

Effect of Some Process Parameters on the Properties of Edible Film Produced from Lizard Fish (*Saurida undosquamis*) Muscle

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Abstract

This study was conducted to investigate the effect of some process parameters on the properties of cast edible protein film produced from lizard fish muscle (*Saurida undosquamis*) and the effects of pH (2.5, 3.0 and 3.5), heating temperature (50, 60 and 70 °C), and heating time (10, 20 and 30 min) of the film-solution on various film properties. These were determined using the Response Surface Methodology (RSM). For all types of films, tensile strength (TS), percentage of elongation (E), water vapor permeability (WVP) and Hunter colour values (L^* , a^* and b^*) after conditioning at 60% RH and 27 ± 2 °C for 72 hrs, and film (FS) and protein solubility (PS) after immersion in water at 25 °C for 24 hrs, were measured. The impact of pH and the heating temperature of the film-solution was more significant, overall, on the film's properties than the heating time. Contour plots of TS and E were highest at pH of 10.0 at 70 °C (2.75-3.02 MPa) but low in E (6.35-9.16%), while WVP was at its lowest (58.55-65.96 g.mm/m².d.kPa). There was a direct correlation between the FS on one hand, and heating temperature of film-solution on the other, which reversed with change in the pH of film-solution. Film color was darker and more yellowish with an increase in the pH of film-solution.

Keywords: edible protein film, lizard fish muscle, response surface methodology, permeability, tensile strength

1. Introduction

Currently, approximately 150 million tons of plastic are produced annually all over the world, and production and consumption continue to increase. Most of these plastics are crude oil based, and any increase in their production results in an increase of oil use and this causes serious environmental pollution, due to wasted and un-degraded polymer [1]. The degradation of plastics is limited and requires a long time and most of them are eventually destined to be burned or buried in landfills [2]. Edible and biodegradable polymer films must be considered as an alternative to more traditional recycling procedures and this has stimulated researchers to synthesize new polymers that can be returned to the biological cycle after use. Therefore, the use of agricultural biopolymers that are easily biodegradable would not only solve these problems, but would also

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provide a potential new use for surplus farm products [3-5]. Components used for the preparation of edible films can be classified into three categories: hydrocolloids (such as protein, polysaccharide, and alginate); lipids (such as fatty acids, acylglycerol, and waxes); and composites [6]. Hydrocolloid films have good barrier properties to oxygen, carbon dioxide, and lipids but not to water vapor. Most hydrocolloid films also possess superb mechanical properties, which are quite useful for fragile food products. Among them, proteins-based edible films are the most attractive. These films have impressive gas barrier properties compared with those prepared from lipids and polysaccharides. When 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 [7]. The mechanical properties of protein-based edible films are also better than those 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 [8]. Protein-based edible films can form bonds at different positions and offer high potential for forming numerous linkages [8].

Low value fish, such as lizard fish (*Saurida undosquamis*), are usually rejected from surimi manufacturing because of poor surimi gel strength. Hence, they have hitherto been mostly used as animal feed and/or sold for low prices because of the lack of techniques for using them as foodstuffs. In order to obtain effective utilization of low value fish, including lizard fish, there is need for more information on films produced from low value fish meat, their mechanical properties and their applications. The purpose of this study was to investigate the effect of process parameters on the mechanical and barrier properties of edible films prepared from low value fish meat (*Saurida undosquamis*).

2. Materials and Methods

2.1 Preparation of raw material

Fresh whole lizard fish (*Saurida undosquamis*) was purchased from a local fish dealer and transported on ice to the Prince of Songkla University in Songkhla Province. They were hand-skinned and filleted on the day they were received. The meats were then removed and washed with fresh water and freeze dried for 24 hrs (Dura-Top/Dura- Dry MP, Model TD97A001, FTS Systems, Inc) and stored in plastic bags at -20° C until needed. The proximate composition was determined by A.O.A.C. (1995) found to be 85.02, 1.37, 4.26 and other 9.35 %, made up of crude protein, crude fat and ash respectively.

2.2 Experimental design

General Response Surface Methodology (GRSM) was used to determine the optimum combinations of pH, heating temperature and time. GRSM is given in terms of a coded variable, x_i [10-12]. Selection of levels for independent variables was based on the results from preliminary tests. The levels of input variables in coded (x_i) and un-coded (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 prepared in random order. Each of the thirteen dependent Y variables (responses) was assumed to be affected by the three independent variables. 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.

2.3 Preparation of edible film

Freeze-dried fish proteins (80.88 g/100g sample) were dissolved in distilled water (3 g/100 ml) to prepare film-solutions. The pH level (2.5, 3.0 and 3.5) was adjusted prior to adding plasticizer (sorbitol) on a protein to sorbitol ratio of 2:1. All components were homogenized (IKA Labortechnik, Selangor, Malaysia) and heated (50, 60 and 70 °C) on a hot plate using a magnetic stirrer for the given time (10, 20 and 30 min). The film-solution was filtered through a polyester screen (mesh no. 140 with mesh opening of 106µm) to remove any small lumps, and cooled to room temperature. This was 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 30 °C undisturbed, and then cooled to an ambient temperature before peeling the films off the plates. Film samples were stored in plastic bags and held in desiccators at 60 % RH for further testing. The thickness of the resulting films were 60-65 µm.

2.4 Film Testing

2.4.1 Conditioning. All films were conditioned prior to subjecting them to permeability and mechanical tests according to Standard method, D618-61 [13]. Films tested for water vapor permeability (WVP), tensile strength (TS) and elongation (E) were conditioned at 60% RH and 27±2 °C by placing them in a desiccator over a saturated solution of Mg (NO₃)₂ ·6H₂O for 72 hrs or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

2.4.2 Film Thickness. The 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.

2.4.3 Film Solubility. Film pieces, 20 mm x 20 mm, were dried at 70° C in a vacuum oven for 24 hrs, 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 a shaking water bath for 24 hrs at 25±2 °C. A portion of the solution was removed and set aside for use later in protein solubility tests as described below. The remaining solution and film pieces were poured onto (Whatman #1) quality filter paper, rinsed with 10 ml distilled water, and dried at 70° C in a vacuum oven for 24 hrs to determine the dry mass of film. Five measurements were taken for each treatment in triplicate. The total soluble matter was calculated from the initial gross mass and final dry mass using the following equation [14]:

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

2.4.4 Water Vapor Permeability (WVP). The gravimetric Modified Cup Method based on ASTM E96-92 was used to determine the WVP of films [15]. The test cups were filled with 20 g of Silica gel (desiccant) to produce a 0% RH below the film. A sample was placed 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 taking the initial weight of the test cup, it was placed in 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 hrs for 18 hrs. The weight gained versus time was plotted and 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). The WVTR was expressed in gram units, per square meter, per day. Steady state over time (slope) yielded a regression coefficient of 0.9994 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.

2.4.5 Tensile Strength and Elongation at Break (TS and E). The TS was measured with a Universal Testing Machine (LLOYD Instruments, Hampshire, England) as per the ASTM D882-91 Standard Method [16]. 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 the percentage 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 breaking of the specimen.

2.4.6 Colour. A CIE colourimeter (Hunter associates laboratory, Inc., VA, USA) was used to determine the film L*, a* and b* colour 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.

2.5 Statistical Analysis

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

$$Y = \beta_{ko} + \sum_{i=1}^3 \beta_{ki} x_i + \sum_{i=1}^3 \beta_{kii} x_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{kij} x_i x_j \dots\dots\dots (1)$$

where: β_{ko} , β_{ki} , β_{kij} are constant coefficients and x_i is the coded independent variable. The STATISTICA version 5.5 program was used for analysis of variance and calculation of the regression coefficient. Contour plots of responses for these models were also drawn using the Statistica for Windows, Version 5.5 by plotting them as a function of two variables, while keeping other variables at a constant value.

3. Results and Discussion

3.1 Statistical analysis and contour plot

The RSREG procedure of the STATISTICA version 5.5 program used to fit the second order polynomial equation (1) to the film properties' data is shown in Table 1. The regression coefficients (β_{ki}) and analysis of variance are presented in Table 2. The results (not presented) indicated that the model developed for the TS, elongation E, WVP, FS, PS and colour (L*, a* and b*) were adequate, and had no significant lack of fit. Further statistical analysis (not presented) was then performed. Results revealed that pH, heating temperature and heating time had a significant overall effect on the all responses. The pH and heating temperature of film-solutions

Table 1 Experimental data for the three-factor, three level response surface analysis^a

pH	Temp	Time	Tensile	Elongation	Water vapor	Films	Proteins					
					strength	at break	permeability	solubility	solubility		Colour	
					(MPa)	(%)	(g.mm/m ² .day.kPa)	(%)	(%)			
Treatment	x ₁	x ₂	x ₃	TS	%E	WVP	FS	PS		L*	a*	b*
1	1(3.5) ^b	1(70)	0(20)	5.39	5.83	48.59		32.40	21.29	23.28	-0.88	3.97
2	1(3.5)	-1(50)	0(20)	4.95	3.23	49.59		32.75	22.42	24.51	-0.81	3.41
3	-1(2.5)	1(70)	0(20)	6.00	26.41	47.73		25.26	14.26	22.73	-0.61	5.32
4	-1(2.5)	-1(50)	0(20)	7.61	9.62	42.64		19.13	15.16	22.20	-0.66	4.89
5	1(3.5)	0(60)	1(30)	6.83	3.67	47.01		19.62	16.49	23.69	-1.02	4.02
6	1(3.5)	0(60)	-1(10)	5.60	12.20	48.41		30.27	19.64	23.08	-0.94	3.64
7	-1(2.5)	0(60)	1(30)	8.06	21.02	38.71		16.50	13.04	22.24	-0.91	4.93
8	-1(2.5)	0(60)	-1(10)	7.74	15.31	43.93		17.48	14.35	23.19	-0.85	4.73
9	0(3.0)	1(70)	1(30)	6.46	30.58	47.71		21.65	20.21	22.64	-0.93	4.67
10	0(3.0)	1(70)	-1(10)	6.29	16.90	49.22		21.17	20.86	22.61	-0.95	4.55
11	0(3.0)	-1(50)	1(30)	7.42	9.15	39.27		17.62	16.92	25.48	-0.80	3.94
12	0(3.0)	-1(50)	-1(10)	7.02	8.84	44.80		20.20	23.10	25.23	-0.83	4.65
13	0(3.0)	0(60)	0(20)	7.19	27.67	44.66		18.22	22.46	24.86	-0.99	4.17
14	0(3.0)	0(60)	0(20)	6.89	26.98	46.94		19.31	19.23	24.32	-0.91	4.24
15	0(3.0)	0(60)	0(20)	6.67	22.00	45.24		21.71	23.13	23.73	-0.95	4.55

^aMean of three replication and the experimental runs were performed in a random order.

^bValues in parentheses are the uncoded of independent variables.

had the most significant effect on TS, E, while heating time had the least effect. WVP, FS and PS were most affected by pH. However, pH, heating temperature and heating time did not have a significant effect on L^* , a^* , and b^* values.

3.2 Tensile strength and Elongation at break

Tensile strength is the maximum tensile stress sustained by the sample during the 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 [17]. Elongation at break is an indication of a film's flexibility and stretchability (extensibility), which is determined as the point when 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 main factors that influenced the film's properties were pH and the heating temperature of the film-solutions, while heating time had less effect. Contour plots of tensile strength and elongation at break as affected by pH and heating temperature are given in Figure. 1. Depending upon the film conditions, the TS showed a high variation between 4.66-4.70 and 7.25-7.92 MPa and 1.22 and 10.69 and 38.20 and 50.77 % for Elongation at break (Figure 1). When compared at the same heating temperature of the film solutions, the results indicated that the TS increased as the pH of film solutions decreased. This implies that the 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 acid pH away from the isoelectric point (pH 4.5), denaturation of proteins was promoted and this resulted in the unfolding and solubilizing of proteins. During the solubilization of proteins, the cohesive forces between the proteins macromolecules were neutralized by unions with the solvent molecules [18]. The weakest films showed that at the highest pH of film solutions a very low TS (4.66 MPa) was obtained at pH 3.5. This was most likely due to less protein-protein interaction. The TS was enhanced as the heating temperature of the film solutions increased from 50-60 °C. This might be due to the fact that higher heating temperature of film solutions induced the denaturation of proteins and resulted in an increase in the number and/or a better localization of bonds between protein chains provided through higher interaction between protein polymers. For the weakest films obtained at the lowest heating temperature; a very low TS (4.66 MPa) was observed at a heating temperature of the film solutions around 50 °C. However, an increasing of the heating temperature of film solutions higher than 60 °C, resulted in a decrease in TS. According to the contour plots, the experimental condition involving lower pH (2.5) and a high heating temperature (60 °C) of film solutions, resulted in higher film formations with a high TS. Elongation at break (E) value was also affected most by the pH and heating temperature of film solutions. All linear, quadratic and interaction terms for pH, heating temperature and heating time were significant. The contour plots of elongation at break (Figure 1) indicated that edible films exhibit the properties of an elastic material with elongation at break values between 1.22-10.69% and 38.20-50.77 %, and presented the highest E when a lower pH and higher heating temperature of film solutions were employed. An increase in elasticity induced by heat was suggested to be due to an increased number of intermolecular disulfide (SS bond) bonds [19]. Prolonged heating time, however, resulted in an increase in E. The experiments showed that the TS and E of the films are almost inversely related.

Tensile strength (MPa)

Elongation at break (%)

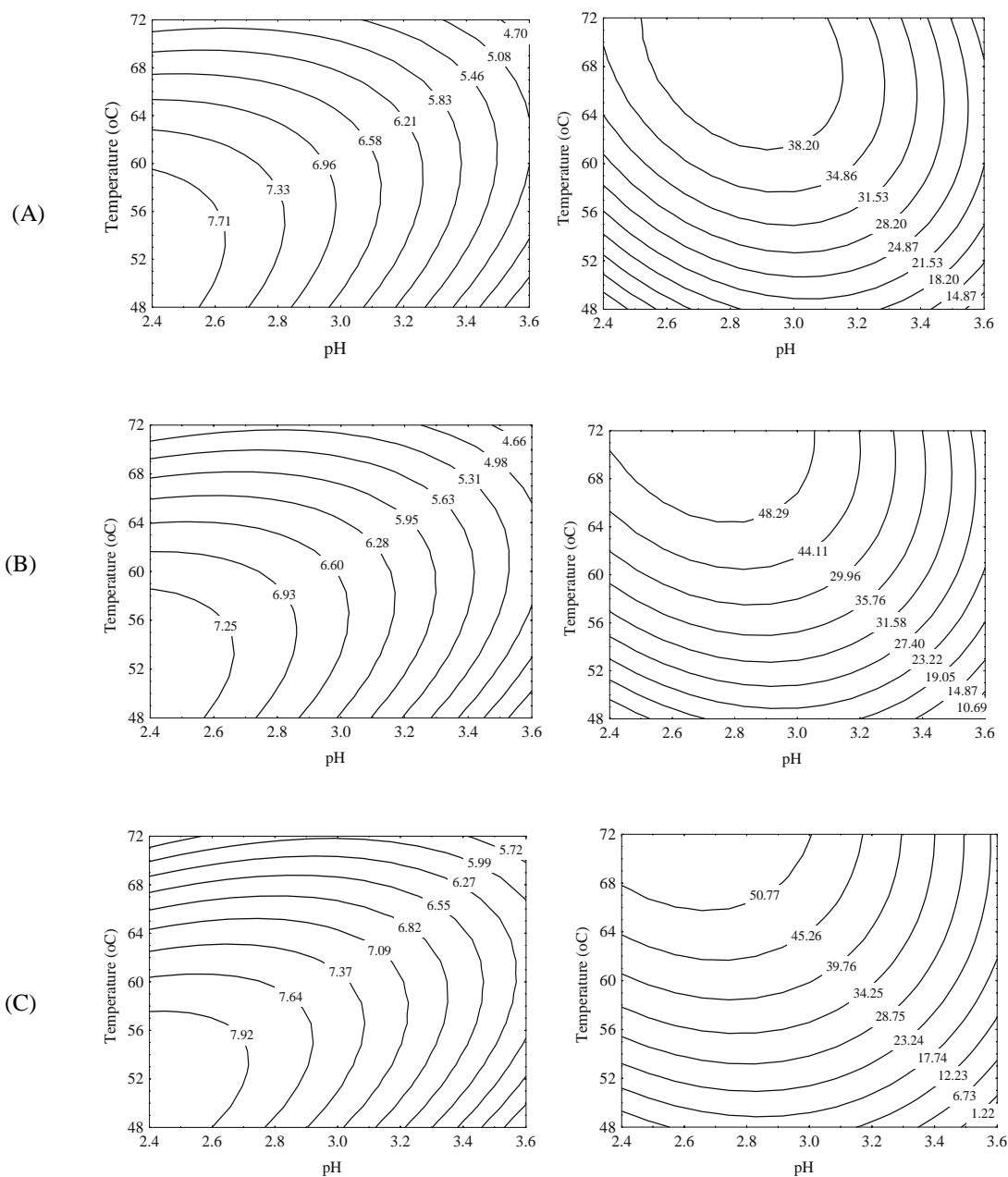


Figure1 Contour plots showing response behavior of pH and heating temperature of film-solutions under constant heating time. The numbers inside the contours represent tensile strength (kPa) and elongation at break (%) of film at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

3.3 Water vapor permeability

Water vapor permeability (WVP) is proportionality constant and assumed to be independent of the water vapor pressure gradient applied across the films. However, hydrophilic (edible or non-edible) materials, such as protein films, deviate from this ideal behavior due to the interactions of permeating water molecules with polar groups in the films' structure [17]. Since a main function of an edible film or coating is often to impede moisture transfer between food and the surrounding atmosphere, or between two components of a heterogeneous food product, WVP should be low as possible. The main factors influencing the WVP of edible film produced from lizard fish meat the pH of film solutions. The contour plots (Figure 2) were characteristic of the effect of these variables and showed that WVP value demonstrated the highest with the pH of film solutions around 3.5 (47.23-48.97 g.mm/m².day.kPa). However, the WVP decreased again when the pH of film solutions was adjusted to 2.5 (35.12-42.71 g.mm/m².day.KPa). Again, at lower pH (away from the isoelectric point) protein denatures, unfolds and solubilizes, facilitating favorable molecule orientation and pronounced higher formation of intermolecular disulfide bonding by thiol-disulfide interchange and thiol oxidation reactions. Thiol-disulfide interchanged by thiol oxidation has also been implicated in whey protein gelation studies [19, 21]. The higher pH (pH >2.5) of film solutions in this study might inhibit the lizard fish muscle film formation. Most likely, due to less denaturation and unfolding, solubilizing results in a low molecule orientation with pronounced lower molecule forms when associated with the formation of films. The highest WVP was observed at the highest pH of this study. The WVP of edible films was affected by the heating temperature of films' solutions. Basically, proteins must be denatured (by heating) in order to form the more extended structures that are required for the formation of film. 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 [22]. However, the results of this experiment showed that increasing of heating temperature of film solutions (50-70°C) resulted in higher water vapor permeability (Figure 2). This most likely resulted from the increasing of the heating temperature of film solutions which might cause a high protein denaturation resulting in precipitation of protein and loss of protein network and structure. The highest water vapor permeability of edible films was found at both highest the pH and heating temperature of film solutions. The effect of the heating time of film solutions on water vapor permeability of edible films showed an inverse trend with the heating temperature.

3.4 Film solubility and protein solubility

Water resistance is an important property of edible films for applications as food protection where water activity is high, or when the film must be in contact with water during processing of the coated food (e.g. to avoid exudation of fresh or frozen products) [23]. Generally, higher solubility would indicate lower water resistance. However, a high solubility may an advantage for some applications [24]. The pH of film solutions significantly affected the FS and PS, while the heating temperature and heating time had less effect. The contour plots of FS and PS showed that both had increased significantly when the pH of film solutions was increased (Figure 3). It was observed that edible film showed higher solubility values when the pH of the film solution was higher than 2.5. Increased solubility may be due to increased protein solubility. Higher pH of film solutions (pH >2.5), with enhanced capability for water dispersion, might result in the loosening of the film structure, causing dissolution of the non-protein materials [25]. It was observed that FS and PS were lowest at pH around 2.5, most likely due to better formation of films. The contour plots of the effect of heating temperature of the film solution on FS and PS are shown in Figure 3. Comparing them at the same pH of the film solution demonstrates that an increase in heating temperature of the film solution from 50 to 70°C resulted in a decrease in films' solubility. This was attributed to more pronounced heat-induced protein denaturation at higher temperatures [26].

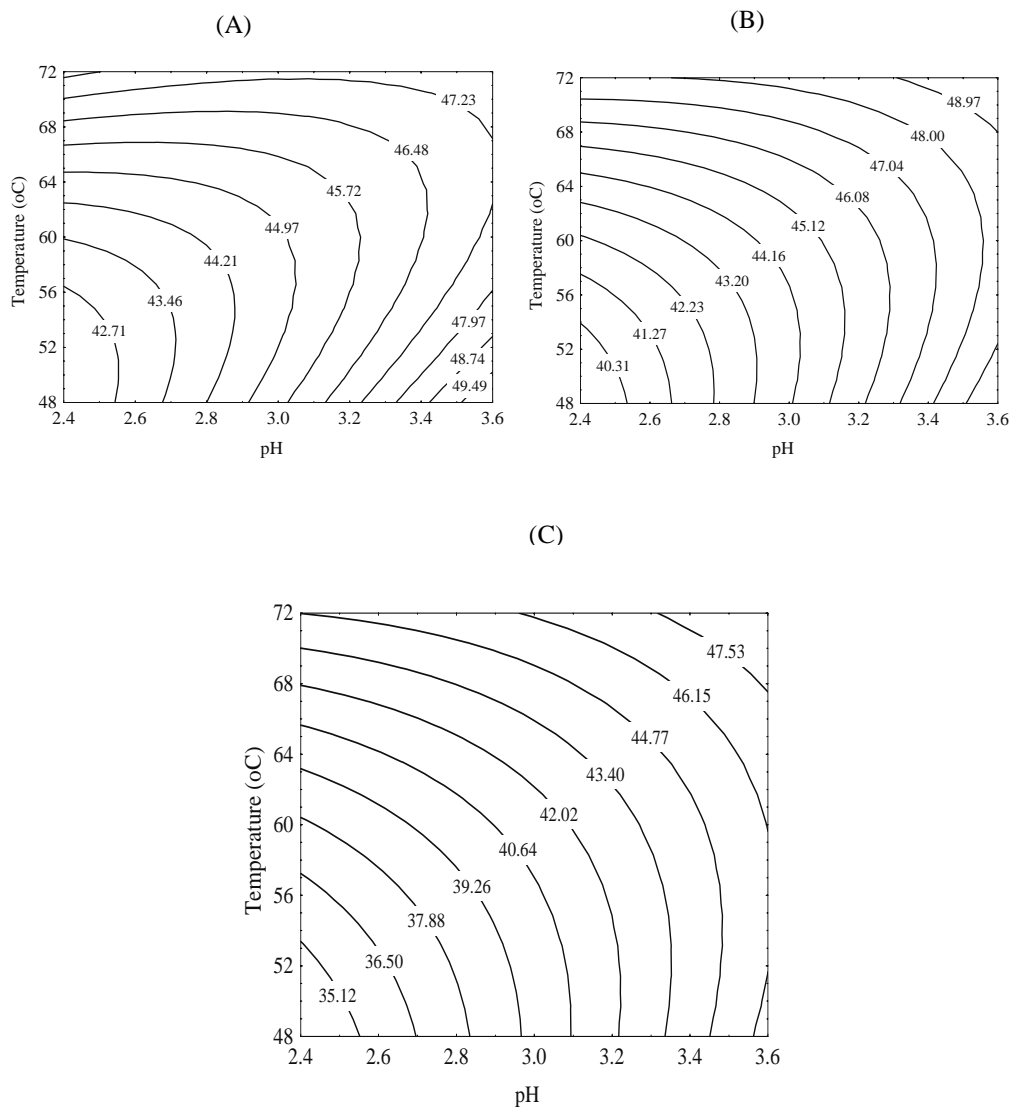


Figure 2 Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent water vapor permeability (g.mm/m².d.kPa) of film at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

Heat induced protein denaturation (unfolds) resulted in exposing previously "buried" groups such as hydrophobic and sulfhydryl (SH) groups which produced a strong film [27,28,29,30]. However, when the heating temperature of film solutions was raised higher than 60 °C it yielded an increase in FS and PS. The heating time of film solutions in this study seemed to have no significant effect on FS and PS.

3.5 Film color

The results of the measurements performed on the films were expressed in accord with the Hunter system, and the rectangular coordinates (L^* , a^* and b^*) were defined. The color of a film was most affected by the pH of the film solution, while heating temperature and heating time were little affected. Film formed at both higher pH and heating temperature were of a lighter yellow color than films formed at a lower pH and heating temperature. Instrumental color parameters L^* and a^* showed a little increase with increase in pH and heating temperature of the film solution (Figure 4.) ,However, value b^* dramatically increased with a decrease in pH, which reversed with change in the heating temperature of film solutions and this made the film appear more yellowish (Figure 5). The value a^* decreased as the pH and heating temperature of the film solution increased (Figure 4), resulting in a greenish yellow film.

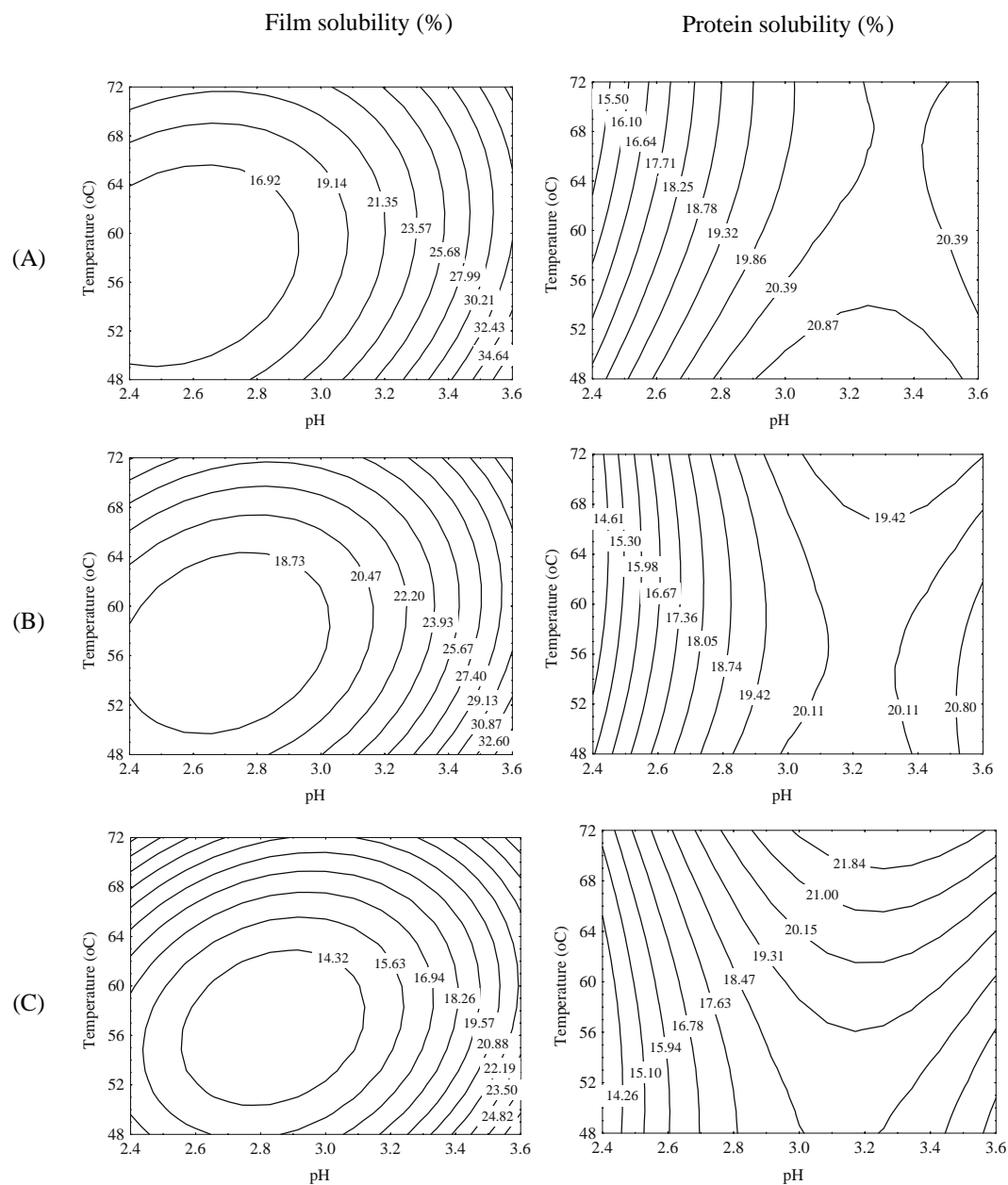


Figure 3 Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent film solubility (%) and protein solubility (%) of film at given heating; (A) = 10 min, (B) = 20 min and (C) = 30.

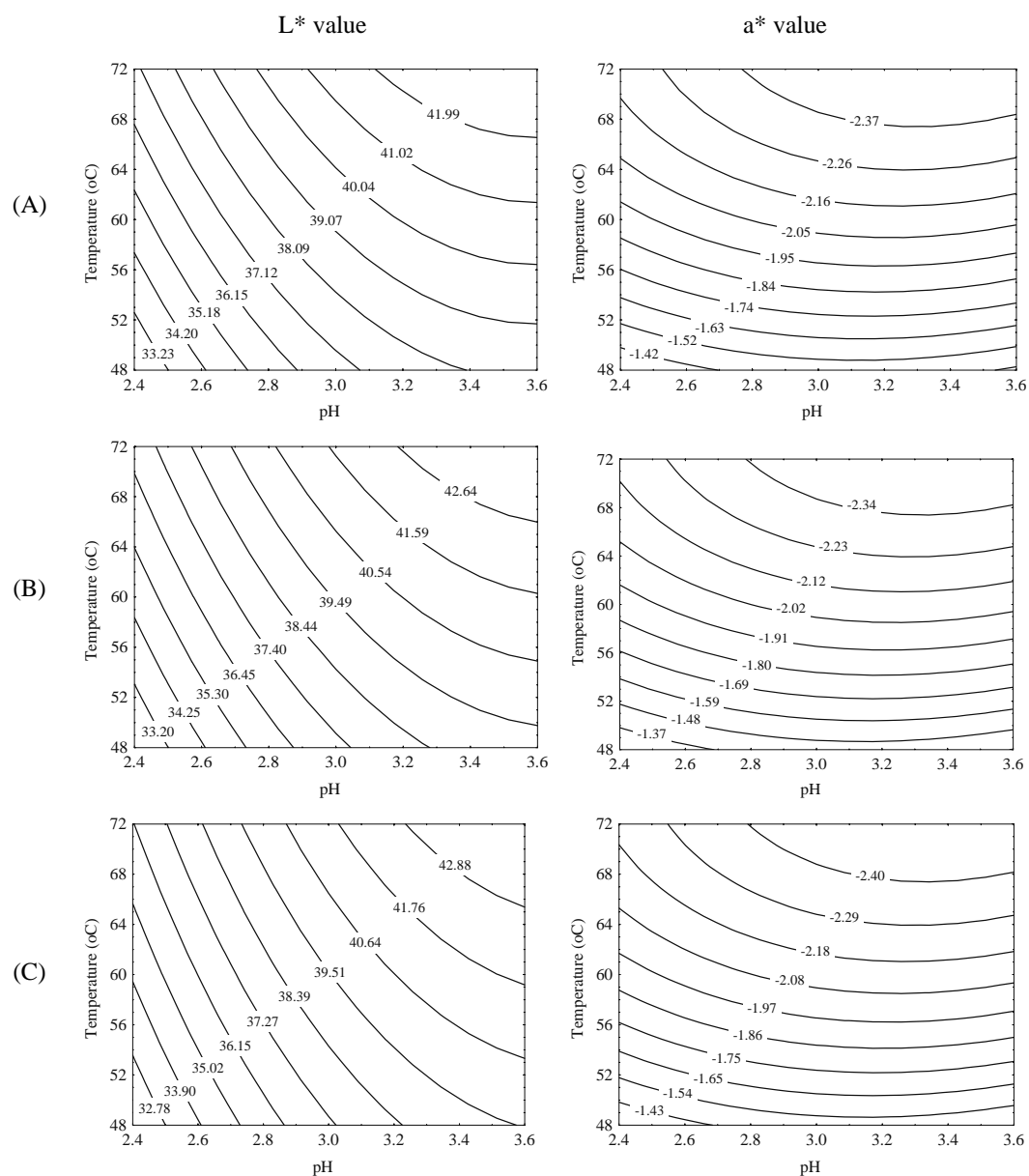


Figure 4 Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent L^* and a^* of film at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

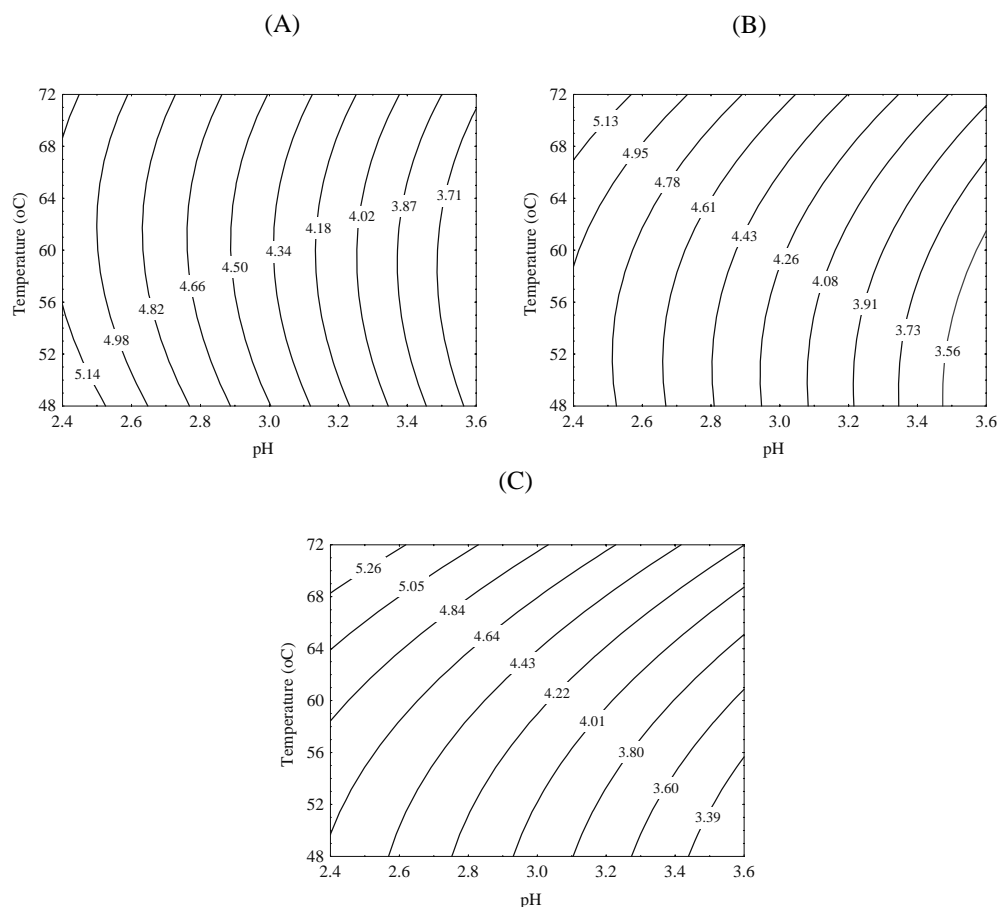


Figure 5 Contour plots showing response behavior of pH and heating temperature of film solutions under constant heating time. The numbers inside the contours represent b^* of film at given heating; (A) = 10 min, (B) = 20 min and (C) = 30 min.

4. Conclusions

The pH and heating temperature of film solutions had the greatest impact on the properties of edible films from lizard fish muscle. The films produced at 2.50 and a heating temperature of 54°C for 30 min of heating time exhibited the highest TS and E, while WVP was at its lowest. There was a direct correlation between the films' solubility and pH, which reversed with changes in the heating temperature and heating time. The films' color turned darker and more yellowish with a decrease in the pH.

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Table 2 Regression Coefficients and analysis of variance of the second order polynomial for thirteen response variables

Coefficient	Tensile Strength	%Elongation	Water vapor permeability	Film solubility	Protein solubility	L*	a*	b*
	Y ₁	Y ₂	Y ₃	Y ₄	Y ₅	Y ₆	Y ₇	Y ₈
β_{k0}	6.91	25.55	46.61	19.74	21.61	24.30	-0.95	4.32
<i>Linear</i>								
β_{k1}	-0.83**	-5.93**	2.57**	4.58**	2.88**	0.52	-0.07*	-0.60
β_{k2}	-0.36**	6.11**	2.12*	1.35	-0.12	-0.77	-0.03	0.20
β_{k3}	0.26*	1.40	-1.71*	-1.72	-1.41	-0.007	-0.01	-0.001
<i>Interaction</i>								
β_{k11}	-0.33*	-8.80**	0.39	4.22*	-3.86**	-1.03	0.08	-0.02
β_{k12}	0.51**	-3.55	-1.52	-1.62	-0.58	-0.44	-0.03	0.03
β_{k22}	-0.59**	-5.48*	1.23	3.41*	0.53	-0.09	0.13*	0.10
<i>Quadratic</i>								
β_{k13}	0.23	-3.56	0.96	-2.42	-0.46	0.39	-0.005	0.04
β_{k23}	-0.06	3.34	1.00	0.76	1.38	-0.05	-0.002	0.21
β_{k33}	0.47*	-3.70	-1.49	-3.00*	-1.87	-0.22	-0.06	0.03
%variability explained (R ²)	97.44	94.02	90.25	94.96	91.64	76.43	88.91	90.72
F	0.76	1.86	3.02	1.50	0.75	3.07	3.58	
2.23								
Probability of F	0.61	0.37	0.26	0.42	0.44	0.24	0.22	0.32

* Significant at 5% level
 ** Significant at 1% level

