

Factors Affecting the Properties of Edible Film Prepared from Mung Bean Proteins

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Abstract: This research investigated the factors affecting the properties of edible film prepared from mungbean protein (*Vigna radiate* (L.) Wilczek). The effects of pH, heating temperature and heating time of the film-solution on various film properties were determined using Response Surface Methodology (RSM). Generally, the impact of pH and heating temperature were the most significant on the film's properties in comparison to heating time. The tensile strength was highest at pH 9.5 at 75°C (5.70- 6.51 MPa) but low in elongation at break (32.06- 40.08%). Under these conditions, the water vapor permeability, films solubility and proteins solubility were at their lowest (11.37- 16.91 g.mm/m².day.kPa, 37.53- 39.43% and 19.26- 27.00%, respectively). Film color was darker and more yellowish with increase in the pH and heating temperature of film-solution.

Keywords: Edible film, mung bean protein, response surface methodology, tensile strength, water vapor permeability

INTRODUCTION

Packaging is necessary for preserving the organoleptic, nutritional and hygienic characteristics of food during storage and marketing. The wide variety of packaging films available can be divided into synthetic and edible or biodegradable. Constant progress in the technology of synthetic film preparation has expanded and supported their utilization in the food industry. However, most synthetic films are petrochemical-based and non-biodegradable, leading to environmental pollution and serious ecological problems (Tharanathan, 2003). In contrast, edible films use renewable resources as raw materials and are biodegradable, making them more environment friendly. Additionally, other adjuncts such as antimicrobials, antioxidants, nutrients, colorants, etc. are easier to add to edible films, thus further enhancing their protective functions. Edible films can be prepared from protein, polysaccharide and lipid materials (Parris *et al.*, 1995). Among them, protein-based edible

films are the most attractive. These films have impressive gas barrier properties compared with those prepared from lipids and polysaccharides. When they are not moist, the 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 that of polysaccharide and fat-based films because proteins have a unique structure (based on 20 different monomers) which confers a wider range of functional properties, especially a high intermolecular binding potential (Cuq *et al.*, 1995). Protein-based edible films can form bonds at different positions and offer high potential for forming numerous linkages (Ou *et al.*, 2005). The interest in the study of plant protein films has increased during the past decade, and research on the properties of such films has been outlined in recent literature including soy proteins (Gennadios and Weller, 1991; Gennadios *et al.*, 1994; Stuchell and Krochta, 1994; Kunte *et al.*, 1997; Rhim *et al.*,

2000), corn zein (Yamada *et al.*, 1995; Parris and Coffin, 1997), wheat proteins (Gennadios and Weller, 1990; Gennadios *et al.*, 1993; Gontard *et al.*, 1992, 1993; Sanchez *et al.*, 1998), cotton seed proteins (Marqui'e *et al.*, 1995), pea proteins (Gu'eguen *et al.*, 1998), peanut protein (Jangchud and Chinnan, 1999), and sunflower proteins (Orliac *et al.*, 2002, 2003). Mung beans are of interest as a potential component of biopolymeric films because of their high protein content. The whole seed of mung bean contains approximately 25-30% protein (Magee, 1996). Mung beans are the primary crops produced in Thailand. In industrial mung bean starch- noodle manufacturing process, mung bean starch is washed with alkaline solution to remove proteins to produce a colorless and characteristic noodle. As a result of washing, approximately 20-30% of proteins are lost in the process. Using mung bean protein not only reduces the negative environmental impact and costs of waste disposal, but may generate potential profits especially in the form of edible film from mung bean proteins. At the moment, there is very limited information on films produced from mung bean protein, their mechanical properties and application is due to the unavailability of commercial mung bean protein concentrate. It is important to have knowledge about suitable film forming methods, the effect of film forming parameters, and film characteristics for further research into their food application. The objective of this study was to investigate the factors affecting the properties of edible film prepared from mung bean protein.

MATERIALS AND METHODS

Preparation of Raw Material

Mung bean protein was prepared by using the classical method of alkaline extraction and acid precipitation from mung bean flour. Mung bean flour was mixed with distilled water in the ratio of 1:10, stirring and adjusting the final pH to 9.0 using 1 M NaOH. The suspension was extracted for 1 h using magnetic stirrer. The filtrate was centrifuged for 30 min at 4°C, 8000 rpm (Model J2-21M, Beckman Instruments Inc., Palo Alto, CA). The pH of the supernatant was adjusted to 4.5 using 1 M HCl to allow precipitation and then centrifuged for 20 min at 4°C, 8000 rpm. The isoelectric form of wet protein concentrate was then freeze-dried for 24 h (Dura-Top/Dura- Dry MP, Model TD97A001, FTS Systems, Inc), ground and placed in a plastic box and stored at -20°C until used. The nitrogen of protein concentrate was analyzed using

the Kjeldahl method. A conversion factor of 5.46 was used to calculate the protein content.

Experimental Design

General Response Surface Methodology (GRSM) was used to determine the optimum combinations of pH, heating temperature and time. GRSM are given in terms of coded variable, x_i (Cochran and Cox, 1957; Box and Draper, 1987; Thomson, 1982) Selection of levels for independent variables was based on the results from preliminary tests and observations of Jangchud and Chinnan (2000). The levels of input variables in coded (x_i) and uncoded (ξ_i) forms are given in Table 1. The experimental design consisted of fifteen experimental points, which included three replications of the center point. The fifteen films were randomly prepared. Each of the thirteen dependent Yvariables (responses) was assumed to be affected by the three independent variables. The responses under observation were: tensile strength (Y_1), percentage of elongation (Y_2), water vapor permeability (Y_3), film solubility (Y_4), protein solubility (Y_5), L* value (Y_6), a* value (Y_7) and b* value (Y_8). Each value represented the mean of three determinations. The product thus obtained was analyzed and experimental values were compared with model predictions.

Preparation of Mung Bean Proteins' Film

Freeze-dried mung bean proteins (93.52%) were dissolved in distilled water (3 g/100 ml) to prepare film-solutions. The pH (8.0, 9.0 and 10.0) was adjusted prior to adding plasticizer (sorbitol) on a protein to sorbitol ratio of 2:1. All components were homogenized (10000 rpm for 2 min) (IKA Labortecnik, Selangor, Malaysia) and heated (60, 70 and 80°C) for the given time (10, 20 and 30 min). The film-solution was cooled to room temperature, followed by vacuum application to remove any dissolved air before pouring onto leveled non-stick trays to set. Once set, the trays were held overnight at 55°C undisturbed, and then cooled to ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccator at 60% RH for further testing.

Film Testing Conditioning

All films were conditioned prior to subjecting them to permeability and mechanical tests according to Standard method, D618-61 (ASTM, 1993a). Films used for testing water vapor permeability (WVP), tensile strength (TS) and elongation (E) were conditioned at 60% RH and $27 \pm 2^\circ\text{C}$ by placing them in a desiccators over a saturated solution of

Table 1: The experiment design and data for the response surface analysis^a

Independent variables					Dependent variables						
Treatment	pH	Temp	Time	TS (MPa)	ε (%)	WVP (g.mm/m ² .day.kPa)	FS (%)	PS (%)	L*	a*	b*
1	1(10)	1(80)	0(20)	6.31	22.21	10.84	42.98	32.96	20.39	-1.39	5.55
2	1(10)	-1(60)	0(20)	5.66	35.23	10.24	41.04	31.02	19.81	-1.71	5.25
3	-1(8)	1(80)	0(20)	5.21	13.02	13.44	43.45	33.25	20.84	-1.23	4.25
4	-1(8)	-1(60)	0(20)	4.50	20.93	16.20	48.38	38.38	18.31	1.45	5.33
5	1(10)	0(70)	1(30)	6.17	37.80	10.04	38.52	28.65	19.59	-1.65	5.18
6	1(10)	0(70)	-1(10)	6.14	20.24	9.07	34.74	24.75	19.43	-1.55	5.14
7	-1(8)	0(70)	1(30)	4.96	11.70	10.11	39.63	29.63	18.56	-1.47	5.01
8	-1(8)	0(70)	-1(10)	4.61	8.83	15.29	46.11	36.10	21.60	-1.23	4.77
9	0(9)	1(70)	1(30)	6.72	21.36	10.97	43.21	33.23	18.75	-1.35	4.76
10	0(9)	1(70)	-1(10)	6.64	30.39	11.18	44.96	34.97	18.80	-1.39	4.47
11	0(9)	-1(60)	1(30)	5.25	28.49	12.25	46.49	36.49	19.74	-1.35	5.27
12	0(9)	-1(60)	-1(10)	5.01	37.70	13.04	43.31	33.32	18.77	-1.41	5.32
13	0(9)	0(70)	0(20)	6.62	31.89	10.83	42.85	32.85	18.84	-1.35	5.08
14	0(9)	0(70)	0(20)	6.63	30.41	10.83	43.02	33.01	17.85	-1.42	5.18
15	0(9)	0(70)	0(20)	6.59	33.87	10.49	42.78	32.75	18.22	-1.43	5.29

Values in parentheses are the uncoded independent variables.

A mean of three replication and the experimental runs were performed in a random order.

Mg (NO₃)₂ · 6H₂O for 72 h or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

Film Thickness

Thickness of the films was measured with a precision digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan) to the nearest 0.0001 (±5%) at five random locations on the film. Mean thickness values for each sample were calculated and used in water vapor permeability (WVP) and tensile strength (TS) calculations.

Film Solubility

A modified method of Jangchud and Chinnan (1999) was used to determine film solubility. Film pieces, 20 mm x 20 mm, were dried at 70°C in a vacuum oven for 24 h, and then weighed to the nearest 0.0001 g for the initial dry mass. Films were immersed into 20 ml of distilled water in 50 ml screw cap tubes containing 0.01g/100 g sodium benzoate. The tubes were capped and placed in shaking water bath for 24 h at 25±2°C. A portion of the solution was removed and set aside for later use during protein solubility tests as described later. The remaining solution and film piece were poured onto (Whatman #1) qualitative filter paper, rinsed with 10 ml distilled water, and dried at 70°C in a vacuum oven for 24 h to determine dry mass of film. Five measurements were taken for each treatment triplicate. Total soluble matter was calculated from the initial gross mass and final dry mass using the following equation:

$$\% \text{ FS (db)} = \frac{(\text{film mass before test} - \text{film mass after test})}{\text{Film mass before test}} \times 100$$

Water Vapor Permeability (WVP)

The gravimetric Modified Cup Method based on ASTM E96-92 was used to determine the WVP of films (McHugh *et al.*, 1994). The test cups were filled with 20 g of Silica gel (desiccant) to produce a 0% RH below the film. A sample was placed in between the cup and the ring cover of each cup coated with silicone sealant (high vacuum grease, Lithelin, Hannau, Germany) and held with four screws around the cup's circumference. The air

gap was at approximately 1.5 cm between the film surface and desiccant. The water vapor transmission rates (WVTR) of each film was measured at 60 ± 2% RH and 25±2°C. After recording the initial weight of the test cup, it was placed into a growth chamber with an air velocity rate of 450 ft/min (Model KBF115, Contherm Scientific, Lower Hutt, New Zealand). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001g with an electronic scale (Sartorius Corp.) every 3 h for 18 h. A plot of weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time (g/h). WVTR was expressed in gram units, per square meter, per day. Steady state over time (slope) yielded a regression coefficient of 0.99 or greater. Six samples per treatment were tested. The WVP of film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the film.

Tensile Strength and Elongation at Break (TS and E)

TS was measured with a Universal Testing Machine (LLOYD Instruments, Hampshire, England) as per ASTM D882-91 Standard Method (ASTM, 1993b). Ten samples, 2.54 cm x 12 cm, were cut from each film. Initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum force by initial specimen cross-sectional area, and percent elongation at break was calculated as follows:

$$E = 100 \times (d_{\text{after}} - d_{\text{before}}) / d_{\text{before}}$$

Where, d was the distance between grips holding the specimen before or after the break of the specimen.

Color

A CIE colorimeter (Hunter associates laboratory, Inc., VA, USA) was used to determine the film L*, a* and b* color value (L* = 0 (black) to 100 (white); a* = -60 (green) to +60 (red); and b* = -60 (blue) to +60 (yellow). The standard plate (calibration CR-A47, L* = 85.45, a* = -0.15 and b* = 54.55) was used as a standard.

Statistical Analysis

Data were analyzed to fit the following second order polynomial equation to all dependent Y variables:

$$Y = \beta_{ko} + \sum_{i=1}^3 \beta_{hi} x_i + \sum_{i=1}^3 \beta_{hii} x_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{hij} x_i x_j \dots \dots \dots (1)$$

where: β_{ko} , β_{hi} , β_{hij} are constant coefficients and x_i is the coded independent variable. The STATISTICA programs were used for analysis of variance and regression coefficient calculation. Contour plot of responses for these models were also drawn using the Statistica for Windows, by plotting as a function of two variables, while keeping other variables constant.

RESULTS AND DISCUSSION

Tensile Strength (TS) and Elongation at Break (ϵ)

An edible film must withstand the normal stress encountered during its application, subsequent shipping and handling of the food, to maintain its integrity and barrier properties. High tensile strength is generally required but deformation values must be adjusted according to the intended application of the films. That is whether it is undeformable material to provide structural integrity or reinforce structure of the food (Gontard *et al.*, 1992). Tensile strength is the maximum tensile stress sustained by the sample during tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile strength at yield or at break, respectively (ASTM, 1991). Elongation at break is an indication of films flexibility and stretch ability (extensibility), which is determined at the point where the film breaks under tensile testing and is expressed as the percentage of change of the original length of the specimen between the grips of a film to stretch (extend). The pH and heating temperature of the film solution were the most important factors affecting the mechanical properties, while heating time had lesser effect. Contour plots for tensile strength and elongation at break as affected by pH and heating temperature are shown in Figure 1. Depending upon the film conditions, TS showed a high variation between 3.73-6.06, 3.59-5.68 and 4.36-6.51 MPa and 10.28-35.79, 14.38-43.74 and 4.60-38.16% for Elongation at break (Figure 1). The results for comparisons at the same heating temperature of film solution

showed that TS increased as pH of film solutions increased. This implies that lower pH of film solutions induced formation of resistant films. Banker (1966) reported that pH plays an important role in protein films made from water-soluble materials. At alkaline condition, pH away from the isoelectric point promoted denaturation of proteins, unfolds and, solubilize. During solubilization, the cohesive forces between the protein macromolecules are neutralized by complexing with the solvent molecules (Banker, 1966). The same charged groups repelled each other and produced a stretching of the polymer chain when functional groups on a linear polymer ionized during dissolution facilitating favorable molecule orientation and fine-stranded network (Banker, 1966). The resulting interaction between polymers may have been responsible for this result. Anker *et al.* (1999) reported that, when the pH of the film solutions from β -lactoglobulin was increased above 8, SH/S-S interchange reactions or thiol/thiol (SH/S) oxidations could occur upon heating and intermolecular disulfide (S-S) bonds formed. The highest tensile strength value was obtained at pH 9.5-9.8 (Figure 1). However, increasing the pH of film solutions higher than 10.0, resulted in decrease of tensile strength due to strongly repulsive forces that occurred between the negative (extreme pH) charges along protein chain which could have decreased the occurrence of molecular associations within the protein matrix and formation of the films (Rhim *et al.*, 2002). Gennadios *et al.* (1993) studied the effect of pH on soy protein isolate film and found that highly alkaline conditions (pH > 12) inhibit soy protein isolate film formations. The weakest films occurred in solutions having the lowest pH (pH 8). This was most likely due to less protein-protein interaction (Gennadios *et al.*, 1993). The TS was enhanced as heating temperature of film solutions increased from 60 - 80°C. According to the contour plots, the experimental condition involved higher pH (9-10) and a high heating temperature (70 - 80°C) of film solutions. This might be due to the fact that higher heating temperature of film solutions induced proteins denaturation and yielded an increase in the number and/or a better localization of bonds between protein chains providing in higher interaction between protein polymers and resulting in a high TS (Bourtoom *et al.*, 2006). Heating time seemed to have no effect on tensile strength. The elongation at break (ϵ) value was also most affected by pH and heating temperature of film solution. The contour plots of elongation at

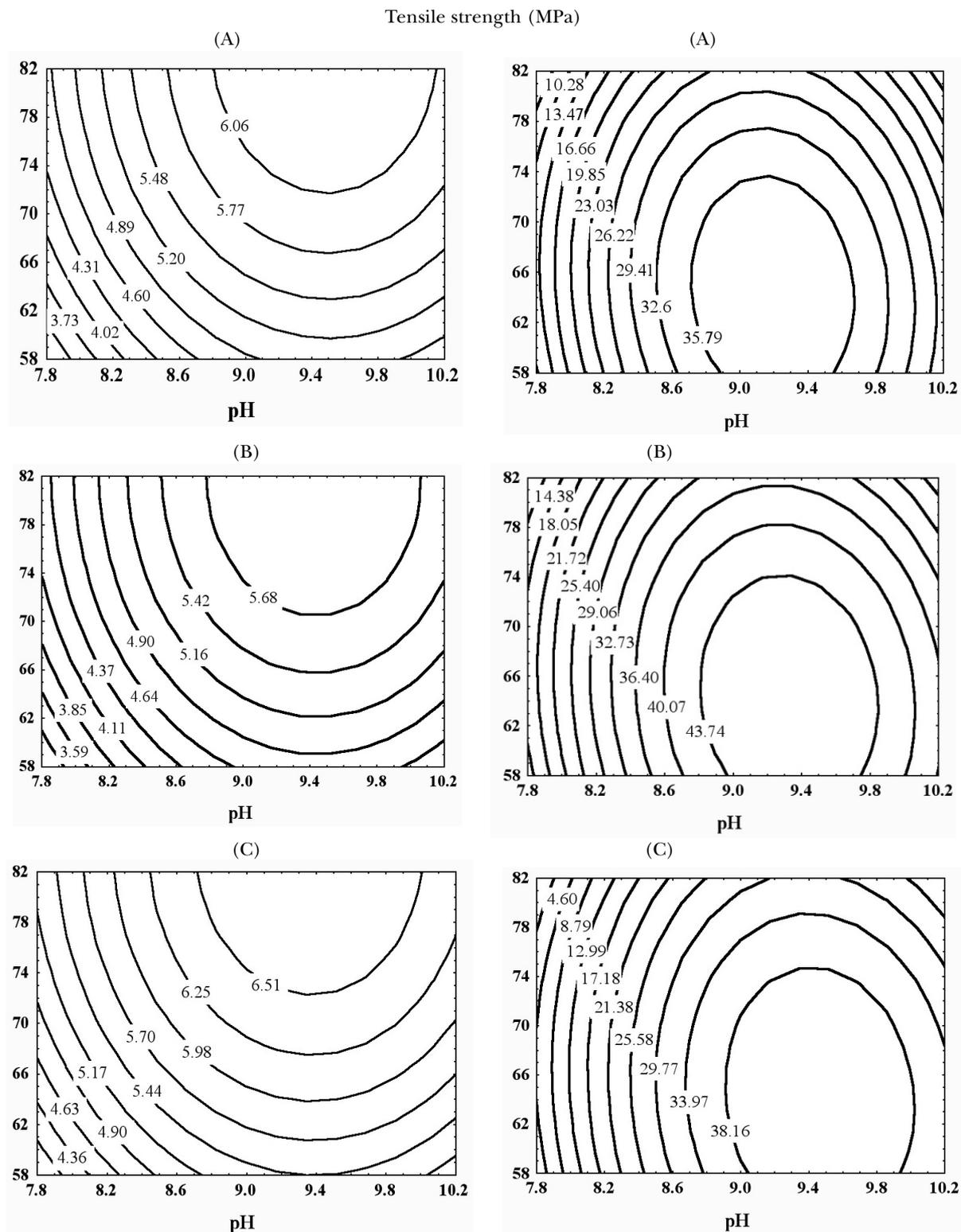


Figure 1: Contour plots showing response behavior of pH and heating time of film solutions on tensile strength and elongation at break under constant heating time; (A) = 10 min, (B) = 20 min and (C) = 30 min

break (Figure 1) indicated that edible films exhibit properties of an elastic material; the highest ϵ occurred when higher pH and higher heating temperature of film solutions were employed. An increase in heat-induced elasticity was suggested to be due to an increased number of intermolecular disulfide (SS bond) bonds (Shimada and Cheftel, 1988).

Water Vapor Permeability (WVP)

Water vapor permeability is proportionally constant and assumed to be independent of the water vapor pressure gradient applied across the films. However, hydrophilic (edible or nonedible) materials, such as protein films, are derivatives from this ideal behavior due to interactions of permeating water molecules with polar groups in the films structure (Hagenmaier and Shaw, 1990). Deviation from the ideal behavior can also be induced by temperature effects on materials (Myers *et al.*, 1962). Since a main function of an edible film or coatings are often to impede moisture transfer between food and the surrounding atmosphere, or between two components of heterogeneous food products, water vapor permeability should be low as possible. The main factor influencing water vapor permeability of edible film from mung bean proteins are pH and heating temperature of film solution. The contour plots (Figure 2) were characteristic of the effect of these variables and showed that water vapor permeability value was the lowest at pH of film solutions around 9.0-10.0 (4.24-6.00 g.mm/m².day.kPa) and tended to increase when pH of film solutions reached 8.0 (9.78-15.55 gram.mm/m².day.KPa). At higher pH protein denatures, unfolds and solubilizes, facilitating favorable molecule orientation and highly pronounced by the formation of intermolecular disulfide bond by thiol-disulfide interchange and thiol oxidation reactions (Shimada and Cheftel, 1988). The function of disulfide bonds on protein insolubilization during drying of soymilk was studied by Fukushima and Van Burea (1970). Thiol-disulfide interchange by thiol oxidation has also been shown to be involved in whey protein gelation (Donovan and Mulvihill, 1987; Shimada and Cheftel, 1988). The water vapor permeability of edible films was also affected by heating temperature of films solution. Basically, proteins must be denatured (by heating) in order to form the more extended structures that are required for film formations. Once extended, protein chains can associate through hydrogen, ionic, hydrophobic and covalent bonding. The chain-to-chain interaction that produces cohesive films

is affected by the degree of chain extension and the nature and sequence of amino acid residues. Uniform distribution of polar, hydrophobic, and/or thiol groups along the polymer chain increase the likelihood of the respective interactions. (Kester and Fennema, 1986). The results showed that increasing of heating temperature of film solutions (60 - 80°C) resulted in lower water vapor permeability (Figure 2) and most likely resulted from an increase of heating temperature of film solutions which promoted greater cross-link between protein-protein chains resulting in a tight and compact protein network and structure. Shimada and Matsushita (1980) have reported that the first step of ovalbumin aggregation involved the formation of SS bonds and the exposure of hydrophobic groups, and that, during further heating, ovalbumin was then polymerized intermolecular sulfhydryl/disulfide (SH/SS) to form a higher protein network structure. The highest water vapor permeability of the films was found at lowest heating temperature of film solutions. The effect of heating time of film solutions on water vapor permeability of edible films from mung bean proteins showed a similar trend with the heating temperature.

Film Solubility (FS) and Protein Solubility (PS)

Film solubility is advantageous in situations when the films will be consumed with a product that is heated prior to consumption and may also be an important factor that determines biodegradability of film when used as packaging wrap. The edible film from mung bean proteins maintained their integrity (i.e., did not dissolve or break apart) even after 24 hr of incubation with gentle motion. This indicates that the protein polymer network remained intact and only the monomers, small peptides and non-protein material were soluble (Stuchell and Krochta, 1994). The pH and heating temperature of film solutions significantly affected the film and protein solubility, while heating time had less effect. The contour plots of film and protein solubility showed that both decreased significantly when the pH of the film solution was increased (Figure 3). It was observed that both film and protein solubility showed lower solubility when the pH of film solution was higher than 9.0. Decreased soluble matters may be due to decreased protein solubility. Lower pH of film solutions (pH < 9.0), with enhanced dispersion capacity in water might have resulted in loosening the film structure, causing dissolution of the non-protein materials (Gnanasambandam *et al.*, 1997). It was observed that film and protein solubility were lowest at pH 9.5-10.0, most likely due to better films formation.

Water vapor permeability (g.mm/m².day.kPa)

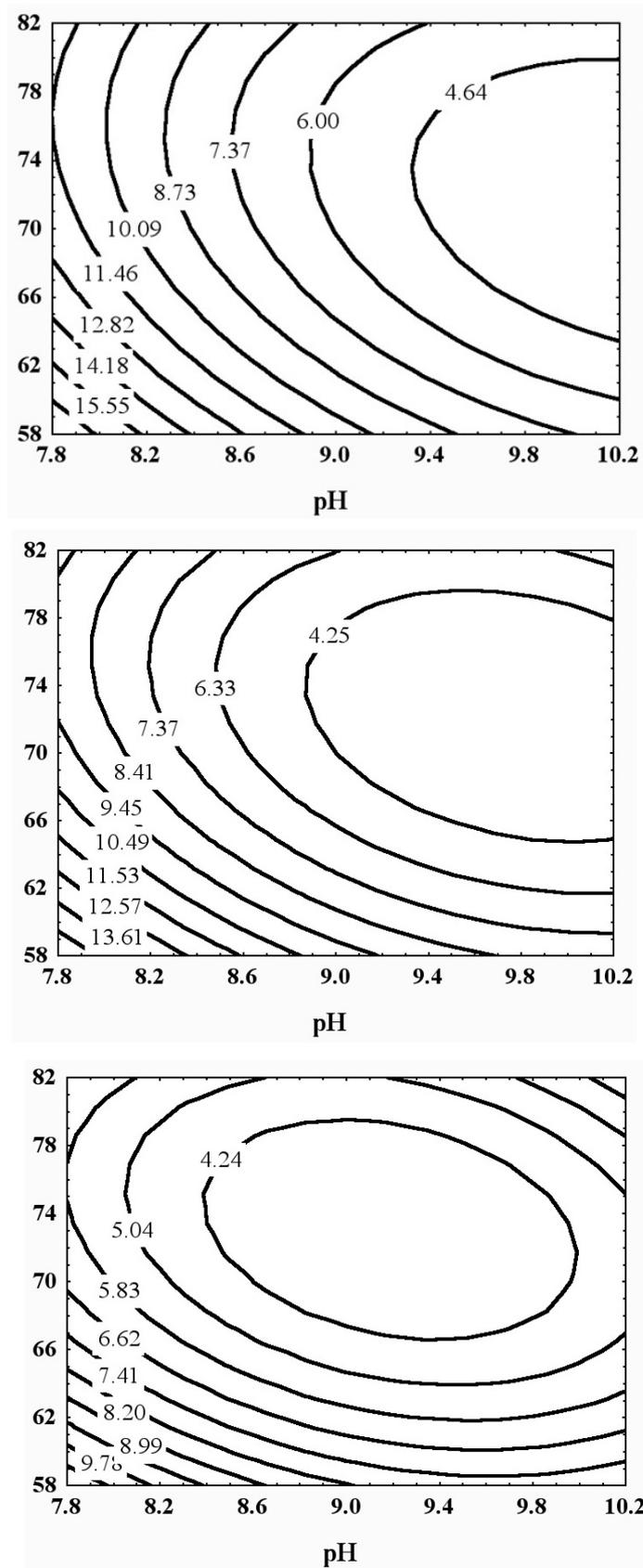


Figure 2: Contour plots showing response behavior of pH and heating time of film solutions on water vapor permeability under constant heating time; (A) = 10 min, (B) = 20 min and (C) = 30 min

The contour plots of the effect of heating temperature of film solutions on film and protein solubility are shown in Figure 3. Increasing the heating temperature of film solution from 60 to 80°C resulted in a decrease in film and protein solubility. This was attributed to more pronounced heat-induced protein denaturation at higher temperatures (Roy *et al.*, 1999). Heating time of film solutions in this study seemed to have no significant effect on film and protein solubility.

Film Color

The results of the measurements performed on the films were expressed in accordance with the Hunter system, and the rectangular coordinates (L*, a* and b*) were defined. The color of film

was most affected by pH of film solutions, while heating temperature and heating time had little effect. Films formed at lower pH and heating temperature showed lighter yellow color than films formed at higher pH and heating temperature. Instrumental color parameters L* had a* decreased little with increasing pH and heating temperature of film solution (Figure 4). However, value b* markedly increased with increasing pH and heating temperature of film solution (Figure 5), and this caused films to appear more yellowish. At alkali pH, proteins were observed to form complexes with polyphenolic compounds. Such complexes might have contributed to discoloration of films prepared at higher pH (Grananasambandam *et al.*, 1997).

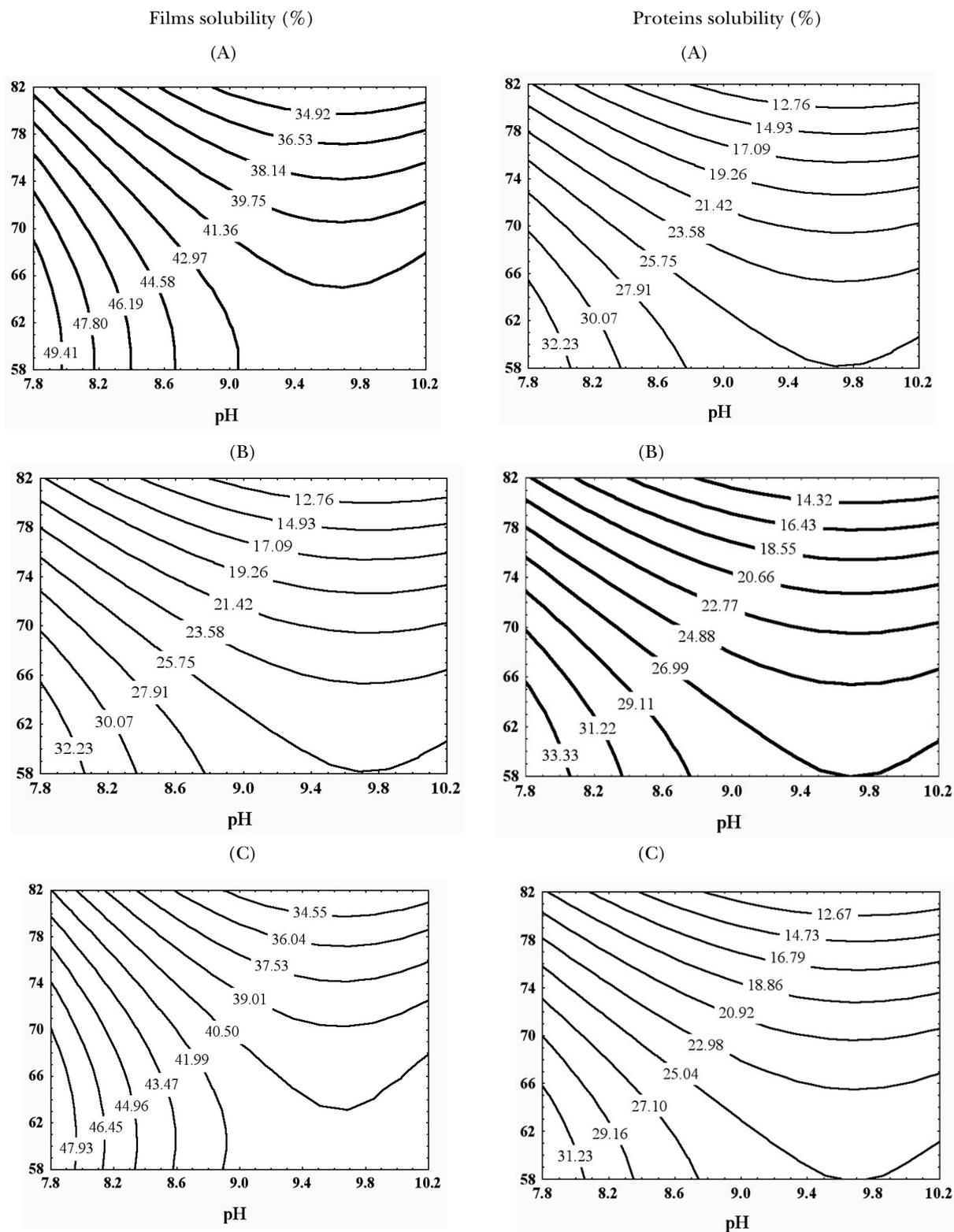


Figure 3: Contour plots showing response behavior of pH and heating time of film solutions on films solubility and proteins solubility under constant heating time; (A) = 10 min, (B) = 20 min and (C) = 30 min

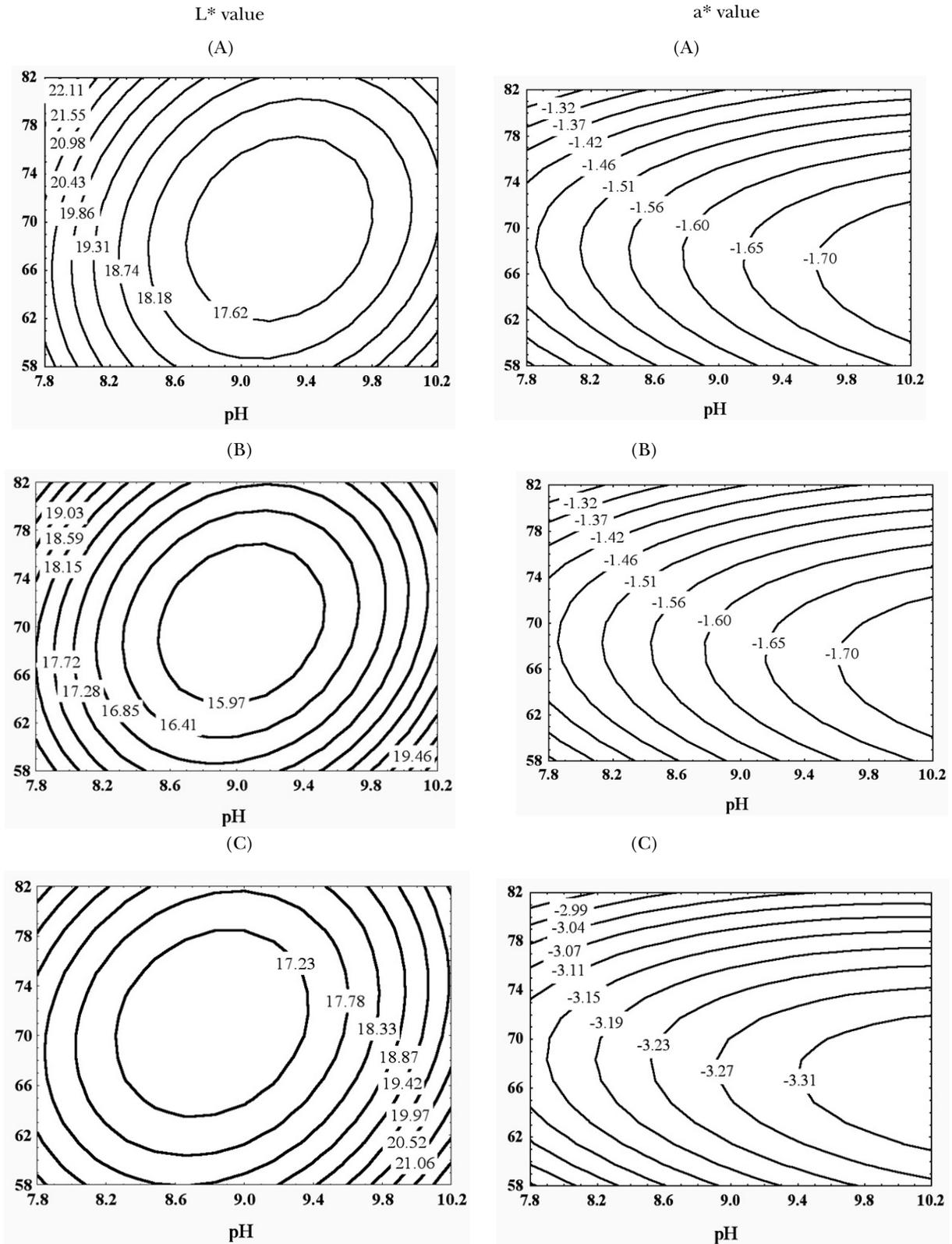


Figure 4: Contour plots showing response behavior of pH and heating time of film solutions on L* and a* values under constant heating time; (A) = 10 min, (B) = 20 min and (C) = 30 min

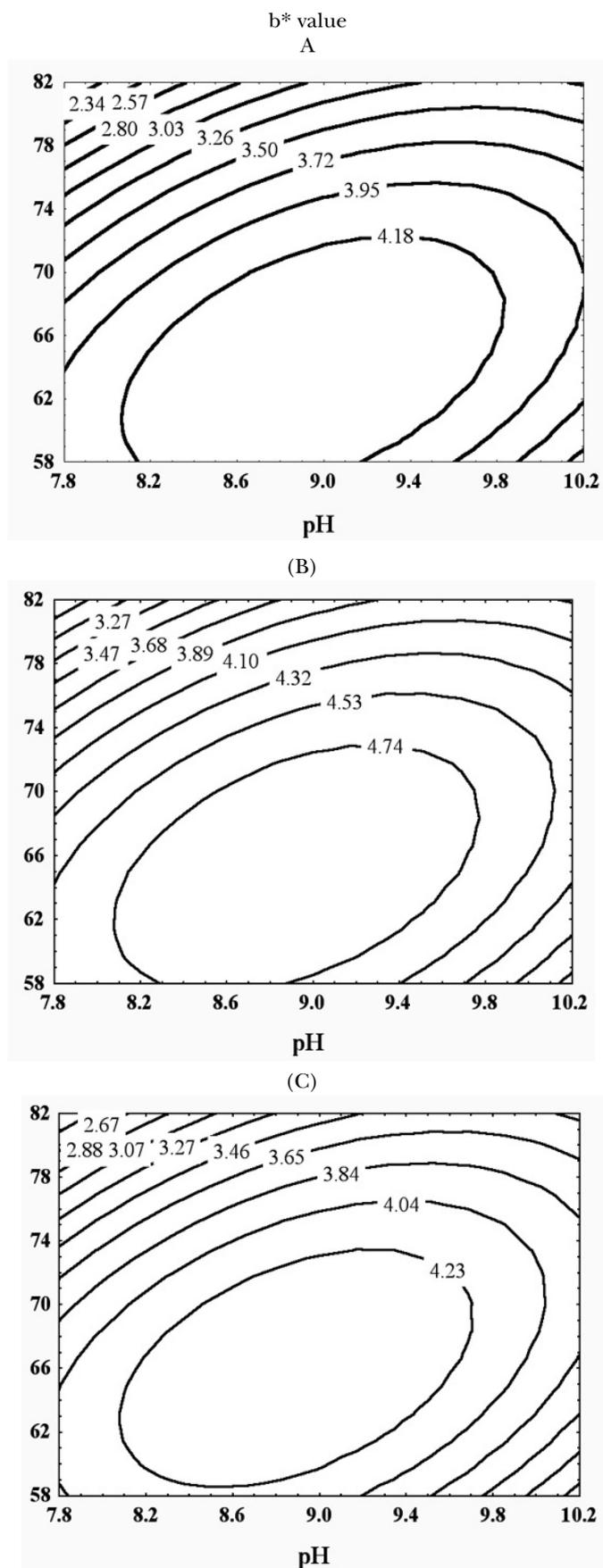


Figure 5: Contour plots showing response behavior of pH and heating time of film solutions on b* value under constant heating time; (A) = 10 min, (B) = 20 min and (C) = 30 min

CONCLUSION

The pH and heating temperature of film solutions had the greatest effect on the physico-chemical and permeability properties of edible films from mung bean protein. The films produced at pH around 9.50 and heating temperature of 75°C for 20 min of heating time exhibited high TS and ϵ , while WVP FS and PS was lowest. Film color was darker and more yellowish with increase in the pH and heating temperature of film-solution.

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