Study of mechanical properties of soy protein based edible film as affected by its composition and process parameters by using RSM

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Abstract The effect of process parameters on mechanical properties of Soy protein Isolate based edible film was studied by using response surface methodology. The process variables selected were Soy Protein Isolate (SPI) concentration, plasticizer concentration and pH whereas responses under consideration were thickness of film, tensile strength, Young's modulus and elongation at break. It was observed that as amount of SPI concentration increases in formulation, the thickness and tensile strength increased while it decreased young's modulus and elongation at break. However increase in plasticizer amount decreased thickness and tensile strength but it increased young's modulus and elongation at break. The optimum formulation for meeting the set criteria of response functions was; SPI concentration 8.65%, plasticizer concentration 60%, and pH 8.99.

Keywords Soy protein isolate · Response surface methodology · Edible film · Tensile strength · Elongation at break

Introduction

Edible films and coatings, such as wax on various fruits, have been used for centuries to prevent loss of moisture, enhancing shelf life and to create a shiny fruit surface for aesthetic purposes. These practices were accepted long before their associated chemistries were understood, and are still carried out in the present day. The term, *edible* film, has been related to food applications only in the past 50 years. There are ancient reports that spies' instructions were written on edible

films, so that if they were captured, they could easily destroy their secrets by eating them. In most cases, the term film and coating are used interchangeably to indicate that the surface of a food is covered by relatively thin layer of material of certain composition. However, a film is occasionally differentiated from a coating by the notion that it is a stand-alone wrapping material, whereas a coating is applied and formed directly on food surface itself. As recently as 1967, edible films had very little commercial use, and were limited mostly to wax layers on fruits. During intervening years, a significant business grew out of this concept (i.e., in 1986, there were little more than ten companies offering such products, while by 1996, numbers grew to 600 companies). Today, edible film use has expanded rapidly for retaining quality of a wide variety of foods.

The main components of our everyday foods (e.g., proteins, carbohydrates and lipids) can fulfill requirements for preparation of edible films. As a general rule, fats are used to reduce water transmission; polysaccharides are used to control oxygen and other gas transmission, while protein films provide mechanical stability. These materials can be utilized individually or as mixed blends to form composite films provided that they do not adversely alter food flavor. A major objective in preparing films for many foods (e.g., fresh fruit and vegetables) is to ensure that the generated films afford physical and chemical properties necessary to maintain transmission of various gases and liquids at the same rates as they occur within their native systems. Chemical structures of the three major components used to prepare films differ widely, and therefore attributes that each component contributes to overall film properties are different too.

Edible films can be prepared from proteins, polysaccharides, lipids or the combination of these components

(Gontard and Guilbert 1994). Among them, edible films of proteins are most attractive. Firstly, they are supposed to provide nutritional value (Gontard and Guilbert 1994).

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Secondly, protein-based films have impressive gas barrier properties compared with those from lipids and polysaccharides. When they are not moist, O₂ permeability of soy protein-based film was 500, 260, 540 and 670 times lower than that of low-density polyethylene, methylcellulose, starch and pectin, respectively (Cuq, et al. 1998). The mechanical properties of protein-based edible films are also better than those of polysaccharide and fat-based films because proteins have a specific structure (based on 20 different monomers) which confers a wider range of functional properties, especially high intermolecular binding potential (Cuq, et al. 1995).

Because film formation of protein is based on crosslinking of amino acids in protein and higher pH (8–13) and temperature (80–90 °C) is necessary to prepare protein-based films (Rhim, et al. 2000), the nutritional properties of proteins may be affected. However, there are few reports on evaluation of changes in nutritional properties of proteins during and after film preparation. Sabato et al. (2001) found that cross linking with irradiation and thermal treatment improved the mechanical properties of soy protein and whey protein based films. Proteins treated by alkali, acid, solvents or heat are partially denatured and when these extended structures associate through hydrogen, ionic, hydrophobic and covalent bonding they form a film matrix (Pol et al. 2002). Brandenburg et al. (1993) reported the effect of pH on mechanical and barrier properties of soy protein films. Alkali treatment increased film elongation at breakpoint and also water vapor and oxygen permeability. Soy protein is a side-product of soybean oil industry and production of biodegradable films has the potential to add value to soy protein (Rhim et al. 2000). In general, SPI films provide limited resistance to water vapor transfer due to the inherent hydrophilicity of proteins (Gennadios et al. 1994; Krochta and Mulder-Johnston 1997). However, there is a considerable interest in edible SPI films due to their potential novel packaging. Therefore the present investigation was carried out to optimize the formulation and process parameters for preparation of soy protein based edible film.

Materials and methods

Materials

Soy Protein Isolate (90 % protein) used in the present experiment was purchased from Clarion Caseins Ltd. Glycerol (LR grade) used as plasticizer, Carboxymethyl cellulose, oleic acid, sodium hydroxide (LR grade) used for pH adjustment, were purchased from local chemical supplier.

Experimental design and statistical analysis

Response Surface Methodology (RSM) was used to generate the experimental designs, statistical analysis and regression

model with the help of Design Expert Software Version 8 (Statease Inc.). The Central Composite Rotatable Design (CCRD) with a quadratic model (Box and Draper 1987) was employed. Three independent variables namely Soy Protein Isolate (SPI) concentration (x_1), plasticizer concentration (x_2) and pH (x_3) were chosen. Each independent variable had 3 levels which were −1, 0 and +1. A total of 20 different combinations (including six replicates of the centre point each signed the coded value (0) were chosen in random order according to a CCRD configuration for three factors divided in three blocks (Cochran and Cox 1957). The α -values in the design outside the ranges were selected for rotatability of the design (Thompson Joe et al. 1982).

The centre points for these designs were selected with ingredients at levels expected to yield satisfactory experimental results. The experimental design in the coded (x) and actual (X) levels of variables is shown in Table 1. The responses function (y) measured were Thickness, Tensile strength, Young's Modulus and Elongation at break of the edible film. These values were related to the coded variables (x_i , $i = 1, 2$ and 3) by a second degree polynomial using the equation below.

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (1)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1, b_2 and b_3 (linear effects), b_{11}, b_{22} and b_{33} (quadratic effects), and b_{12}, b_{13} and b_{23} (interaction effects). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significances of all terms in the polynomial were judged statistically by computing the F-value and compared with standard significance level of 0.1 %, 1 % and 5 %. The regression coefficients were then used to make statistical calculation to generate contour maps from the regression models.

Film formation and formulation

Soy protein isolate with desired concentration was dispersed in 100 ml distilled water, then glycerol was added as plasticizer followed by pH adjustment with 1 N sodium hydroxide solution to the desired value as per formulations given in Table 1. The solutions were heated on heating mantle with continuous stirring at $85 \pm 5^\circ$ Celsius temperature for 15 ± 5 min and kept at room conditions for 5 min to allow bubbling to dissipate prior to pouring. All of the solutions in the beakers were poured onto glass plate of 24" × 24" to control film thickness, the quantity of each film forming solutions poured onto these plate were always and dried overnight at room temperature.

Table 1 The Central Composite Experimental Design Employed for Preparation of Edible Film

Sr. No.	SPI Concentration (%)	Plasticizer Concentration (% of SPI)	pH
	$X_1 (x_1)$	$X_2 (x_2)$	$X_3 (x_3)$
1	8 (0)	50 (0)	9 (0)
2	8 (0)	50 (0)	9 (0)
3	8 (0)	50 (0)	9 (0)
4	8 (0)	50 (0)	9 (0)
5	8 (0)	50 (0)	9 (0)
6	8 (0)	50 (0)	9 (0)
7	8 (0)	33 ($-\alpha$)	9 (0)
8	10 ($+\alpha$)	50 (0)	9 (0)
9	8 (0)	67 ($+\alpha$)	9 (0)
10	8 (0)	50 (0)	11 ($+\alpha$)
11	6 ($-\alpha$)	50 (0)	9 (0)
12	8 (0)	50 (0)	7 ($-\alpha$)
13	9 (+1)	60 (+1)	8 (-1)
14	9 (+1)	40 (-1)	10 (+1)
15	7 (-1)	60 (+1)	10 (+1)
16	7 (-1)	40 (-1)	8 (-1)
17	7 (-1)	60 (+1)	8 (-1)
18	9 (+1)	40 (-1)	8 (-1)
19	7 (-1)	40 (-1)	10 (+1)
20	9 (+1)	60 (+1)	10 (+1)

The values in bracket i.e. x_1 , x_2 and x_3 represents the coded values for respective factors

Methodology for measurement of edible film properties

Film thickness was measured with a micrometer (No. 7327, Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan) to the nearest 0.001 mm around the film testing area at 5 random positions. Tensile strength, elongation at break and Young's modulus are the most commonly reported responses to describe mechanical properties of edible films and coatings. These parameters were determined according to the standard method D882-95 (ASTM, 1995), taking an average of three determinations in each case. The films were cut into 25 mm wide and 125 mm long strips using a scalpel, and mounted between the grips of the texture analyzer TA *plus* (Lloyds, England). The initial grip separation was set at 100 mm and the crosshead speed at 50 mm/min. The tensile strength and elongation at break were determined directly from the stress - strain curves using the software Nexgen V 4.5.

Result and discussion

Effect of SPI concentration, plasticizer concentration and pH on thickness of edible film

The data presented in Table 2 showed that thickness was positively related to linear effects of SPI concentration and

negatively related to linear effects of plasticizer concentration and pH ($p < 0.01$). Similarly it was positively related to quadratic effects of SPI concentration and pH ($p < 0.01$) and plasticizer concentration ($p < 0.1$). It is evident from Fig. 1 that, as SPI concentration increased thickness of edible film also increased whereas as plasticizer concentration increased thickness of film decreased. Similar trend was observed with respect to pH and these results are in agreement with the findings of Cho and Rhee (2004) who noted that as concentration of SPI increases, the thickness of film also increases at constant plasticizer level. The maximum thickness of 165 μm was observed at 10 % SPI and minimum thickness of 143 μm was observed with 6 % SPI. Increasing the SPI concentration increases the amount of protein molecules available for interactions and thereby resulting in more thickness. On the other hand increase in plasticizer decreases film thickness due to increased plasticization effect. The above results are in accordance with data observed with composite film from 2 % chitosan suspensions (García et al. 2004). Sobral (2000) reported that to obtain protein films by casting with higher thicknesses, either higher mass of the same solution or a higher concentration of film-forming solution has to be used. The final equations in terms of actual factors for thickness are as follows

Table 2 Regression Coefficients, R^2 and P Or Probability Values For Dependant Variables

Regression coefficient	Thickness (micron)	Tensile Strength (MPa)	Young's Modulus (MPa)	Elongation at break (%)
b_0	150.90	2.52	25.09	97.92
b_1	5.25*	0.47*	0.19*	4.00*
b_2	-4.32*	0.037	-1.34*	6.30*
b_3	-1.75*	-0.063***	-6.250E ⁻⁰⁰³	0.62
b_{12}	0.25	0.025	-0.16**	-0.75
b_{13}	0.000	0.050	-0.21**	-0.25
b_{23}	0.25	0.025	-0.11	-5.50*
B_{11}	0.83*	0.027	-0.17*	-3.81*
b_{22}	0.67***	-0.17*	-0.31*	-1.18
B_{33}	0.83*	-0.061***	0.059	-2.56*
R^2	0.9869	0.9681	0.9908	0.9589
p or probability	0.0001	0.0001	0.0001	0.0001

Subscripts 1 = SPI concentration, 2 = Plasticizer concentration, 3 = pH

* Significant at 0.01 level

** Significant at 0.05 level

*** Significant at 0.1 level

$$\begin{aligned} \text{Thickness} = & +305.07 - 9.32 * x_1 - 1.53 * x_2 - 17.98 * x_3 \\ & + 0.025 * x_1 * x_2 - 1.57E-015 * x_1 x_3 + 0.025 * \\ & x_2 * x_3 + 0.83 * x_1^2 + 6.73E-003 * x_2^2 + 0.83272 * x_3^2 \end{aligned} \quad (2)$$

Effect of SPI concentration, plasticizer concentration and pH on tensile strength of edible film

Tensile strength or more accurately the ultimate tensile strength is the maximum tensile strength that a film can sustain thus; it

Fig 1 Response Surface for Thickness of Edible Film as a Function of SPI and Plasticizer Concentration at pH 9

expresses the maximum stress developed in the film during the tensile testing. Contour map of Fig. 2 showed the effect of independent variables on the tensile strength of edible film. The tensile strength of edible film was influenced by linear effects of SPI concentration ($p < 0.01$) and pH ($p < 0.1$). The other factor contributing to tensile strength were quadratic effects of plasticizer concentration ($p < 0.01$) and pH ($p < 0.1$). Tensile strength of film increased rapidly with the increase in SPI concentration whereas it decreased slowly with the increase in pH. There was gradual increase in tensile strength at low pH followed by decline in tensile strength with increase in plasticizer concentration. The maximum and minimum tensile strength of 3.5 and 1.6 MPa was found at SPI concentration of 10 and 6 %, respectively. Banker (1966) reported that pH played an important role in protein films made from water soluble materials. At alkaline pH away from the isoelectric point, denaturation of proteins was promoted and resulted in unfolding and solubilizing of the proteins. During solubilization, the cohesive forces between the protein macromolecules were neutralized by complexing with the solvent molecules. (Pérez-Gago et al. 1999) mentioned that heat denatured whey protein films had higher tensile properties than native whey protein films.

As plasticizer concentration increased tensile strength also increased slowly and again there was fall in tensile strength with increased plasticizer concentration (Fig. 2). This behavior can be explained by the fact that glycerol could exert an antiplasticizing effect on film beyond the 'glycerol threshold' or 'glycerol antiplasticizing range'. A similar phenomenon has been reported for wheat starch films with a small amount of sorbitol incorporated (Gaudin et al. 2000). The final equations for tensile strength in terms of actual factors are as follows

Fig 2 Response Surface for Tensile Strength of Edible Film as a Function of Plasticizer Concentration and pH at SPI Concentration 8.0 %

$$\begin{aligned} \text{Tensile Strength} = & -9.86 + 0.373 * x_1 + 0.131 * x_2 + 1.30 * x_3 \\ & + 2.50E-003 * x_1 * x_2 - 0.05 * x_1 * x_3 + 2.50E \\ & -003 * x_2 * x_3 + 0.026 * x_1^2 - 1.70E-003 * \\ & x_2 - 0.060 * x_3^2 \end{aligned} \quad (3)$$

Effect of SPI concentration, plasticizer concentration and pH on Young's modulus of edible film

Young's modulus indicates the stiffness of the material and it will describe how the flexibility and mechanical properties of film relate to the chemical structure. It is evident from Table 2, that the Young's modulus of edible film was affected by positive linear effect of SPI concentration ($p < 0.01$) and negative linear effects of plasticizer concentration ($p < 0.01$). The other factors having significant effects were interaction of SPI concentration and plasticizer and SPI concentration and pH ($p < 0.05$). The quadratic effects of SPI concentration and plasticizer concentration ($p < 0.01$) were also found to influence the young's modulus of edible film.

Young's modulus of edible film increased rapidly with increase in SPI concentration but decreased linearly with plasticizer concentration (Fig. 3). The maximum and minimum value of 26.5 and 21.8 MPa for young's modulus was observed with plasticizer concentration of 33 and 67%, respectively.

At lower plasticizer concentration, the young's modulus increased with increase in pH while at lower pH, the young's modulus decreased with increase in plasticizer concentration (Fig. 3). McHugh and Krochta (1994) reported that increasing plasticizer (sorbitol and glycerol) concentration in whey protein edible films have decreased the young's modulus, and

Fig 3 Response Surface for Young's Modulus of Edible Film as a Function of SPI and Plasticizer Concentration at pH 9

increased the film flexibility. The final equation for young's modulus in terms of actual factors is as follows

$$\begin{aligned} \text{Young's Modulus} = & -10.26 + 5.57 * x_1 + 0.41 * x_2 + 1.19625 * \\ & x_3 - 0.016 * x_1 * x_2 - 0.212 * x_1 * x_3 - 0.011 * \\ & x_2 * x_3 - 0.166 * x_1^2 - 3.13E003 * x_2^2 + 0.058 * x_3^2 \end{aligned} \quad (4)$$

Effect of SPI concentration, plasticizer concentration and pH on elongation at break of edible film

Elongation at break is a measure of the film's capacity for stretching, also refers to the maximum change in length of the

Fig 4 Response Surface for Elongation at Break of Edible Film as a Function of Plasticizer Concentration and pH at SPI Concentration 8.0 %

test specimen before breaking. The effect of various process parameters on elongation at break is given in Table 2. It is evident from data that elongation at break was influenced by linear effects of SPI and plasticizer concentration ($p < 0.01$). Interaction effect of plasticizer concentration and pH was also found significant ($p < 0.01$). In addition, the quadratic effects of SPI concentration and pH negatively affected elongation at break values of edible film. The elongation at break of edible film increased gradually with SPI concentration and then decreased slightly due to effect of plasticizer concentration. As plasticizer concentration increases the elongation values also increased. The results supports the finding of Butler et al. (1996) who stated that plasticizer could easily be inserted between polymer chains, producing a “cross-linker” effect that would decrease the free volume and the segmental mobility of the polymer, decreasing the mechanical strength of the films and enhancing their extensibility.

The interaction effect of plasticizer concentration and pH (Fig. 4) revealed that at lower pH value, elongation was found to be the function of plasticizer concentration and it increased with increase in plasticizer amount. At higher pH value, elongation at break initially increased with plasticizer concentration and then decreased with further increase in plasticizer concentration. The maximum elongation at break value of 110% was obtained with plasticizer concentration of 67% while minimum value of 73% at plasticizer concentration of 40%. The final equation for elongation at break in terms of actual factors is as follows

$$\begin{aligned} \text{Elongation at break} = & -747.70 + 70.99 * x_1 + 7.35 * x_2 + 76.24 * \\ & x_3 - 0.075 * x_1 * x_2 - 0.250 * x_1 * x_3 - 0.550 * \\ & x_2 * 3.81248 * x_1^2 - 0.011 * x_2^2 - 2.56 * x_3^2 \end{aligned} \quad (5)$$

Conclusion

It can be concluded from present investigation that increase in amount of SPI in formulation increased thickness of edible film whereas increase in plasticizer and pH decreased the film thickness. As SPI concentration increased tensile strength of film increased rapidly however, as pH increased tensile strength decreased slowly. At lower pH value tensile strength of film gradually increased with increase in plasticizer concentration and further it decreased. The young's modulus and elongation at break values of edible film increased with increase in SPI but decreased with increase in plasticizer concentration.

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