the barrier and optical properties (THI, WVP, OP, MC, SOL, SWE and TR) of the tapioca starch based edible films.

The Box-Behnken design (BBD) was specifically selected since it requires fewer runs than a central composite design (CCD) in cases of three or four variables. This cubic design is characterized by set of points lying at the midpoint of each edge of a multidimensional cube and center point replicates ($n = 5$) whereas the 'missing corners' help the experimenter to avoid the combined factor extremes. This property prevents a potential loss of data in those cases (Box & Behnken, 1960). A BBD with four independent variables at three levels was performed to develop model and study the effect of process parameters on the barrier and optical properties of the tapioca starch based edible films. On the basis of single-factor experiment for the development of tapioca starch based edible films, proper ranges of tapioca starch, glycerol, agar and span80 were preliminarily determined. Each independent variable was coded at three levels between +1 and −1. The details are listed in Table 1. For statistical calculation, the relations between coded and actual values are described as the following equation:

$$X_i = \frac{Z_i - Z_{cp}}{\Delta Z_i} \quad (6)$$

where X_i was dimensionless value of an independent variable; Z_i was the real value of an independent variable; Z_{cp} was the real value of an independent variable at the center point; and ΔZ_i, step change of the real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i.

In Box-Behnken method, a total number of 29 experiments including five centre points were carried out and the experimental conditions and corresponding results (responses) are presented in Table 2. The performance of the process was evaluated by analyzing the responses (Y), which depend on the input factors $x_1, x_2 \ldots, x_k$, and the relationship between the response and the input process parameters is described by

$$Y = f(x_1, x_2 \ldots x_k) + e \quad (7)$$

where f is the real response function the format of which is unknown and e is the error which describes the differentiation.

A second-order polynomial model corresponding to the BBD was fitted to correlate the relationship between the independent variables and the responses and also to identify the relevant model terms using statistical software (Design Expert 8.0.7.1, Statease Inc., Minneapolis, USA). A quadratic model, which also includes the linear model, can be described as

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ij} x_i x_j + e_i \quad (8)$$

where Y is the response; x_i and x_j are variables (i *and* j *range from* 1 to k); β_0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are interaction coefficients of linear, quadratic and the second-order terms, respectively; k is the number of independent parameters ($k = 4$ in this study); and e_i is the error (Prakash Maran & Manikandan, 2012).

2.11. Statistical analysis

Experimental design data were analyzed using multiple regressions through the least square method. The significance of the regression coefficients was also tested by F-test and the F-test was used to verify the statistical significance of models at $p \leq 0.05$ using the statistical software Design Expert 8.0.7.1 (Statease Inc., Minneapolis, USA). The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2 and the values of adjusted-R^2 of models were evaluated to check the model adequacies. The significance of each term in the equation is to estimate the goodness of fit in each case. The results were analyzed using the Pareto analysis of variance (ANOVA) and ANOVA tables were generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. In order to visualize the relationship between the response and experimental levels of each factor, the regression coefficients were used to make statistical calculation to generate 3-D surface plots and contour plots from the fitted polynomial equation. These graphs are drawn by maintaining two factors constant (in turn at its central level) and varying the other two factors in order to understand their main and interactive effects on the dependent variables.

Table 1
Experimental range and levels of independent variables.

Variables	Coded variables	Variable levels			Step change value ΔZ_i
		−1	0	1	
Starch (g)	X_1	1.00	2.00	3.00	1.00
Glycerol (ml)	X_2	0.50	0.75	1.00	0.25
Agar (g)	X_3	0.50	0.75	1.00	0.25
Span80 (ml)	X_4	0.10	0.30	0.50	0.20

3. Results and discussion

3.1. Chemical composition of tapioca starch

The chemical composition of the tapioca starch was: ash (0.15 (± 0.01%), moisture (12.23 (± 0.51) and starch (98.06 (± 0.18). The amylose and amylopectin contents were found to be 27 and 73%, respectively. This amylose present in the starch is responsible for the film forming capacity. Because, the formation of starch based edible films involves two process namely, gelatinization (swelling, disruption and leaching of soluble components (amylose) of the starch) and retrogradation (reduction in the solubility of dissolved starch). In gelatinization process, the continuous phase of the viscous mass is constituted by amylose and discontinuous phase is constituted by amylopectin content, respectively. In retrogradation process, amylose showed a much more rapid rate than amylopectin. The gelatinization and retrogradation can be interpreted as the result of forming a helical network and then aggregates into gels or retrogradaed materials become more rigid and difficult to disperse (Flores et al., 2007). The amylose and amylopectin contents are found to be higher than the reported value (Chen et al., 2009).

3.2. Box-Behnken analysis

According to the Box Behnken experimental design, experiments were performed to study the individual and interactive effects of the independent variables (Starch, glycerol, agar and span80) on the barrier and optical properties (THI, WVP, OP, MC, SOL, SWE and TR) of the tapioca starch based biodegradable edible films and the results were shown in Table 2.

Fitting of the data to various models (linear, interactive, quadratic and cubic models) were carried out to obtain the regression equations. In general, exploration of a fitted response surface may produce poor or misleading results, unless the model exhibits a good fit, which makes checking of the model adequacy essential (Liyana-Pathirana & Shahidi, 2005). To decide about the adequacy of models among various models to represent barrier and optical properties of the starch based biodegradable edible films, two different tests namely the sequential model sum of squares and model summary statistics were carried out in the present study and the results are given in Table 3.

The fit summary of the output indicates that the quadratic model is statistically highly significant and the p-value was lower than 0.0001. This means that at least one of the terms in the regression model has a significant correlation with the response variables. Cubic model was found to be aliased. Model summary statistics showed that the excluding cubic model which was aliased, quadratic model was found to have maximum "Adjusted R-Squared" and the "Predicted R-Squared" values.

3.3. Fitting of second-order polynomial equation and statistical analysis

The second-order polynomial equation was fitted with the experimental results obtained on the basis of Box-Behnken experimental design. Six empirical models were developed to understand the interactive correlation between the responses and process variables. The final equation obtained in terms of coded factors is given below

$$\begin{aligned}\mathrm{THI} = {} & 0.029 + 7.083E-003X_1 - 4.167E-004X_2 + 5.00E \\ & -004X_3 + 5.00E-004X_4 + 0.001X_1X_2 - 500E \\ & -004X_1X_3 + 3.00E-003X_1X_4 - 1.5E-003X_2X_3 \\ & -7.5E-004X_2X_4 + 1.75E-003X_3X_4 + 1.917E \\ & -003X_1^2 + 4.917E-003X_2^2 + 4.542E-003X_3^2 \\ & +5.042E-003X_4^2 \end{aligned} \quad (9)$$

$$\begin{aligned}\mathrm{WVP} = {} & 0.41 + 2E-003X_1 - 0.048X_2 + 0.04X_3 - 0.048X_4 \\ & +0.027X_1X_2 + 2.5E-004X_1X_3 - 1.75E-003X_1X_4 \\ & +0.01X_2X_3 + 6.5E-003X_2X_4 - 0.023X_3X_4 + 0.054X_1^2 \\ & +0.03X_2^2 + 5.375E-003X_3^2 - 9.25E-003X_4^2 \end{aligned} \quad (10)$$

$$\begin{aligned}\mathrm{OP} = {} & 4.12 + 0.027X_1 + 0.23X_2 + 0.13X_3 + 0.051X_4 - 0.18X_1X_2 \\ & +0.08X_1X_3 + 0.02X_1X_4 + 2.5E-003X_2X_3 + 0.21X_2X_4 \\ & +0.052X_3X_4 + 0.25X_1^2 + 0.21X_2^2 + 0.11X_3^2 - 0.093X_4^2 \end{aligned} \quad (11)$$

$$\begin{aligned}\mathrm{MC} = {} & 13.6 - 0.033X_1 + 1.15X_2 + 0.092X_3 - 1.09X_4 - 0.25X_1X_2 \\ & +0.35X_1X_3 - 0.5X_1X_4 - 0.38X_2X_3 + 0.67X_2X_4 + 1.00X_3X_4 \\ & -0.34X_1^2 + 0.41X_2^2 - 0.029X_3^2 - 0.15X_4^2 \end{aligned} \quad (12)$$

$$\begin{aligned}\mathrm{SOL} = {} & 26.9 + 0.12X_1 + 1.26X_2 - 0.058X_3 + 0.083X_4 - 0.025X_1X_2 \\ & +0.025X_1X_3 - 0.2X_1X_4 - 0.3X_2X_3 - 0.25X_2X_4 - 0.3X_3X_4 \\ & -0.2X_1^2 - 0.44X_2^2 - 0.16X_3^2 - 0.05X_4^2 \end{aligned} \quad (13)$$

$$\begin{aligned}\mathrm{SWE} = {} & 77.8 + 0.15X_1 + 1.28X_2 - 0.11X_3 + 0.067X_4 - 0.15X_1X_2 \\ & +0.1X_1X_3 + 0.001X_1X_4 - 0.35X_2X_3 - 0.13X_2X_4 - 0.23X_3X_4 \\ & -0.27X_1^2 - 0.35X_2^2 + 0.12X_3^2 - 0.067X_4^2 \end{aligned} \quad (14)$$

$$\begin{aligned}\mathrm{TR} = {} & 86.7 + 0.23X_1 + 1.3X_2 - 0.28X_3 + 0.05X_4 - 0.1X_1X_2 \\ & +0.13X_1X_3 - 0.35X_1X_4 - 0.63X_2X_3 - 0.52X_2X_4 - 0.13X_3X_4 \\ & -0.096X_1^2 - 0.43X_2^2 + 0.13X_3^2 + 0.17X_4^2 \end{aligned} \quad (15)$$

Analysis of Variance (ANOVA) was used for graphical analysis of the data to find the interaction between the process variables and the responses. The adequacy and fitness of the models were tested

Table 2
Box-Behnken experimental design and observed responses.

Run	Starch (g)	Glycerol (ml)	Agar (g)	Span80 (ml)	Thickness (mm)	Water vapor permeability (g/m s Pa) × 10^{-10}	Oxygen permeability (cm^3/m s Pa) × 10^{-10}	Moisture content (%)	Solubility (%)	Swelling capacity (%)	Transparency (%)
1	2	0.75	0.75	0.3	0.029	0.406	4.12	13.6	26.9	77.8	86.7
2	1	0.75	0.75	0.1	0.032	0.493	4.23	13.7	26.2	77.3	86.1
3	1	0.75	0.75	0.5	0.027	0.411	4.28	12.4	26.7	77.5	86.9
4	1	0.75	1	0.3	0.029	0.504	4.49	13.1	26.4	77.4	86.2
5	2	0.75	0.5	0.5	0.037	0.335	4.02	11.4	27.2	78.4	87.5
6	3	0.75	0.75	0.1	0.039	0.501	4.22	14.7	26.9	77.5	87.4
7	2	0.75	1	0.5	0.042	0.365	4.34	13.3	26.4	77.5	86.6
8	2	1	0.5	0.3	0.039	0.348	4.54	15.3	27.8	79.2	88.6
9	1	0.5	0.75	0.3	0.03	0.561	4.13	12.3	24.9	75.5	84.6
10	3	1	0.75	0.3	0.042	0.464	4.65	14.6	27.6	78.6	87.6
11	3	0.75	0.75	0.5	0.046	0.412	4.35	11.4	26.6	77.7	86.8
12	1	0.75	0.5	0.3	0.027	0.417	4.39	13.5	26.6	77.6	86.9
13	1	1	0.75	0.3	0.028	0.412	4.96	15.2	27.4	78.4	87.3
14	2	1	0.75	0.5	0.039	0.331	4.75	14.6	27.6	78.6	87.3
15	2	0.75	0.75	0.3	0.029	0.406	4.12	13.6	26.9	77.8	86.7
16	2	0.5	0.75	0.5	0.04	0.418	3.86	11.1	25.5	76.1	85.6
17	2	0.75	0.75	0.3	0.029	0.406	4.12	13.6	26.9	77.8	86.7
18	2	0.5	1	0.3	0.041	0.521	4.33	13.3	25.3	76.7	85.5
19	2	0.75	0.75	0.3	0.029	0.406	4.12	13.6	26.9	77.8	86.7
20	2	0.5	0.75	0.1	0.038	0.534	4.18	14.5	24.8	75.8	84.4
21	2	1	1	0.3	0.038	0.448	4.82	14.9	27.2	78.3	86.8
22	2	0.75	0.75	0.3	0.029	0.406	4.12	13.6	26.9	77.8	86.7
23	2	0.75	0.5	0.1	0.039	0.387	4.02	15.6	26.4	77.8	87.2
24	2	0.5	0.5	0.3	0.036	0.462	4.06	12.2	24.7	76.2	84.8
25	3	0.75	1	0.3	0.043	0.513	4.73	13.7	26.6	77.8	86.7
26	3	0.5	0.75	0.3	0.044	0.507	4.55	12.7	25.2	76.3	85.3
27	3	0.75	0.5	0.3	0.044	0.425	4.31	12.7	26.7	77.6	86.9
28	2	1	0.75	0.1	0.039	0.421	4.21	15.3	27.9	78.8	88.2
29	2	0.75	1	0.1	0.037	0.508	4.13	13.5	26.8	77.8	86.8

Table 3
Sequential model sum of squares and model summary statistics tested for responses.

Source	Sum of squares	DF	Mean square	*F* value	Prob > *F*	R^2	Adjusted R^2	Predicted R^2	PRESS	Remarks
Thickness										
Mean	0.037	1	0.0372							
Linear	0.0006	4	0.0002	9.19	0.0011	0.605	0.539	0.494	0.00051	
2FI	6.18E−05	6	1.03E−05	0.55	0.7633	0.666	0.481	0.406	0.0006	
Quadratic	0.0003	4	8.27E−05	207.45	<0.0001	0.994	0.989	0.968	0.00003	Suggested
Cubic	5.33E−06	8	6.67E−07	16	0.0016	1.000	0.999	0.964	0.00004	Aliased
Residual	2.50E−07	6	4.17E−08							
Total	0.038	29	0.0013							
Water vapor permeability										
Mean	5.59	1	5.59							
Linear	0.07	4	0.02	14.52	0.0018	0.708	0.659	0.567	0.05	
2FI	0.01	6	0	0.65	0.6928	0.759	0.626	0.316	0.07	
Quadratic	0.02	4	0.01	172.36	<0.0001	0.995	0.99	0.972	0.002	Suggested
Cubic	0.0003	8	0.00004	1.11	0.4643	0.998	0.991	0.721	0.03	Aliased
Residual	0.0002	6	0.00003							
Total	5.69	29	0.2							
Oxygen permeability										
Mean	540.09	1	540.09							
Linear	0.89	4	0.22	4.64	0.0064	0.436	0.342	0.163	1.71	
2FI	0.36	6	0.06	1.35	0.2880	0.611	0.395	−0.081	2.21	
Quadratic	0.79	4	0.20	582.33	<0.0001	0.998	0.995	0.987	0.03	Suggested
Cubic	0.00	8	0.00	2.90	0.1059	1.000	0.998	0.931	0.14	Aliased
Residual	0.00	6	0.00							
Total	542.13	29	18.69							
Moisture content										
Mean	5325.83	1	5325.83							
Linear	30.29	4	7.57	16.81	0.0016	0.737	0.693	0.588	16.93	
2FI	8.13	6	1.35	9.09	0.0011	0.935	0.898	0.784	8.88	
Quadratic	2.45	4	0.61	37.17	<0.0001	0.994	0.989	0.968	1.33	Suggested
Cubic	0.19	8	0.02	4.08	0.0518	0.999	0.996	0.874	5.16	Aliased
Residual	0.04	6	0.01							
Total	5366.92	29	185.07							
Solubility										
Mean	20439.5	1	20439.5							
Linear	19.29	4	4.82	44.44	0.0036	0.881	0.861	0.828	3.77	
2FI	1.14	6	0.19	2.32	0.078	0.933	0.896	0.841	3.49	
Quadratic	1.38	4	0.35	57.59	<0.0001	0.996	0.992	0.978	0.48	Suggested
Cubic	0.05	8	0.01	1.19	0.427	0.999	0.993	0.786	4.68	Aliased
Residual	0.03	6	0.01							
Total	20461.4	29	705.57							
Swelling capacity										
Mean	174476	1	174476							
Linear	19.97	4	4.99	45.84	0.0028	0.884	0.865	0.825	3.96	
2FI	0.88	6	0.15	1.54	0.223	0.923	0.881	0.773	5.12	
Quadratic	1.41	4	0.35	15.76	<0.0001	0.986	0.972	0.92	1.81	Suggested
Cubic	0.28	8	0.03	5.83	0.0227	0.998	0.993	0.772	5.16	Aliased
Residual	0.04	6	0.01							
Total	174498	29	6017.19							
Transparency										
Mean	217505	1	217505							
Linear	21.83	4	5.46	24.41	0.0019	0.803	0.77	0.692	8.38	
2FI	3.32	6	0.55	4.87	0.004	0.925	0.883	0.754	6.68	
Quadratic	1.88	4	0.47	39.65	<0.0001	0.994	0.988	0.965	0.96	Suggested
Cubic	0.13	8	0.02	2.3	0.1635	0.998	0.993	0.784	5.88	Aliased
Residual	0.04	6	0.01							
Total	217532	29	7501.09							

by Pareto analysis of variance and the results indicated that the equation adequately represented the actual relationship between the independent variables and responses (Table 4). The significance of each coefficient was determined using *p*-value, which is used as a tool to check the significance of each coefficient and are necessary to understand a pattern of mutual interactions between the process variables. If *p*-value is the smaller, it is the bigger significance of the corresponding coefficient and *p* values lower than 0.05 indicates that the model is statistically significant (Segurola, Allen, Edge, & Mahon, 1999). The regression coefficients and *p*-value for the second-order polynomial equation is presented in Table 4 and it shows that the model or intercept *p* value is very low ($p < 0.0001$), which indicates the developed model is significant and adequately represented the actual relationship between the response (THI, WVP, OP, MC, SOL, SWE and TR) and their significant variables.

The significance of the *F*-value depends on the number of degrees of freedom (DF) in the model, and is shown in the *p*-value column (95% confidence level). In general, the effects lower than 0.05 are significant. The ANOVA result for the THI, WVP, OP, MC, SOL, SWE and TR shows *F*-value of 179.61, 207.82, 429.3, 177.02, 259.11, 70.89 and 162.96, which implies that the developed quadratic models have a significant effect on the responses. The large value of *F* indicates that most of the variation in the response can be explained by the regression models. The associated *p* value

Table 4
ANOVA and significance of regression coefficients.

Source	THI		WVP		OP		MC		TSM		SWE		TR	
	CE	*P*-value	CE	*P*-value	CE	*P*-value	CE	*P*-value	CE	*P*-value	CE	*P*-value	CE	*P*-value
Model	0.290	<0.0001	0.406	<0.0001	4.12	<0.0001	13.600	<0.0001	26.900	<0.0001	77.800	<0.0001	86.700	<0.0001
X_1	0.071	<0.0001	0.002	0.2687	0.0275	0.0001	−0.033	0.3837	0.117	0.0001	0.150	0.0038	0.225	<0.0001
X_2	−0.004	0.0384	−0.048	<0.0001	0.235	<0.0001	1.150	<0.0001	1.258	<0.0001	1.275	<0.0001	1.300	<0.0001
X_3	0.005	0.0159	0.040	<0.0001	0.125	<0.0001	0.092	0.0268	−0.058	0.0207	−0.108	0.0252	−0.275	<0.0001
X_4	0.005	0.0159	−0.048	<0.0001	0.05083	<0.0001	−1.092	<0.0001	0.083	0.0023	0.067	0.1455	0.050	0.1338
X_{12}	0.001	1.0000	0.027	<0.0001	−0.1825	<0.0001	−0.250	0.0016	−0.025	0.5294	−0.150	0.0650	−0.100	0.0874
X_{13}	−0.008	0.0324	0.000	0.9349	0.08	<0.0001	0.350	<0.0001	0.025	0.5294	0.100	0.2031	0.125	0.0376
X_{14}	0.030	<0.0001	−0.002	0.5699	0.02	0.0474	−0.500	<0.0001	−0.200	0.0001	0.001	1.0000	−0.350	<0.0001
X_{23}	−0.015	0.0003	0.010	0.0042	0.0025	0.7898	−0.375	<0.0001	−0.300	<0.0001	−0.350	0.0004	−0.625	<0.0001
X_{24}	−0.007	0.0324	0.007	0.0485	0.215	<0.0001	0.675	<0.0001	−0.250	<0.0001	−0.125	0.1173	−0.525	<0.0001
X_{34}	0.018	<0.0001	−0.023	<0.0001	0.0525	<0.0001	1.000	<0.0001	−0.300	<0.0001	−0.225	0.0095	−0.125	0.0376
X_1^2	0.019	<0.0001	0.054	<0.0001	0.245	<0.0001	−0.342	<0.0001	−0.200	<0.0001	−0.267	0.0005	−0.096	0.0416
X_2^2	0.049	<0.0001	0.030	<0.0001	0.21375	<0.0001	0.408	<0.0001	−0.437	<0.0001	−0.354	<0.0001	−0.433	<0.0001
X_3^2	0.045	<0.0001	0.005	0.0391	0.10625	<0.0001	−0.029	0.5721	−0.163	0.0001	0.121	0.0591	0.129	0.0091
X_4^2	0.050	<0.0001	−0.009	0.0016	−0.0925	<0.0001	−0.154	0.0085	−0.050	0.1228	−0.067	0.2761	0.167	0.0016

CE—Coefficient estimate.

is used to estimate whether *F* is large enough to indicate statistical significance.

The coefficient of determination (R^2) gives the proportion of the total variation in the response predicted by the model, indicating ratio of sum of squares due to regression (SSR) to total sum of squares (SST). A high R^2 value, close to 1, is desirable and a reasonable agreement with adjusted R^2 is necessary (Nordin, Venkatesh, Sharif, Elting, & Abdullah, 2004). A high R^2 coefficient ensures a satisfactory adjustment of the quadratic model to the experimental data. Coefficient of determination (R^2) and adj-R^2 were calculated to check the adequacy and fitness of the model. The values of R^2 were calculated to be 0.995, 0.995, 0.997, 0.994, 0.996, 0.986 and 0.993 for THI, WVP, OP, MC, SOL, SWE and TR, respectively, which implies that 95% of experimental data was compatible. The adj-R^2 value corrects the R^2 value for the sample size and for the number of terms in the model. The value of adj-R^2 (0.989 for THI, 0.990 for WVP, 0.995 for OP, 0.989 for MC, 0.992 for SOL, 0.972 for SWE and 0.988 for TR) is also high to advocate for a high significance the model.

The coefficient of variance (CV) is the ratio of the standard error of estimate to the average value of the observed response defined by the reproducibility of the model. If the CV of the model is greater than 10%, then the model is reproducible (Ghafari, Aziz, Isa, & Zinatizadeh, 2009). The CV were found to be 1.76%, 1.37%, 0.43%, 0.95%, 0.29%, 0.19% and 0.13% for thickness, water vapor permeability, moisture content, total soluble matter, swelling capacity and transparency, respectively. The very low values of CV clearly indicated a very high degree of precision and a good reliability of the experimental values. Adequate precision is the measure of signal to noise ratio and the ratio is greater than 4 is desirable in support of the fitness of the model (Muthukumar, Mohan, & Rajendran, 2003). For the present study, the signal to noise ratio was found to be 44.40, 52.08, 85.12, 48.54, 55.89, 34.30 and 51.74, which indicates adequate signal and confirm that all predicted models can be used to navigate the design space.

3.4. Adequacy of the models

Generally, it is important to confirm the fitted model to make sure that it gives a sufficient approximation to the actual values. Unless the model shows a satisfactory fit, proceeding with an investigation and optimization of the fitted response surface likely gives poor or misleading results (Murugesan, Dhamija, Nam, Kim, & Chang, 2007). Diagnostic plots such as the predicted versus experimental values (Fig. 1 a–g) help us to judge the model satisfactoriness and exhibit the relationship between predicted and experimental values. The data points on this plot lie reasonably close to the straight line and indicate an adequate agreement between real data and the data obtained from the models.

Data were also analyzed to check the normality of the residuals. Normal probability plot represents the normal distribution of the residuals and the residual gives the difference between the observed value of a response measurement and the value that is fitted under the theorized model, and the small residual value indicates that model prediction is accurate (Mohajeri, Aziz, Isa, & Zahed, 2010). By constructing a normal probability plot of the residuals, a check was made for the normality assumption and the normal probability plots of the residuals are shown in Fig. 2 a–g and the data points on this plot lie reasonably close to a straight line. But, some scatter is expected even with normal data, therefore, it could be concluded that data was normally distributed.

3.5. Effect of process variables

By visual observation, the films were found to be transparent and homogeneous. The thickness of the liquid film-forming dispersion affects the drying kinetics, which may cause differences in the film structure (Debeaufort & Voilley, 1995). Therefore, controlling this parameter is crucial for the physical and barrier properties of the dried films. The same volume of film-forming solutions was cast in Petri dishes, due to the differences in film-forming solution formulations increases the dry matter of solutions, which caused the differences in thickness of the films. From Fig. 3 a and b, it was found that, the film thickness increased from 0.029 to 0.045 mm due to the increased concentration of starch, glycerol and agar in the film forming solution.

Water vapor permeability is one of the important characteristics of the film since it greatly influences the utility of the film in food industries (Mali, Grossmann, García, Martino, & Zaritsky, 2006). The results showed that glycerol (0.5–1.0 ml), agar (0.5–1.0 g) and span80 (0.1–0.5 ml) have a distinct influence on WVP of tapioca starch based films (Fig. 3 c and d). Agar creates strong inter-molecular links with tapioca starch and forms a dense three-dimensional structure. Increase in concentration of agar and starch combined with glycerol causes fluctuations in film density and it may be lead to the formation of films with pores and cracks and hence facilitates the permeation of water vapor (Wu et al., 2009).

The addition of glycerol (hydrophilic molecule, molecular weight of 92.09 g/mol) leads to increase the WVP of the films as it increases the free volume and chain movements, reduces rigidity and increases the molecular mobility of the films, which facilitates the diffusion of water vapor into the films. Similar results have

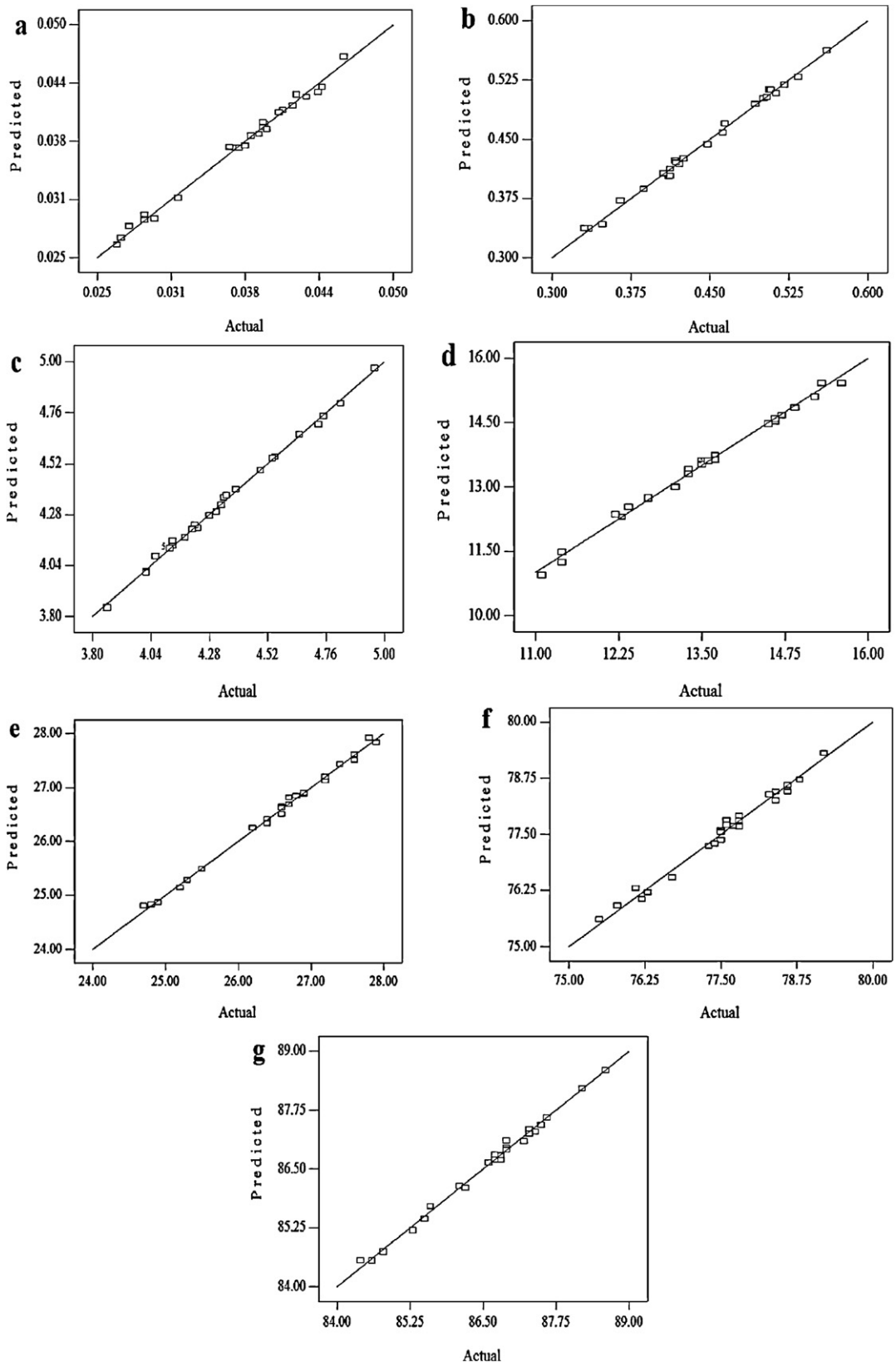

Fig. 1. Comparison between predicted and experimental values of thickness (a), water vapor permeability (b), oxygen permeability (c), moisture content (d), solubility (e), swelling capacity (f) and transparency (g).

been reported for films developed from chitosan (Caner, Vergano, & Wiles, 1998; Ziani et al., 2008), sodium caseinate and soluble starch (Arvanitoyannis & Biliaderis, 1998) and whey protein (McHugh & Krochta, 1994; Sothornvit & Krochta, 2000). The presence of span80 decreases the affinity towards water by reducing the number of polar groups available to interact with water molecules and decreases the WVP of the films. It might be due to the hydrophilic/lipophilic balance (HLB) ratio, hydrophobicity and

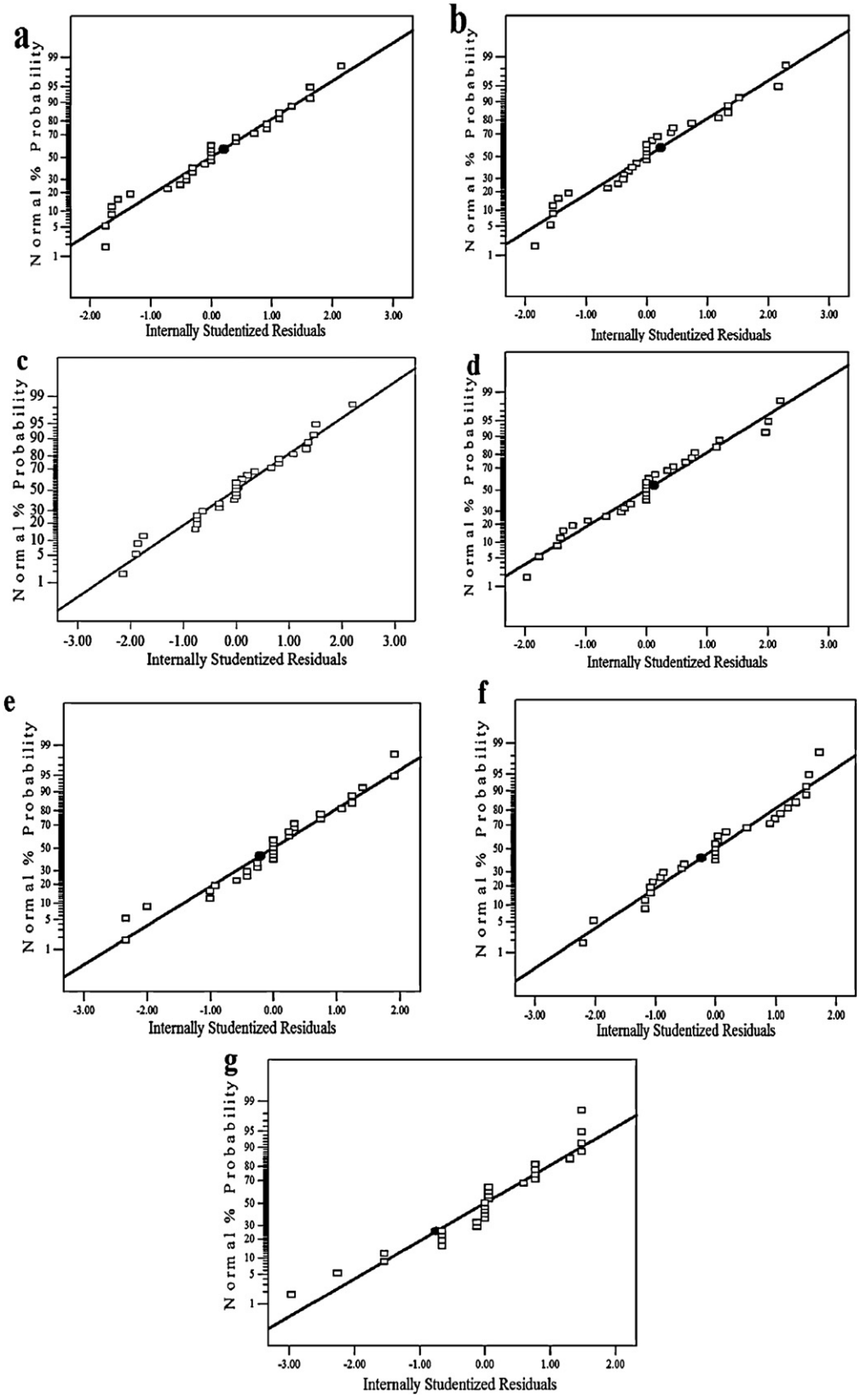

Fig. 2. Normal probability plots of studentized residuals for thickness (a), water vapor permeability (b), oxygen permeability (c), moisture content (d), solubility (e), swelling capacity (f) and transparency (g).

stereo-hinderance structure of the span80. Villalobos, Hernández-Muñoz, and Chiralt (2006) found that the WVP of hydroxypropyl methylcellulose films with surfactants (which was a mixture of span60 and sucrose ester P-1570, providing HLB values between 4.7 and 8.0) was lower than that of films prepared without surfactants.

The small hydrophilic glycerol molecule can easily interact with the starch chains and reducing the bindings between the chains. I

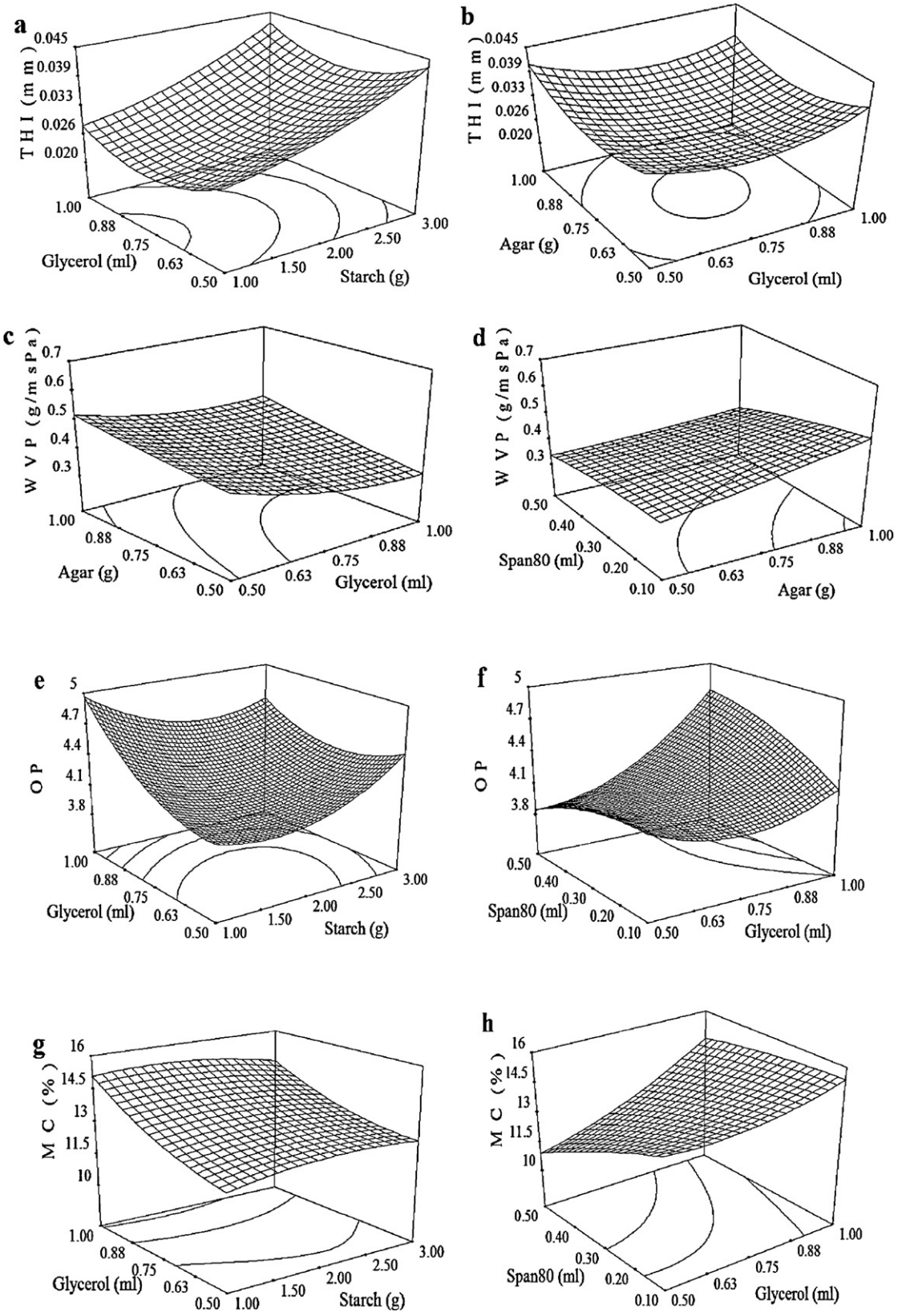

Fig. 3. Response surface plots showing the interaction effects of process variables on thickness (a and b), water vapor permeability (c and d), oxygen permeability (e and f) and moisture content (g and h).

t leads to a less dense and more disorganized polymeric matrix is formed in the presence of glycerol, allowing for greater oxygen diffusion through the film (Fig. 3e). The increasing content of the starch increases the OP of the film, because of hydrophilic nature as well as lower amylose content of the starch. On the other hand, the increase in surfactant concentration decreases the gas permeability due (Fig. 3f) to the formation of an interconnecting polymer network within the film matrix, decreasing the film

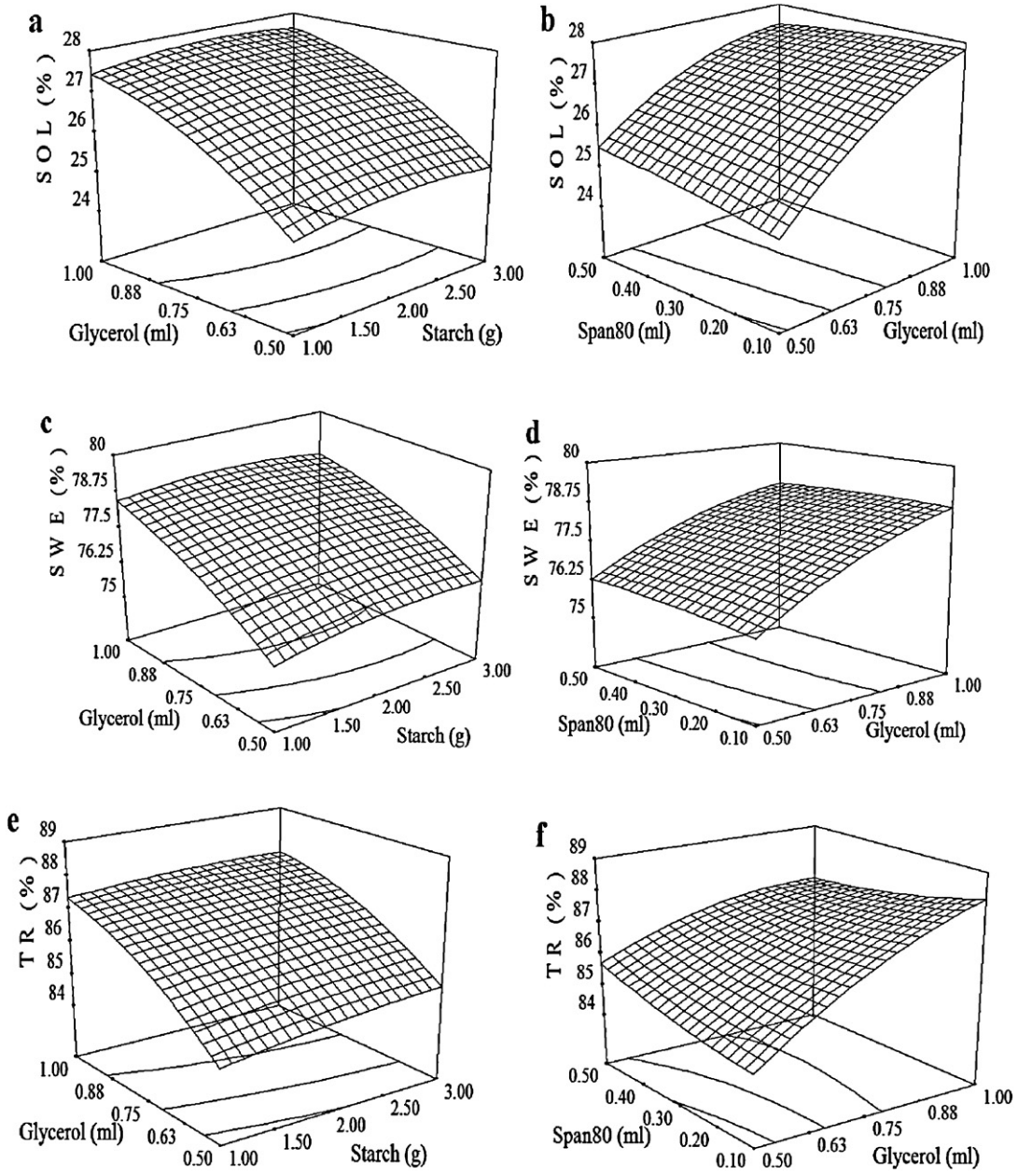

Fig. 4. Response surface plots showing the interaction effects of process variables on solubility (a and b), swelling capacity (c and d) and transparency (e and f).

free volume. High water resistance of a film is one of the most important properties from a food packaging point of view, especially for high water activity foods. The determination of moisture content gives information on the amount of water present in the film. Moisture content of tapioca starch films is shown in Table 2. Fig. 3g and f shows the variation of moisture content in tapioca starch films and the influence of plasticizer concentration on water content in starch film. High concentrations of plasticizer favor the adsorption of water molecules due to its hydrophilic nature, which retains water in the film matrix and form hydrogen bonds (O–H) (Cerqueira, Souza, Teixeira, & Vicente, 2012). On the other hand, the moisture content decreases for films with higher concentrations of span80 (Fig. 3h), because surfactant incorporation reduces the mobility of the polysaccharide matrix and decreases the moisture content of the films. The moisture content of tapioca starch based films was found to be lower than those reported (up to 27%) by Chang, Cheah, and Seow (2000).

Solubility in water and swelling capacity of edible films denote the applicability of film to pack water-rich foods such as peeled fruits and are related to the biodegradability of films (Cerqueira et al., 2012). Solubility and swelling capacity of starch films increase with higher glycerol concentrations (Fig. 4a and c). The increase in concentration of the surfactants decreases the solubility and swelling of the films (Fig. 4b and d) because of its hydrophobic character. This hydrophobic character decreases the number of O–H bonds and the presence of aliphatic groups, which changes the film structure leading to the formation of film with less solubility in water. The results obtained are in agreement with the solubility values reported for the polysaccharide-based films (Mehyar & Han, 2004; Piermaria, Pinotti, García, & Abraham, 2009). The higher swelling nature is due to higher amylopectin and lower amylose contents of tapioca starch compared to cereal starch such as wheat and corn starch (Chen et al., 2009).

Transparency of films is relatively important when edible films applied for food packaging or food coating since it directly influences consumer acceptability (Chen, Kuo, & Lai, 2010). With various proportions of glycerol, agar and span80, tapioca starch based film-forming solution produced homogeneous and transparent films and the transparency obtained for each film samples are summarized in Table 2. Tapioca starch films had high transparency, about 85% transmittance in visible light district. Higher transparency indicates that the tapioca starch blends have good compatibility and interaction effects among the constituent components. The transparency of films increased showed an increasing tendency (Fig. 4e and f), when the glycerol and span80 concentration was increased ($p < 0.05$), probably due to the dilution effect of glycerol and span80.

3.6. Optimization and verification of the model

Second order polynomial models obtained in this study were utilized for each response in order to obtain specified optimum conditions. Simultaneous optimizations of the multiple responses were carried out using Derringer's desirability function method. This function searches for a combination of factor levels that simultaneously satisfies the requirements for each response in the design. This numerical optimization evaluates a point that maximizes the desirability function. Applying the methodology of desired function, the optimum level of various parameters were obtained and it indicates that an tapioca starch of 1.95 g, glycerol of 0.8 ml, agar of 0.7 g and span 80 of 0.3 ml gives 0.029 mm of THI, 0.389×10^{-10} g/m s Pa of WVP, 4.12×10^{-10} cm^3/m s Pa of OP, 13.8% of MC, 27.1% of TSM, 78.1% SWE and 87% of TR, respectively, with an overall desirability of 0.915.

This set of optimum conditions are used to validated experimentally. Triplicate experiments were carried out to compare the experimental results with the predicted values of the responses using the developed empirical model equations. The experimental values (0.028 mm for THI, 0.384×10^{-10} g/m s Pa for WVP, 4.15×10^{-10} cm^3/m s Pa for OP, 13.2% for MC, 26.9% for TSM, 77.6% for SWE and 86.4% for TR) were found to be in agreement with the predicted values and clearly indicated the suitability of the developed quadratic models. The results obtained through confirmation experiments indicate the suitability of the developed quadratic models and it may be noted that these optimal values are valid within the specified range of process parameters.

4. Conclusion

Box-Behnken experimental design was employed to develop model and study the barrier and optical properties of edible films formed from tapioca starch, glycerol, agar and span 80. Varying the proportion of glycerol (0.5–1.0 ml) and span80 (0.1–0.5 ml) in edible films has a significant influence on the barrier and optical properties of tapioca starch based films. Due to the hygroscopic nature and plasticizing effect of glycerol increases the free volume and chain movements on the polymer matrix, reduces rigidity and increases the molecular mobility of the films, which facilitates the diffusion of water vapor on the biopolymer matrix and increases the barrier properties of the films. Hydrophilic/Lipophilic balance ratio, hydrophobicity and stereo-hinderance structure of the span80 decreases the affinity towards water by reducing the number of polar groups available to interact with water molecules and decreases the barrier properties of the films. The transparency of films increased when the glycerol and span80 concentration increased ($p < 0.05$), probably due to the dilution effect of glycerol and span80. Analysis of variance showed a high coefficient of determination value (R^2) of 0.995 for thickness, 0.995 for water vapor permeability, 0.997 for oxygen permeability, 0.994 for moisture content, 0.996 for solubility, 0.986 for swelling capacity and 0.993 for transparency, thus ensuring that the developed second order polynomial regression models were good satisfactory fit with the experimental data. The optimum formulations of tapioca starch based films was found to be tapioca starch of 1.95 g, glycerol of 0.8 ml, agar of 0.7 g and span 80 of 0.3 ml, respectively. Therefore, the present work suggested that, Box-Behnken response surface design to be an effective tool for this study, because of the complexity of film forming formulations involving interrelated variables.

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