Development of chitosan based edible films: process optimization using response surface methodology

Tarun Pal Singh · Manish Kumar Chatli · Jhari Sahoo

Revised: 12 January 2014 / Accepted: 6 March 2014 / Published online: 13 April 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract Three-factors Box-Behnken design of response surface methodology (RSM) was used to optimize chitosan level (1.5, 2.0, 2.5 %w/v), glycerol level (0.5, 0.75, 1.0 %w/v) and drying temperature (35, 40, 45 °C) for the development of chitosan based edible films. The optimization was done on the basis of different responses viz. thickness, moisture, solubility, colour profile (L^* , a^* , b^* value), penetrability, density, transmittance and water vapor transmission rate (WVTR). The linear effect of chitosan was significant ($p < 0.05$) on all the responses. However, density was only significantly ($p < 0.05$) affected by glycerol in a negative linear fashion. Drying temperature also significantly ($p < 0.05$) affected thickness, penetrability, transmittance and WVTR in linear terms. The quadratic regression coefficient of chitosan showed a significant effect ($p < 0.05$) on moisture, solubility and WVTR; glycerol level on moisture, L^* value and transmittance; and drying temperature on a^* value, penetrability, transmittance and WVTR. The effect of interaction of glycerol x temperature as well as chitosan x temperature was also significant ($p < 0.05$) on a^* value and WVTR of edible films. The optimized conditions were: 2.0 % w/v chitosan level, 0.75 % w/v glycerol level and drying temperature 40 °C at a constant time of 48 h. All the response variables were in favourable range including thickness; 108.59 μm , penetrability; 16.41 N, transmittance; 75.60 %, WVTR; 0.00174 $\text{g}/\text{m}^2\text{-t}$ for the optimized edible film. Results concluded that edible films with desirable bio-mechanical properties can be successfully developed and effectively utilized in the food packaging industry.

Keywords Chitosan · Edible films · Response surface methodology · Box-behnken design

Introduction

The continuously increasing interest of consumers in the quality, convenience and safety of food has encouraged research into edible films. An edible film is a thin, continuous layer of edible material formed or placed on or between foods or food components (Bravin et al. 2006). The application of edible films permit very diverse objectives such as the control of moisture loss, microbial growth, preservation of the structural integrity of the product or gradual release of antioxidant and antimicrobials into the food and food products (Arvanitoyannis et al. 1996). Edible and biodegradable films are always not meant for total replacement of the synthetic packaging films (Krochta and Johnston 1997). The most commonly occurring and applied natural polymers include polysaccharides (starch, cellulose and its derivatives, chitosan, alginate, gellan gum), proteins (collagen, zein, soybean and gluten proteins, milk proteins) and fats (bee wax, candelilla wax, carnauba wax, fatty acids, glycerols) (Donhowe and Fennema 1993; Park et al. 1994; Nussinovitch and Hershko 1996; Ayranci and Tunc 1997; Xie et al. 2002; Pomet et al. 2003; Bravin et al. 2006; Casariego et al. 2008 and Saucedo-Pompa et al. 2009). Films prepared with these polymers, are generally biodegradable, nontoxic, and some of them are effective barriers to oxygen and carbon dioxide. Edible films made from carbohydrates generally exhibit lower moisture barriers due to their hydrophilicity (Hiemenz and Rajagopalan 1997). Moreover, there is a growing interest to develop newer packaging materials with antimicrobial properties to prevent microbial deteriorative changes in foods. Various materials were explored and chitosan is considered as interesting film forming material (Dutta et al. 2009).

T. P. Singh · M. K. Chatli (✉) · J. Sahoo
Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, Punjab, India
e-mail: manishchatlilpt@gmail.com

Chitosan is a natural polymer consisting of (1,4)-linked 2-amino-deoxy-b-D-glucan, deacetylated derivative of chitin, which is the major constituent of the exoskeleton of crustaceans and is the second most abundant polysaccharide found in nature after cellulose. Chitosan has been found to be nontoxic, biodegradable, biofunctional, biocompatible in addition to having antimicrobial characteristics (Darmadji and Izumimoto 1994; Jayakumar et al. 2007). In addition, chitosan is an excellent edible film component due to its film-forming capacity and good mechanical properties and can form transparent films, which can fulfill various packaging needs (Srinivasa et al. 2002). As compared with other bio-based food packaging materials, chitosan-based films have also been claimed as effective carriers of many functional ingredients, such as antimicrobial agents and antioxidants to extend storage quality of food (Tripathi et al. 2008, Pranoto et al. 2005, Park and Zhao 2004). Chitosan films can be prepared by cross-linking with agylcone geniposidic acid (Mi et al. 2006), ternary chitosan-glucomann-nisin (Li et al. 2006), blending of ferulic acid incorporated starch chitosan (Mathew and Abraham 2008), incorporation of garlic oil, potassium sorbate and nisin (Pranoto et al. 2005), chitosan-potato starch film using microwave treatment (Tripathi et al. 2008), starch/chitosan blend film under the action of irradiation (Zhao et al. 2004) and water soluble chitosan and amylose film (Suzuki et al. 2005) etc. However, chitosan films are rigid and need plasticizers to diminish film fragility and increase film flexibility and manageability. These plasticizers reduce the intermolecular forces and increase mobility of polymer chains, thereby improving flexibility and extensibility of the film however, plasticizers generally increase water vapour and solute permeability of the film (Sobral et al. 2001). Therefore, the incorporation of plasticizers in the formulation of the film improve mechanical properties and maintain them during longer storage period. (Srinivasa et al. 2007; Kerch and Korkhov 2011).

Response surface methodology is a set of statistical techniques, designing experiments, for building models, searching the optimum conditions and evaluating the effects of factors (Manivannan and Rajasimman 2011). This methodology presupposes the use of experimental design techniques to investigate and learn about the functional form of a process that involves factors or independent variables. Various authors have used this technique to developed the protein based film from lizard fish muscle (*Saurida Undosquamis*) (Wittaya and Sopanodora 2009), standardization of the concentrations of chitosan and glycerol for coating Berangan banana (*Musa sapientum* cv. *Berangan*) (Malmiri et al. 2011), standardization of the formulation and processing technology of whey protein films (Ozdemir and Floros 2001) and the interactive effects of glycerol and chitosan on tapioca starch-based edible film properties (Chillo et al. 2008).

The goal of the present work was to prepare chitosan based films and to improve mechanical properties of the films by the

addition of glycerol as plasticizers. This paper mainly focused on the influence of the effects of three different levels of chitosan, glycerol and drying temperature on response variables which are relevant for food packaging applications.

Materials and methods

Preparation of chitosan based edible films

Chitosan edible films were prepared by dissolving 1.5–2.5 % w/v of chitosan (Bio basic Canada Inc., Canada) level in a stirred mixture of 1 % (v/v) acetic acid (SDFCL, Mumbai India) aqueous solution containing 0.5–1.0 % w/v glycerol (Fisher Scientific Pvt. Ltd. Mumbai, India) as plasticizer by heating for 20 min at 90 °C on hot plate magnetic stirrer (Macro Scientific Works, India). The above levels of chitosan, glycerol and drying temperature were selected on the basis of preliminary trials and on consultation with literature (Dutta et al. 2009; Rhim et al. 2006; Srinivasa et al. 2007; Hosseini et al. 2008). Thereafter, the solution was filtered through a cheese cloth to remove undissolved material. These solutions (250 ml) were then casted on fiber glass plates (18×12 cm) and dried at 35–45 °C for 48 h in an incubator (Macro Scientific Works, India). The dried films obtained were peeled off manually and stored in a humidity chamber at 50 % RH and 25 °C and thereafter analysed for different responses viz. thickness, moisture content, solubility, L^* value, a^* value, b^* value, penetrability, density, transmittance and water vapor transmission rate (WVTR). Box-Behnken experimental design was used in which a total of 17 different trials were conducted with three different levels of each of chitosan levels, glycerol levels and drying temperature with number of centre points 5 (2.0,0.75,40) as presented in Table 1.

Processing parameters of chitosan films

Thickness

Film thickness was measured with an electronic digital micrometer (Forbes Gokak Ltd., Measuring Instrument, Aurangabad, Mumbai, India). The thickness was measured

Table 1 Coded and uncoded values of processing variables

Independent variables	Levels		
	–1	0	1
Chitosan level (% w/v) X_1	1.5	2.0	2.5
Glycerol level (% w/v) X_2	0.50	0.75	1.0
Drying temperature (°C) X_3	35	40	45

at five different points at center and on the sides covering the complete length of film and mean value was calculated.

Moisture content

The moisture content (MC) of films was determined as per the gravimetric method. Briefly, 500 mg of film was dried at $105 \pm 1^\circ\text{C}$ for 24 h (till the equilibrium weight was attained). The weight loss of the sample was determined and MC was calculated as the percentage of water removed from the system.

Water solubility

The water solubility of films was determined as the content of dry matter solubilized after 24 h of immersion in water. The films weighing 500 mg was immersed in 50 ml of water (at $23 \pm 1^\circ\text{C}$) for 24 h of immersion with agitation at regular interval. After that pieces of film were taken out and dried till constant weight achieved in a hot air oven at $105 \pm 1^\circ\text{C}$. Solubility (%) was calculated as, $\text{Solubility (\%)} = [(\text{Wt. of initial dry film} - \text{Wt. of undissolved film}) / \text{Wt. of initial dry film}] \times 100$.

Colour profile analysis

Colour profile was measured using Lovibond Tintometer (Lovibond RT-300, Reflectance Tintometer, United Kingdom) set at 2° of cool white light (D_{65}) and known as L^* , a^* , and b^* values. L^* value denotes (brightness 100) or lightness (0), a^* (+ redness/- greenness), b^* (+ yellowness/- blueness) values were recorded on chitosan film kept in a plate. The instrument was calibrated using light trap (black hole) and white tile provided with the instrument. The instrument was directly put on the surface of three individual chitosan films and values displayed were noted.

Penetrability

Penetrability was determined by simulating the conditions to measure the force required to pierce the edible film. The probe used had a diameter of 5 mm and suitable sample size of edible film was subjected to run at 30 mm/min with a displacement of 20 mm and load cell of 100 N. Penetrability was calculated automatically by the preloaded software in the texture analyzer (TMS-PRO, Food Technology Corporation, USA) from the force-time plot.

Density

Density of the film was determined using a floatation method at 25°C using CCl_4 (1.5935 g/ml) and heptane (0.71 g/ml) as solvents. The film (1.5×1.5 cm) was immersed in 5 ml heptane taken in a small beaker. CCl_4 was taken in a burette and

added drop wise to the beaker until the film floats in the middle of the solution and density of the film was calculated (Qurashi et al. 1992) as,

$$\text{Density (g/ml)} = (V_1 d_1 + V_2 d_2) / (V_1 + V_2)$$

Where

- V_1 Volume of heptane in ml
- V_2 Volume of CCl_4 in ml
- d_1 Density of heptane in g/ml
- d_2 Density of CCl_4 in g/ml

Transmittance

Transmittance of chitosan films was determined by placing the film strips ($3 \text{ cm} \times 1 \text{ cm}$) in the cuvette containing water and was measured the percentage transmittance at fixed wavelength of 660 nm using UV-VIS spectrophotometer (Elico SL-159, Mumbai, India).

Water vapor transmission rate (WVTR)

Water vapor transmission rate was measured using a modified ASTM 96–00 method (ASTM 2000). The film was sealed on a modified test cell containing 15 mL of distilled water. The test cell was then kept in a desiccator containing pre-dehydrated silica gel. Silica gels were dried at 180°C for 3 h for these measurements. The whole assembly was kept at 25°C and weight loss of the test cell was measured after storage for 24 h. WVTR of the film was calculated according to the equation, $\text{WVTR} = \Delta W / (\Delta t \times A)$ where ΔW is the weight loss of test cell, Δt is the time of storage, and A is the area of exposed film.

Statistical analysis

Response surface methodology (RSM) using 3-factor-3-level Box-Behnken experimental design (Montgomery 2005, Box and Behnken 1960) with five replicates at the centre point was employed to develop predictive models for different responses. In this design, X_1 , X_2 and X_3 are the coded variables, which are related to uncoded variables in actual units linearly by the relation, $X_i = 2(\xi_i - \bar{\xi}) / d_i$, where ξ_i is the variable value in actual units of the i^{th} observation. $\bar{\xi}$ is the mean of highest and lowest variable value of ξ_i and d_i is the difference between the highest and lowest variable value of ξ_i . The levels of 3 factors (processing variables), experimental design in terms of coded and uncoded values and the experimental responses are presented in Tables 1 and 2. The data was analysed employing multiple regression technique to develop a response surface model. After conducting the runs, a second

order polynomial of the following form was fitted to each response using the “Design Expert” software (Version 8.0.4.1, Stat-Ease, Inc., Minneapolis, USA) statistical package.

$$y' = \beta_o + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i=1}^3 \sum_{j=1, j \neq i}^3 \beta_{ij} x_i x_j + \varepsilon$$

$$Y = \beta_o + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i=1}^3 \sum_{j=1, j \neq i}^3 \beta_{ij} x_i x_j + \varepsilon$$

Where Y is the estimated response; β_o is the constant coefficient, β_i is the linear coefficient, β_{ii} the quadratic coefficient and β_{ij} the second order interaction coefficient. x_i , x_j are coded independent variables and ε is the error involved in estimating the coefficients β from the experimental data. After fitting the equation several targets of the responses were given through the software for achieving the best combination of variables which resulted in required product. Target values were given in the form of ranges of values of all the process variables and responses. All the models were tested for their adequacy using ANOVA technique. The F-values and R^2 values were computed for all the responses (Table 3). Surface plots and equations were developed as a function of the variables on each response. The full

second order model of the form was fitted to data and regression coefficients were computed the results of which are reported in Table 4.

Results and discussion

Perusal of Table 3 revealed that for all the responses, F-values for the “model” were significant and that for “lack of fit” were non-significant ($p < 0.05$) thereby confirming the validity of the models. Also these models adequately explained the variation of the responses with satisfactory R^2 values, which indicated that most variations well explained by different, models (Linear, 2FI or Quadratic).

Thickness

The following second order polynomial equation showed a relationship between the independent variables chitosan level (X_1), glycerol level (X_2) and drying temperature (X_3) and the dependent variable thickness as, Thickness = $221.09 + 18.25X_1 - 80X_2 - 2.73X_3 + 16X_1X_2 - 0.5X_1X_3 + 1.2X_2X_3$; $R^2 = 0.9889$

From the above equation, it was seen that thickness was found to have 2FI relationship with the three variables. This shows that interaction of chitosan and glycerol

Table 2 Second order design matrix used to evaluate the effects of process variables and values of experimental responses for chitosan based edible film

Runs	Variables			Responses									
	Chitosan (X_1) % w/v	Glycerol (X_2) % w/v	Temp. (X_3) °C	Thickness (mm)	Moisture (%)	Solubility (%)	L^*	a^*	b^*	Penetrability (N)	Density (g/ml)	Transmittance (%)	WVTR (g/m ² -t)
1	2.00	0.50	45	93.00	13.47	24.46	46.36	0.87	7.28	13.79	1.431	79.62	0.00203
2	2.50	0.50	40	111.00	12.20	19.63	46.37	0.91	7.38	16.31	1.433	75.49	0.00174
3	2.50	0.75	35	131.00	13.43	20.24	45.02	0.97	7.36	16.92	1.404	73.89	0.00151
4	2.00	0.75	40	111.00	14.57	25.12	44.51	0.81	7.08	16.33	1.389	75.57	0.00176
5	1.50	0.50	40	105.00	19.30	28.18	44.69	0.77	7.99	16.18	1.421	76.67	0.00168
6	2.00	0.75	40	109.00	15.06	25.21	44.78	0.85	7.29	16.42	1.395	75.73	0.00176
7	2.00	0.75	40	108.00	14.50	25.45	44.39	0.82	6.98	16.46	1.395	75.43	0.00172
8	2.00	0.75	40	108.00	14.70	24.93	44.03	0.86	6.99	16.48	1.393	75.64	0.0017
9	2.00	1.00	35	120.00	14.50	25.35	45.17	0.84	7.34	16.81	1.365	74.79	0.0016
10	1.50	1.00	40	101.00	19.00	28.57	45.48	0.76	7.31	15.96	1.359	77.07	0.0017
11	2.00	0.75	40	107.00	15.00	25.05	44.62	0.83	7.39	16.35	1.394	75.61	0.00176
12	2.00	1.00	45	96.00	13.47	24.64	44.48	0.95	7.76	14.01	1.361	79.51	0.00204
13	1.50	0.75	35	118.00	19.50	28.34	42.65	0.75	7.43	16.77	1.384	75.12	0.00159
14	2.50	0.75	45	99.00	13.43	20.51	44.44	0.99	7.21	14.12	1.406	78.91	0.00211
15	2.00	0.50	35	123.00	13.63	25.38	45.59	0.92	7.89	16.84	1.427	74.34	0.00164
16	1.50	0.75	45	91.00	19.13	28.21	45.28	0.78	7.81	13.65	1.382	79.77	0.00186
17	2.50	1.00	40	115.00	11.87	19.77	45.65	0.94	7.22	16.21	1.371	75.32	0.00172

X_1 is coded independent variable for chitosan level, X_2 is coded independent variable for glycerol level, X_3 is coded independent variable for temperature, L^* , Lightness, a^* , Redness, b^* , Yellowness, N, Newton, WVTR, Water Vapor Transmission Rate, Temp., Temperature

Table 3 Significance of the regression models (F values) and the effects of processing variables on the chitosan based edible film

Source of variance	Thickness $R^2=0.9889$ F value	Moisture $R^2=0.9864$ F value	Solubility $R^2=0.9939$ F value	L^* value $R^2=0.8799$ F value	a^* value $R^2=0.9641$ F value	b^* value $R^2=0.8386$ F value	Penetrability $R^2=0.9973$ F value	Density $R^2=0.9854$ F value	Transmittance $R^2=0.9974$ F value	WVTR $R^2=0.9895$ F value
Linear										
β_1	101.92 ^a	429.72 ^a	1114.39 ^a	6.58 ^a	149.37 ^a	7.05 ^a	15.14 ^a	58.01 ^a	145.41 ^a	12.09 ^a
β_2	0.00	0.037	0.47	2.86	0.11	3.11	0.26	822.18 ^a	1.87	0.17
β_3	774.15 ^a	1.55	2.25	2.61	3.21	6.01×10^{-3}	2096.99 ^a	0.00	2232.49 ^a	558.84 ^a
Cross product										
β_{12}	7.76 ^a	1.14×10^{-3}	0.13	2.62	0.85	2.03	0.44	-	3.75	0.62
β_{13}	3.03	0.17	0.32	11.86 ^a	0.05	2.11	3.10	-	1.58	42.12 ^a
β_{23}	4.37	0.96	0.09	2.45	13.60 ^a	7.98 ^a	1.89	-	3.62	0.97
Quadratic										
β_{11}	-	63.03 ^a	26.06 ^a	4.08×10^{-3}	0.30	1.45	7.37 ^a	-	7.74 ^a	13.19 ^a
β_{22}	-	16.93 ^a	1.98	22.07 ^a	2.51	6.24 ^a	7.68 ^a	-	22.73 ^a	1.47
β_{33}	-	0.26	0.07	0.34	17.51 ^a	5.04	434.15 ^a	-	246.86 ^a	32.24 ^a
Lack of fit	0.83	5.87	6.19	5.00	1.22	0.94	3.08	1.88	2.85	0.55
Total model	148.54 ^a	56.59 ^a	127.39 ^a	5.70 ^a	20.89 ^a	4.04 ^a	287.03 ^a	293.40 ^a	298.05 ^a	73.55 ^a

^a $p < 0.05$; R^2 , Coefficient of determination, DF , degrees of freedom, L^* , Lightness, a^* , Redness, b^* , yellowness, $WVTR$, Water Vapour Transmission Rate

level significantly ($p < 0.05$) affected thickness of edible films. However, linearly chitosan and drying temperature has significantly ($p < 0.05$) higher effect on thickness of films (Table 3). The “Predicted R-Squared” of 0.9628 is in reasonable agreement with the “Adjusted R-Squared” of 0.9822 was explained by software. The R^2 value (0.9889), being a measure of the goodness of fit of the model, indicated that 98.89 % of the total variation was explained by the model (Table 3). Whereas, adjusted R^2 value (0.9822) showed the significance of the model. Also low value of the coefficient of variation (1.32 %) indicated higher precision and reliability of the experiment. With the increase in chitosan level, there was increase in the thickness of films, but it was not affected by glycerol level (Fig. 1a and b). Film thickness depends on the nature and composition of the films. These observations are in agreement with the views of Sebti et al. (2007). The inference was drawn from the results that glycerol did not influence the film thickness. The effect of glycerol on the thickness is directed by the level of incorporation, which was less than 1.0 % in our study. The equation depicted that there were positive interactions between chitosan and glycerol level, glycerol and drying temperature whereas, negative interactions between chitosan level and drying temperature for film thickness. Hence, it can be predicted that film thickness is directed by concentration of chitosan and drying temperature. Figure 1 b and c displayed that there was decrease in thickness in a linear fashion. It might be due to more drying effect of temperature on chitosan films.

Moisture

Packaging films should maintain moisture levels within the packaged product. Therefore, the knowledge of moisture content and total soluble matter of the films is very important for food packaging applications (Leceta et al. 2013). The amount of water present in films provide an indication of the hydrophobicity of the films, hence, the hydrophilic films have higher moisture content (Bourbon et al. 2011). In the present study, it was found to fit with the three variables as perquadratic relationship. The best model equation for moisture was, Moisture = $39.34 - 35.39X_1 + 28.54X_2 + 0.37X_3 - 0.06X_1X_2 + 0.04X_1X_3 - 0.17X_2X_3 + 6.86X_1^2 - 14.23X_2^2 - (4.37 \times 10^{-3})X_3^2$; $R^2 = 0.9864$

Chitosan content directed significantly ($p < 0.05$) higher effect on moisture both in linear and quadratic terms. The effect of glycerol level (quadratic) on moisture was also significant ($p < 0.05$) (Table 3). The R^2 value (0.9864) indicated that 1.36 % of the total variation was not explained by the present model (Table 3). The Model F-value was significant and there is only a 0.01 % chance that it could occur due to noise. With the increase in chitosan level, moisture content decreased and thereafter increased after reaching to a certain minima and reverse was true with glycerol level (Fig. 2a, b and c). Bonilla et al. (2013) also postulated that positively charged chitosan chains have wide hydration layers with high retention of water molecules which take part in the film structure thus inhibiting the chain approximation and giving rise to thicker films. Fundo et al. (2008) observed that high

Table 4 Values of regression coefficients estimated by multiple linear regression for response variables and their significance

Term	Regression coefficients	Thickness (mm)	Moisture (%)	Solubility (%)	L^* value	a^* value	b^* value	Penetrability (N)	Density (g/ml)	Transmittance (%)	WVTR ($\text{g/m}^2\cdot\text{s}$)
Constant	β_0	221.09	39.34	33.50	3.83	4.23	28.11	-29.94	1.46	134.51	6.80×10^{-3}
Chitosan level (A)	β_1	18.25	-35.39	4.46	15.72	0.26	-0.71	0.71	0.02	-5.07	-4.78×10^{-4}
Glycerol level (B)	β_2	-80.00	28.54	5.44	-9.00	-1.83	-16.10	0.40	-0.13	-1.16	-6.15×10^{-4}
Temperature (C)	β_3	-2.73	0.37	-0.30	-1.34	-0.16	-0.69	2.56	-2.76×10^{-19}	-3.11	-2.63×10^{-4}
Chitosan level * chitosan level (A^2)	β_{11}	NS	6.86	-3.49	0.06	-0.02	0.43	-0.48	NS	0.80	-1.80×10^{-4}
Glycerol level * glycerol level (B^2)	β_{22}	NS	-14.23	-3.86	17.07	-0.27	3.55	-1.96	NS	5.47	2.40×10^{-4}
Temperature * temperature (C^2)	β_{33}	NS	-4.37×10^{-3}	1.86×10^{-3}	-5.32×10^{-3}	-1.77×10^{-3}	7.98×10^{-3}	-0.04	NS	0.05	2.90×10^{-6}
Chitosan * glycerol (AB)	β_{12}	16.00	-0.06	-0.50	3.02	0.08	1.04	-0.24	NS	-1.14	-8.00×10^{-5}
Chitosan * temperature (AC)	β_{13}	-0.50	0.04	0.04	-0.32	-1.00×10^{-3}	-0.05	0.03	NS	0.04	-3.30×10^{-5}
Glycerol * temperature (BC)	β_{23}	1.20	-0.17	0.04	-0.29	-0.03	0.21	0.05	NS	-0.11	-1.00×10^{-5}

β_0 , Intercept, β_i , regression coefficients for linear terms, β_{ij} , regression coefficients for quadratic terms, β_{ijk} , regression coefficients for interactive terms, Significant at $p < 0.05$, NS, Non-significant, L^* , Lightness, a^* , redness, b^* , yellowness, N, Newton, WVTR, Water Vapour Transmission Rate

chitosan/glycerol concentration solutions led to films with significantly ($p < 0.05$) higher water content. This can be related with higher molecular entanglement and viscosity, which lead to higher retention of water molecules during drying of the films. Sobral et al. (2001) observed that the increase in the plasticizer concentration increases the moisture content of the film because of its high hygroscopic character, which also contributes to the reduction of the forces between the adjacent macromolecules. There was non-significant increase in moisture content with the increase in temperature (Fig. 2c and b). It might be due to interaction with chitosan and glycerol.

Solubility

Water solubility is an important property of edible films that gives indication of the film's water affinity (Bourbon et al. 2011), for its applications in food protection, this property become significant when films come in contact with high moisture food products and food product with high water activity and during storage.

Solubility was found to have quadratic relationship with the three process variables as per the following equation,

$$\begin{aligned} \text{Solubility} = & 33.50 + 4.46X_1 + 5.44X_2 - 0.30X_3 - 0.50X_1X_2 \\ & + 0.04X_1X_3 + 0.04X_2X_3 - 3.49X_1^2 - 3.86X_2^2 \\ & + (1.86 \times 10^{-3})X_3^2; R^2 = 0.9939 \end{aligned}$$

Chitosan affected solubility significantly ($p < 0.05$) both in linear and quadratic terms. The R^2 value indicated that regression model could explain 99.39 % of total variations (Table 3). Furthermore, a very high degree of precision and a good deal of the reliability of the experiment was indicated by a low value of the coefficient of variation ($CV = 1.42\%$). With the increase in glycerol level, there was an increase in the solubility of the films, however the solubility increased in a curvilinear fashion with the increase in chitosan level (Fig. 3a and b). It might be due to the presence of plasticizer, which decrease the polymer network interaction density and associated increase in solubility properties (Stuchell and Krochta 1994). It might be due to the three hydrophilic hydroxyl groups present in glycerol that are responsible for its solubility in water (Chillo et al. 2008). Perusal of equation and Fig. 3b revealed that the solubility did not vary with the changes in temperature. However, on interaction with glycerol level, the increases in temperature lead to decrease in solubility (Fig. 3c). Wittaya & Sopanodora 2009 also stated that increase in temperature leads to decrease in the solubility of films. Bourtoom (2008) reported that an increase of film solubility with increasing plasticizer concentration could be briefly explained by hydrophilic plasticizers enhancing film solubility in water.

Fig 1 Surface plot (3-D) for thickness (μm). **a** Effect of glycerol and chitosan levels on thickness of films. **b** Effect of temperature and chitosan levels on thickness of films. **c** Effect of temperature and glycerol levels on thickness of films

Lightness (L^*) value

Packaging films have a significant influence on the appearance and consumer's acceptance of the products. Instrumental colour profile is of prime importance to determine the effect of films on the product appeal and consumer acceptability (Ramos et al. 2013). The following equation showed a quadratic relationship between the independent and dependent variable.

$$L^* = 3.83 + 15.72X_1 - 9.00X_2 - 1.34X_3 + 3.02X_1X_2 - 0.32X_1X_3 - 0.29X_2X_3 + 0.06X_1^2 + 17.07X_2^2 - (5.32 \times 10^{-3})X_3^2; \\ R^2 = 0.8799$$

Fig 2 Surface plot (3-D) for moisture %. **a** Effect of glycerol and chitosan levels on moisture content of films. **b** Effect of temperature and glycerol levels on moisture content of films. **c** Effect of temperature and chitosan levels on moisture content of films

The analysis of variance showed that chitosan (linearly) and chitosan x temperature interaction significantly ($p < 0.05$) affected L^* value of the films. The quadratic effect of glycerol level on L^* value was also significant ($p < 0.05$). The R^2 value (0.8799), indicated that 87.99 % of the total variation was explained by the model (Table 3). Adequate precision ratio (10.55), indicated adequate signal and was used to navigate the design space as suggested by the software. L^* value always improved with the increase in chitosan level and temperature (Fig. 4a, b and c) however, it increased with the decrease in glycerol level (Fig. 4a

Fig 3 Surface plot (3-D) for solubility %. **a** Effect of glycerol and chitosan levels on solubility (%) of films. **b** Effect of temperature and chitosan levels on solubility (%) of films. **c** Effect of temperature and glycerol levels on solubility (%) of films

and **c**). Our results are not in accordance with the study of Ramos et al. (2013). They documented that increase in glycerol level in the formulation of whey protein concentrate films lead to increase in the reflection of light on the film surface, recorded higher L^* values. The variation in the results can be attributed to the variation in the base material used for the development of film as chitosan and whey protein concentrate in later reference.

Fig 4 Surface plot (3-D) for L^* value. **a** Effect of glycerol and chitosan levels on L^* of films. **b** Effect of temperature and chitosan levels on L^* of films. **c** Effect of temperature and glycerol levels on L^* of films

Redness (a^*) value

Redness (a^*) also showed quadratic relationship with independent variables as,

$$a^* = 4.23 + 0.26 X_1 - 1.83 X_2 - 0.16 X_3 + 0.08 X_1 X_2 - (1.00 \times 10^{-3}) X_1 X_3 - 0.03 X_2 X_3 - 0.02 X_2^2 - 0.27 X_2^2 - 1.77 \times 10^{-3} X_3^2; R^2 = 0.9641$$

Chitosan (linear), glycerol $\times$ temperature interaction and temperature (quadratic terms) significantly ($p < 0.05$)

affected a^* value of films (Table 3). The R^2 value (0.9641), indicated that only 3.59 % of the total variation was not explained by the model (Table 3). Chitosan always improved a^* value of the films (Fig. 5a and b), but it varied with change in temperature and glycerol level (Fig. 5a, b and c). Leceta et al. (2013) observed that there was a significant increases ($p<0.05$) in redness value a^* value with the addition of glycerol. Therefore, the optimization of the glycerol level and drying temperature is very important to develop chitosan films with optimum redness for the packaging of fresh red and white meat.

Fig 5 Surface plot (3-D) for a^* value. **a** Effect of glycerol and chitosan levels on a^* of films. **b** Effect of temperature and chitosan levels on a^* of films. **c** Effect of temperature and glycerol levels on a^* of films

Yellowness (b^*) value

Yellowness (b^*) was found to have quadratic relationship with the three process variables as per the following equation,

$$b^* = 28.11 - 0.71X_1 - 16.10X_2 - 0.69X_3 + 1.04X_1X_2 - 0.05X_1X_3 + 0.21X_2X_3 + 0.43X_1^2 + 3.55X_2^2 + (7.98 \times 10^{-3})X_3^2; \\ R^2 = 0.8386$$

Chitosan (linear), glycerol x temperature and glycerol (quadratic terms), all significantly ($p<0.05$) influenced b^* value of the films (Table 3). The R^2 value (0.8386) indicated that 83.86 % of the total variation was explained by the model (Table 3). With the variation in chitosan, glycerol and temperature, b^* value initially showed a decrease and thereafter followed by an increasing trend (Fig. 6a, b and c). Leceta et al. (2013) observed that yellowness value increased ($p<0.05$) with the addition of glycerol. It indicated that temperature had a significant influence on the structure of the films which in turn affect the crosslinking between amino groups in chitosan and reducing aldehyde carbonyl groups leading to a tighter structure, thus yellowish colour of the sample. Bourtoom (2008) also reported that increased yellowness occurred when higher plasticizers were used.

Penetrability

Penetrability is a measure of the resistance of the film to be punctured. When packed product has protuberances, film should show good biaxial mechanical properties in order to maintain integrity during their application and subsequent shipping and handling of food and food products (Bonilla et al. 2013; Leceta et al. 2013). The films with better penetrability strength are useful to pack the bone-in, cut-up-parts of meat animal carcass. In the present study, it was found to fit with the three variables as per quadratic relationship. The best model equation for penetrability was,

$$\text{Penetrability} = -29.94 + 0.71X_1 + 0.40X_2 + 2.56X_3 - 0.24X_1X_2 + 0.03X_1X_3 + 0.05X_2X_3 - 0.48X_1^2 - 1.96X_2^2 - 0.04X_3^2; R^2 = 0.9973$$

This shows that chitosan level (linear and quadratic effect), glycerol level (quadratic effect) and drying temperature (linear and quadratic effect) significantly ($p<0.05$) affected the penetrability of films (Table 3). Furthermore, a very high degree of precision and a good deal of the reliability of the conducted experiment was indicated by a low value of the coefficient of variation (CV=0.57 %). The R^2 value (0.9973) implied that

Fig 6 Surface plot (3-D) for b^* value. **a** Effect of glycerol and chitosan levels on b^* of films. **b** Effect of temperature and chitosan levels on b^* of films. **c** Effect of temperature and glycerol levels on b^* of films

only 0.27 % of the total variation was not explained by the model (Table 3). With glycerol, chitosan and temperature; penetrability increased and thereafter decreased after reaching to certain maxima (Fig. 7a, b and c). The ability of the plasticizer to expand chitosan chain facilitates mobility on

Fig 7 Surface plot (3-D) for penetrability (N). **a** Effect of glycerol and chitosan levels on penetrability (N) of films. **b** Effect of temperature and chitosan levels on penetrability (N) of films. **c** Effect of temperature and glycerol levels on penetrability (N) of films

the plane of the film. Leceta et al. (2013) studied that the addition of glycerol promotes chain mobility in the direction of the deformation, increasing puncture deformation values for films with higher glycerol content.

Density

Density was found to have linear relationship with the three independent variables as described by the following equation,

$$\text{Density} = 1.46 + 0.02X_1 - 0.13X_2 - (2.76 \times 10^{-19})X_3; \\ R^2 = 0.9854$$

The analysis of variance showed that linearly chitosan and glycerol level significantly ($p < 0.05$) affected the density of

films (Table 3). A low value of the coefficient of variation (0.23 %) indicated higher precision and reliability of the experiment. The R^2 value (0.9854), being a measure of the ‘goodness’ of fit of the model, indicated that 98.54 % of the total variation was explained by the model (Table 3). Density showed positive linear relationship with chitosan, but negative linear relationship with glycerol and temperature (Table 4) (Fig. 8a and b). The density of a chitosan film decreases with the increase in concentration of a plasticizer (Srinivasa 2004).

Transmittance

Transmittance indicates about light barrier properties of chitosan films. This property is important to prevent lipid oxidation induced by UV light in food system (Leceta et al. 2013; Ramos et al. 2013). Hence, it is an important parameter to evaluate the efficacy of packaging films for foods (Leceta et al. 2013).

Transmittance was found to have quadratic relationship with the three process variables as per the following equation,

$$\begin{aligned} \text{Transmittance} = & 134.51 - 5.07X_1 - 1.16X_2 - 3.11X_3 - 1.14X_1X_2 \\ & + 0.04X_1X_3 - 0.11X_2X_3 + 0.80X_1^2 + 5.47X_2^2 \\ & + 0.05X_3^2; R^2 = 0.9974 \end{aligned}$$

Fig 8 Surface plot (3-D) for density (g/ml). **a** Effect of temperature and chitosan levels on density of films. **b** Effect of glycerol and chitosan levels on density of films

This shows that chitosan level (linear and quadratic effect), glycerol (quadratic) and drying temperature (linear and quadratic effect) significantly ($p < 0.05$) affected transmittance of films (Table 3). Furthermore, a very high degree of precision and a good deal of the reliability of the conducted experiment was indicated by a low value of the coefficient of variation ($CV = 0.19\%$). The R^2 value (0.9974), being a measure of the goodness of fit of the model, indicated that 99.74 % of the total variation was explained by the model (Table 3). Figure 9a, b and c exhibited that transmittance decreased with the level of chitosan however; it increased with glycerol and temperature. Yan et al. (2012) documented that the films containing 35 % glycerol had the highest light transmittance rate, whereas lowest with 30 % glycerol level. Bangyekan et al. (2006) documented that the transmittance of chitosan

Fig 9 Surface plot (3-D) for transmittance %. **a** Effect of temperature and chitosan levels on transmittance (%) of films. **b** Effect of temperature and glycerol levels on transmittance (%) of films. **c** Effect of glycerol and chitosan levels on transmittance (%) of films

film (95.3–95.7 %) is slightly higher than that of starch film (91.3–94.0 %).

Water vapour transmission rate (WVTR)

WVTR is the most extensively studied property of edible films mainly because of the importance of water in deteriorative reactions in foods and made to impede moisture transfer between food and surrounding atmosphere (Bajpai et al. 2011). High WVTR of the edible film is not desirable with respect to its usage performance (Bangyekan et al. 2006). It is a mass of water vapor transmitted through a unit area in a unit time under specified condition of temperature and humidity (ASTM 2005).

The following equation showed a quadratic relationship between the independent uncoded variables X_1 , X_2 and X_3 and the dependent variable WVTR,

$$\begin{aligned} \text{WVTR} = & (6.80 \times 10^{-3}) - (4.78 \times 10^{-4})X_1 - (6.15 \times 10^{-4}) \\ & X_2 - (2.63 \times 10^{-4})X_3 - (8.00 \times 10^{-5}) \\ & X_1X_2 - (3.30 \times 10^{-5})X_1X_3 - (1.00 \times 10^{-5}) \\ & X_2X_3 - (1.80 \times 10^{-4})X_1^2 + (2.40 \times 10^{-4}) \\ & X_2^2 + (2.90 \times 10^{-6})X_3^2; R^2 = 0.9895 \end{aligned}$$

As per this model, chitosan (linearly and quadratically), temperature (linear and (quadratically) and chitosan x temperature interaction significantly ($p < 0.05$) affected WVTR of films (Table 3). The R^2 value (0.9895), being a measure of the goodness of fit of the model, indicated that 98.95 % of the total variation was explained by the model (Table 3). WVTR initially varied with chitosan level and thereafter it decreased (Fig. 10a and b). The decrease in WVTR could be attributed to the formation of intermolecular hydrogen bonding between NH_3^+ groups of the chitosan and hydroxyl groups of the glycerol. Xu et al. (2005) also observed the similar trend while working with corn starch and chitosan blend films. Li et al. (2006) documented that glucomannan-chitosan-nisin ternary antimicrobial films exhibited a decrease in WVTR with the increase of chitosan concentration. Figure 10b and c showed that there is an increase in WVTR with increase in temperature. Bajpai et al. (2011) stated that permeation of water vapour through films is enhanced with the increase in temperature. Our results are also agreement with the studies of Bonilla et al. (2013). Perusal of (Fig. 10a) indicated that WVTR initially increase with glycerol, but later on decreased after reaching to a certain maxima. It could be due to increase in inter chain spacing due to inclusion of glycerol molecules between the polymer chain which may promote water vapor diffusability through the film and hence accelerate the water vapor transmission (Yang and Paulson 2000). Gontard et al. (1993) stated that the increase of water vapor permeability

Fig 10 Surface plot (3-D) for WVTR ($\text{g/m}^2\text{-t}$). **a** Effect of glycerol and chitosan levels on WVTR of films. **b** Effect of temperature and chitosan levels on WVTR of films. **c** Effect of temperature and glycerol levels on WVTR of films

might be related to the hydrophilicity of all tested plasticizers. It is well known that the presence of plasticizers increases the concentration of polar residues in hydrocolloid based film. Myllarinen et al. (2002) found that the WVTR of polysaccharide films were related to their thickness.

Optimization of process parameters

Responses were optimized individually in combination using Design expert software. In response surface analysis, the selected model was used to calculate the stationary point. A stationary point is a point at which the slope of the response surface is zeroed in all the directions. Since the optimum response for each variable were not all in exactly the same region in the space formed by the processing variables. So, constraints were set in the form of ranges (minimum and

maximum values) for all the dependent and independent variables from Table 2, in such a way that the selected chitosan level (%w/v), glycerol (%w/v) level and drying temperature (°C) were optimum for most important attributes and close to optimum for the others. These constraints were met in the region, where chitosan level was 2 % w/v, glycerol level was 0.75 % w/v and drying temperature was 40°C. The desirability function to get optimum scores for different responses was fitted by the least square method. On the basis of ranges of these different responses, a total of 43 solutions were found out of which, the film with the above mentioned levels was having desirability of 1.0 and it was selected. Mean optimized values for thickness, moisture, solubility, L^* , a^* , b^* , penetrability, density, transmittance and WVTR of chitosan based edible film were 108.59 μm , 14.77 %, 25.15 %, 44.74, 0.83, 7.15, 16.41 N, 1.395 g/ml, 75.60 % and 0.00174 $\text{g/m}^2\text{-t}$ respectively as derived from the software.

Conclusions

Response surface methodology using Box–Behnken design was found to be an effective technique to optimize the process development of chitosan based edible film as a function of 2 % w/v chitosan level, 0.75 % w/v glycerol level and drying temperature of 40 °C for 48 h. From the response surface plots the three independent variables were found to significantly influence all the response variables either independently or interactively. Chitosan level was found to be the most significant factor ($p < 0.05$) in development of films. Using RSM the combined effect of three variables on the responses can be predicted which is difficult to achieve with conventional methods. Chitosan based edible films developed can be exploited by the food packaging industry for its use on commercial level.

References

- Arvanitoyannis E, Psomiadoub E, Nakayama A (1996) Edible films made from sodium caseinate, starches, sugars or glycerol. Part I. Carbohydr Polym 31:179–192
- ASTM (2000) Standard test methods for water vapor transmission of materials. Method E96-00. The Society, West Conshohocken
- ASTM (2005) Standard test methods for water vapour transmission of materials, methods. 1509: E96. ASTM, Philadelphia
- Ayranci E, Tunc S (1997) Cellulose-based edible films and their effects on fresh beans and strawberries. Z Lebensm UntersForsch A205(6): 470–473
- Bajpai SK, Navin C, Ruchi L (2011) Water vapour permeation and antimicrobial properties of sago starch based films formed via microwave irradiation. Int Food Res J 18:417–426
- Bangyekan C, Aht-Ong D, Sriulkit K (2006) Preparation and properties evaluation of chitosan-coated cassava starch films. Carbohydr Polym 63:61–71
- Bonilla J, Atarés L, Vargas M, Chiralt A (2013) Properties of wheat starch film-forming dispersions and films as affected by chitosan addition. J Food Eng 114:303–312
- Bourbon AI, Pinheiro AC, Cerqueira MA, Rocha CMR, Avides MC, Quintas MAC, Vicente AA (2011) Physico-chemical characterization of chitosan-based edible films incorporating bioactive compounds of different molecular weight. J Food Eng 106:111–118
- Bourtoom T (2008) Plasticizer effect on the properties of biodegradable blend film from rice starch-chitosan. Songklanakarin. J Sci Technol 30(1):149–165
- Box GE, Behnken DW (1960) Some new three level designs for the study of quantitative variables. Technom 2:455–475
- Bravin B, Peressini D, Sensidoni A (2006) Development and application of polysaccharide-lipid edible coating to extend shelf-life of dry bakery products. J Food Eng 76:280–290
- Casariogo A, Souza BWS, Vicente AA, Teixeira JA, Cruz L, Diaz R (2008) Chitosan coating surface properties as affected by plasticizer, surfactant and polymer concentrations in relation to the surface properties of tomato and carrot. Food Hydro 22:1452–1459
- Chillo S, Flores S, Mastromatteo M, Conte A, Gerschenson L, Del Nobile MA (2008) Influence of glycerol and chitosan on tapioca starch-based edible film properties. J Food Eng 88:159–168
- Darmadji P, Izumimoto M (1994) Effect of chitosan in meat preservation. MeatSci 38(2):243–254
- Donhowe IG, Fennema O (1993) Water vapor and oxygen permeability of wax films. J Am Oil Chem Society 70:867–873
- Dutta PK, Tripathi S, Mehrotra GK, Dutta J (2009) Perspectives for chitosan based antimicrobial films in food applications. Food Chem 114:1173–1182
- Fundo JF, Quintas MAC, Silva CLM (2008) Influence of film forming solutions on properties of chitosan/glycerol films. Centro de Biotecnologia e Química Fina 21:323–327
- Gontard N, Guilbert S, Cuq JL (1993) Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. J Food Sci 58:206–211
- Hiemenz PC, Rajagopalan R (1997) Surface Tension and Contact Angle, 3rd edn. Marcel Dekker, Inc., New York, pp 248–255
- Hosseini MH, Razavi SH, Mousavi SMA, Yasaghi SAS, Hasansaraei AG (2008) Improving antibacterial activity of edible films based on chitosan by incorporating thyme and clove essential oils and EDTA. J Appl Sci 8(16):2895–2898
- Jayakumar R, Nwe NT, Tokura S, Tamura H (2007) Sulfated chitin and chitosan as novel biomaterials. Int J Biol Macromol 40: 175–181
- Kerch G, Korkhov V (2011) Effect of storage time and temperature on structure, mechanical and barrier properties of chitosan-based films. Eur Food Res Techn 232:17–22
- Krochta M, Johnston CD (1997) Edible and biodegradable polymer films: Challenges and opportunities. Food Technol 51(2):61–74
- Leceta I, Guerrero P, Caba KDL (2013) Functional properties of chitosan-based films. Carbo Poly 93:339–346
- Li B, Kennedy JF, Peng JL, Yie X, Xie BJ (2006) Preparation and performance evaluation of glucomannan-chitosan-nisin ternary antimicrobial blend film. Carbohydr Polym 65(4):488–494
- Malmiri JH, Osman A, Tan CP, Abdul-Rahman R (2011) Development of an edible coating based on chitosan-glycerol to delay ‘Berangan’ banana (*Musa sapientum* cv. Berangan) ripening process. Int Food Res J 18(3):989–997
- Manivannan P, Rajasimman M (2011) Optimization of process parameters for the osmotic dehydration of beetroot in sugar solution. J Food Process Eng 34(3):804–825
- Mathew S, Abraham TE (2008) Characterisation of ferulic acid incorporated starch-chitosan blend films. Food Hydro 5(22):826–835
- Mi FL, Huang CT, Liang HF, Chen MC, Chiu YL, Chen CH (2006) Physicochemical, antimicrobial and cytotoxic characteristics of a

- chitosan film cross-linked by a naturally occurring cross-linking agent, aglycone geniposidic acid. *J Agric Food Chem* 54(9):3290–3296
- Montgomery DC (2005) *Design and Analysis of Experiments*, 6th edn. John Wiley and Sons, New York
- Myllarinen P, Partanen R, Seppala J, Forssell P (2002) Effect of glycerol on behavior of amylose and amylopectin films. *Carbohydr Polym* 50:355–361
- Nussinovitch A, Hershko V (1996) Gellan and alginate vegetable coatings. *Carbohydr Polym* 30(2–3):185–192
- Ozdemir M, Floros JD (2001) Analysis and modeling of potassium sorbate diffusion through edible whey protein films. *J Food Eng* 47(2):149–155
- Park SI, Zhao Y (2004) Incorporation of a high concentration of mineral or vitamin into chitosan-based films. *J Agric Food Chem* 52(7):1933–1939
- Park HJ, Chinnan MS, Shewfelt RL (1994) Edible corn-zein filmcoatings to extend storage life of tomatoes. *J Food Process Pres* 18:317–331
- Pommet M, Redl A, Morel MH, Guilbert S (2003) Study of wheatgluten plasticization with fatty acids. *Poly* 44:115–122
- Pranoto Y, Rakshit SK, Salokhe VM (2005) Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LWT Food Sci Technol* 38:859–865
- Qurashi MT, Blair HS, Allen SJ (1992) Studies on modified chitosan membranes. I. Preparation and characterization. *J Appl Poly Sci* 46(2):255–261
- Ramos OL, Reinas I, Silva SI, Fernandes JC, Cerqueira MA, Pereira RN, Vicente AA, Pocas MF, Pintado ME, Malcata FX (2013) Effect of whey protein purity and glycerol content upon physical properties of edible films manufactured there from. *Food Hydro* 30:110–122
- Rhim JW, Hong SI, Park HM, Perry KWNG (2006) Preparation and characterization of chitosan based nanocomposite films with antimicrobial activity. *J Agric Food Chem* 54:5814–5822
- Saucedo-Pompa S, Rojas-Molina R, Aguilera-Carbó AF, Saenz-Galindo A, de La Garza H, Jasso-Cantú D, Aguila CN (2009) Edible film based on candelilla wax to improve the shelf life and quality of avocado. *Food Res Int* 4(42):511–515
- Sebti I, Chollet E, Degraeve P, Noel C, Peyrol E (2007) Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan. *J Agric Food Chem* 55(3):693–699
- Sobral PJA, Menegalli FC, Hubinguer MD, Roques MA (2001) Mechanical, water vapour barrier and thermal properties of gelatin based edible films. *Food Hydro* 15:423
- Srinivasa PC (2004) Process development of biodegradable chitosan based films and their suitability for food packaging. Ph.D. Thesis, CFTRI, Mysore
- Srinivasa PC, Baskaran R, Ramesh MN, Harish Prashanth KV, Tharanathan RN (2002) Storage studies of mango packed using biodegradable chitosan film. *Eur Food Res Technol* 215:504–508
- Srinivasa PC, Ramesh MN, Tharanathan RN (2007) Effect of plasticizers and fatty acids on mechanical and permeability characteristics of chitosan films. *Food Hydro* 21:1113–1122
- Stuchell YM, Krochta JM (1994) Enzymatic treatments and thermal effects on edible soy protein films. *J Food Sci* 59:1322–1337
- Suzuki S, Shimahashi K, Takahara J, Sunako M, Takaha T, Ogawa K (2005) Effect of addition of water-soluble chitin on amylose film. *Biomacromolecules* 6(6):3238–3242
- Tripathi S, Mehrotra GK, Dutta PK (2008) Chitosan based antimicrobial films for food packaging applications. *E-Polym* 93:1–7
- Wittaya T, Sopanodora P (2009) Effect of some process parameters on the properties of edible film produced from Lizard Fish (*Saurida Undosquamis*) muscle. *J Food Sci Technol* 1(9):27–42
- Xie L, Hettiarachchy NS, Ju ZY, Meullenet J, Wang H, Slavik MF, Janes ME (2002) Edible film coating to minimize egg shellbreakage post-wash bacterial contamination measured by penetration in eggs. *J Food Sci* 67:280–284
- Xu YX, Kim KM, Hanna MA, Nag D (2005) Chitosan–starch composite film: preparation and characterization. *Ind Cro Prod* 21:185–192
- Yan Q, Hou H, Guo P, Dong H (2012) Effect of extrusion and glycerol content on properties of oxidized and acetylated corn starch-based films. *Carbohydr Polym* 87:707–712
- Yang L, Paulson AT (2000) Effects of lipids on mechanical and moisture barriers properties of edible gellan film. *Food Res Int* 55:571–578
- Zhai M, Zhao L, Yoshii F, Kume T (2004) Study on antibacterial starch/chitosan blend film formed under the action of irradiation. *Carbohydr Polym* 57(1):83–88