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## EFFECT OF GLYCEROL CONTENT AND pH VALUE OF FILM-FORMING SOLUTION ON THE FUNCTIONAL PROPERTIES OF PROTEIN-BASED EDIBLE FILMS

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*The work is concerned with the effects of glycerol content and pH value of film forming solution on the functional properties of protein-based films. The films were produced of chicken breast proteins, dissolved under either acidic (pH 3) or alkaline (pH 11) conditions, with different concentrations of glycerol (35%, 50% and 65% w/w of protein content). Glycerol content affected significantly mechanical properties, water vapor permeability, color at pH 3 and film solubility ( $p < 0.05$ ). The pH value had significant influence on light transmission, color, transparency and film solubility ( $p < 0.05$ ). Considering the results of mechanical properties and film solubility, the obtained films are in the acceptable range for the use as a packaging material. It was estimated that water vapor permeability, color, light transmission and transparency need to be improved for the application.*

KEY WORDS: Edible films, packaging material, glycerol, pH value.

### INTRODUCTION

Plastic packaging has come into widespread use, thanks to its good mechanical properties and effectiveness as a barrier to oxygen and water. However, synthetic packaging materials have led to the serious ecological problems due to their non-biodegradability. With the emphasis on limited resources and the environment, in the recent years research attention has turned to developing biodegradable and/or edible packaging (1).

An edible film has been defined as a thin, continuous layer of edible material, which can prevent the food from interaction with its environment, gains or losses of moisture or aroma, taking up oxygen or contamination with microorganisms (2,3). Furthermore, edible and biodegradable films can be used to incorporate various food additives, such as flavorings, antimicrobial and antioxidant agents into foods at specific locations.

The basic materials for the film preparation are biopolymers. The biopolymers to be used as raw material for edible films, such as proteins, polysaccharides and lipids, should be capable of forming continuous matrix and normally are from renewable and abundant

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resources (4). Recently, increasing attention is being paid to the use of biopolymers from agro-industrial residues or their composites as packaging materials, since it could contribute to the reduction of environmental pollution (5). Among these materials, proteins have been extensively used for the development of edible films because of their relative abundance, film-forming ability and nutritional qualities (6). Protein-based films have impressive gas barrier and mechanical properties, compared with those from lipids and polysaccharides, but show the poor water-vapor barrier properties (7, 8, 9).

The objective of this study was to determine the functional properties of chicken protein-based films as influenced by plasticizer content and pH value of film forming solution.

## EXPERIMENTAL

### Preparation of protein-based film

Films were prepared from a film-forming solution based on myofibrillar proteins isolated from chicken breast muscles in distilled water, plasticizer and either HCl or NaOH. The film-forming solution was prepared as described by Shiku et al. (2) with a slight modification.

Chicken breasts, bought in supermarket (Hat Yai, Thailand) were used as raw material. Frozen muscles were thawed using running water (26-27°C) until the core temperature reached 0°C. Protein content of chicken muscles was determined by Kjeldahl's method (10). Meat was chopped into small pieces and connective tissues were removed as much as possible. The right amount of meat, needed to obtain the final protein concentration of 2% (w/v) in 100 ml of film-forming solution, was measured and added with a small amount of distilled water (up to 30 ml). The mixture was homogenized at 13000 rpm for 1 minute, using a homogenizer (Polytron PT - MR 2100, Kinematica AG, Switzerland). The pH value of mixtures was then adjusted to 3 (group of samples A) and 11 (group of samples B), using 1M HCl and 1M NaOH, respectively. The mixture obtained was filtrated through a layer of nylon sheet. Plasticizer (glycerol) was then added at 35%, 50% and 65% (w/w) of protein content (obtained samples - A35%, A50%, A65%, B35%, B50% and B65%). The volume was then adjusted to 100 ml with distilled water, and the solution obtained was used for film casting.

The prepared film-forming solution (4 g) was cast onto a rimmed silicone resin plate (50 x 50 mm) and air blown for 12 h at room temperature, prior further drying at 25°C and 50% relative humidity (RH) for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and used for the analyses.

### Determination of film properties

**Film thickness** was measured using a micrometer (Gotech, GT-313-A, Gotech testing machines Inc, Tawai) at five random positions of each film of ten specimens.

**Mechanical properties.** Prior to the testing of mechanical properties, the films were conditioned for 48 h at 25±0.5 °C and 50±5 % RH environmental chamber. Tensile

strength (TS) and elongation at break (EAB) were determined using Universal testing machine LR 30 K LLOYD Instruments Ltd., Farenham. Ten specimens (2 x 5 cm) with initial grip length of 3 cm were used for testing. Cross-head speed was 3 cm/min. TS was calculated by dividing the maximum force at break by the initial cross-sectional area of specimen (film thickness x 2 cm). EAB was calculated as follows:

$$EAB = \frac{d_{after}}{d_{before}} \cdot 100 \quad [1]$$

where  $d_{before}$  was 3 cm (initial grip length) and  $d_{after}$  was the difference between distance of grips after the break of specimen and initial grip length.

**Water vapor permeability** (WVP) was determined using a modified ASTM method, as reported by Shiku et al. (2). The film in three replicates was sealed on an aluminium cup containing silica gel (0% RH) with silicone vacuum grease and a rubber band to hold the film in place. The cups were weighed, and then placed at 30°C in a desiccator containing the distilled water. Distilled water was placed at the bottom of desiccator for providing RH of 100% at 30°C. The cups were weighed at 1 h intervals over a 8 h period. The water vapor transferred through films was determined from the weight gain of the cups. WVP (g/m·s·Pa) of the film was calculated according to (11):

$$WVP = \frac{w \cdot l}{A \cdot t} \cdot \frac{1}{P_2 - P_1} \quad [2]$$

where  $w$  is the weight gain of the cup (g),  $l$  is the film thickness (m),  $A$  is the area of the exposed film (m<sup>2</sup>),  $t$  is the time of gain (s) and  $(P_2 - P_1)$  is the vapor pressure difference across the film (Pa).

**Color** of the films was determined as  $L$  (lightness, 0=black, 100=white),  $a$  ( $-a$ =greenness,  $+a$ =redness) and  $b$  ( $-b$ =blueness,  $+b$ =yellowness), using CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). The color of the films was also expressed as the total difference in color,  $\Delta E^*$ :

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad [3]$$

where  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta b^*$  are the differentials between the color parameter of the samples and the color parameter of the white standard ( $L_0^*=94.8$ ,  $a_0^*=-0.78$  and  $b_0^*=1.43$ ).

**Light transmission and film transparency.** The ultraviolet (UV) and visible light barrier properties of the films were measured at selected wavelengths between 200 and 800 nm using the UV-16001 spectrophotometer (Shimadzu, Kyoto, Japan). The transparency of the films was calculated according to (12):

$$T = \frac{-\log T_{600}}{l} \quad [4]$$

where  $T_{600}$  is the fractional transmittance at 600 nm and  $l$  is film thickness (mm).

**Film solubility** (FS) is a parameter of biodegradability of films and it was expressed as the percentage of film dry matter solubilized in distilled water (13). Dry matter of the film was determined according to AOAC 2000 method (14). The conditioned film sam-

ples (2 cm x 4 cm) were weighed and placed in 50 ml centrifuge tubes containing 10 ml of distilled water with 0.1 % (w/w) sodium azide and then stored at 30°C for 24 h with continuous gentle stirring (250 rpm, Heidolph Incubator 10000, Schwabach, Germany). Undissolved dry matter was determined by centrifugation at 3000 x g for 20 min (Avanti J-E, Beckman Coulter) and drying them at 105 °C for 24 h. The weight of solubilized dry matter was calculated by subtracting the weight of unsolubilized dry matter from the initial weight of dry matter and expressed as the percentage of total weight.

**Statistical analysis.** Data were subjected to the analyses of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test (15). Analysis were performed using StatSoft package (STATISTICA 9.1 StatSoft Inc., Tulsa, OK, USA).

## RESULTS AND DISCUSSION

### Film formation

The obtained films were strong and flexible enough to be peeled and handled. Films produced of proteins solubilized under the acidic conditions were generally transparent and smooth, while the films produced by proteins solubilized under the alkaline conditions were yellow, thick and sandy. Visually, the differences were noticeable between the films containing 35% and 65% of glycerol, which were rigid and sticky, respectively.

### Thickness

Results for film thickness are shown in Table 1.

**Table 1.** Film thickness for A and B samples, with different content of glycerol

Sample	A35%	A50%	A65%	B35%	B50%	B65%
Thickness [mm]	0.0285 ±0.004*	0.0299 ±0.003	0.0316 ±0.003	0.0297 ±0.002	0.0309 ±0.003	0.0319 ±0.002

(\*) – Mean ± SD from 10 measurements

Samples with higher concentration of glycerol had a higher content of dry mater in film-forming solution, so it is expected for resulting film to be thicker. However, the results showed that this difference was not statistically significant ( $p>0.05$ ).

### Mechanical properties

At the extreme acidic or alkaline pH values, strong electrostatic repulsion of ionized groups occurs in the film-forming solution, which leads to the solubilization of proteins. Solubilization processes are a prerequisite for film preparation and have the impact on the mechanical properties of the resulting films (16). The unfolded proteins obtained using either acidic or alkaline solubilizing process underwent the aggregation through hydrogen, ionic, hydrophobic and covalent bondings, particularly when the water was remo-

ved. (17). The distribution and extents of intra- and inter-molecular interactions, which give rise to three-dimensional network structures of the films, could affect their mechanical properties (6). Results given in Table 2 showed that when the same plasticizer content was used, TS of films prepared at the pH 3 was significantly different ( $p < 0.05$ ) from that prepared at pH 11. However, no significant effect of pH was observed on EAB of protein-based films ( $p > 0.05$ ).

Plasticizers are the basic additives for the film-forming polymers. They reduce inter-molecular forces, which increase the molecular spacing and mobility of biopolymer chains. Addition of a plasticizing agent is necessary in order to overcome brittleness of the film and to improve its flexibility (18). Brandenburg et al. (19) found that films made without plasticizer are extremely brittle and shattered upon handling. Polar groups (-OH) along plasticizer chains are believed to develop polymer-plastic hydrogen bonds that replace the polymer-polymer interactions in the biopolymer films. Due to its small size and high polarity, glycerol is most commonly used as plasticizing agent (20).

Results in Table 2 show that TS decreased and EAB increased significantly ( $p < 0.05$ ) with increase of glycerol content. Thus, the addition of glycerol increased the extensibility of protein-based films, while reduced its mechanical strength.

**Table 2.** Tensile strength and elongation at break for A and B samples, with different content of glycerol

Sample	A35%	A50%	A65%	B35%	B50%	B65%
TS [MPa]	4.3137 ±0.98*	2.4263 ±0.49	1.5445 ±0.31	4.1866 ±0.57	3.3196 ±0.46	2.5571± 0.53
EAB [%]	79.7410 ±6.91	99.0243 ±5.26	117.8600 ±4.31	81.6633 ±15.02	96.2100 ±13.68	101.2367 ±9.78

(\*) – Mean ± SD from 10 measurements

TS of chicken protein-based films is in the same range as TS of conventional polyolefin films: 3-10 MPa (21). EAB values are higher than that of cellophane (by about 20%) and considerably lower than those of the most commercial synthetic polymer films, like LDPE (by about 500%) or HDPE (by about 300%) (22).

### Water vapor permeability

Water vapor permeability is another important and widely studied property of edible films. The barrier properties of the films are influenced by the hydrophobic/hydrophilic nature of the polymer and by the type, level and compatibility of the incorporated plasticizer (23, 24). Results shown in Table 3 indicate that the WVP of edible films are much higher than WVP of plastic films (LDPE  $0.0055 \cdot 10^{-10}$  g/m·s·Pa; PVC  $0.0071 \cdot 10^{-10}$  g/m·s·Pa; (25)). Increased transmission of water vapor through a protein-based film is caused by a high content of polar amino acid residues in the structure of the film, as well as to the presence of hydrophilic plasticizer - glycerol. (26).

The WVP values significantly increase ( $p < 0.05$ ) with increasing of the content of glycerol in the film-forming solution. A higher amount of glycerol gives a higher amount of

polar groups in the film, which could absorb more water from the surrounding atmosphere.

At the same glycerol content used, no differences were found between films prepared at the values pH 3 and pH 11 ( $p > 0.05$ ).

**Table 3.** Water vapor permeability for A and B samples, with different content of glycerol

Sample	A35%	A50%	A65%	B35%	B50%	B65%
$WVP \times (10^{-6} \cdot \frac{g}{m \cdot s \cdot Pa})$	0.2147 $\pm 0.01^*$	0.2664 $\pm 0.01$	0.2874 $\pm 0.01$	0.2267 $\pm 0.01$	0.2455 $\pm 0.01$	0.2709 $\pm 0.01$

(\*) – Mean  $\pm$  SD from 3 measurements

### Color

Color attributes are of prime importance because they directly influence consumers acceptability. The  $L$ ,  $a$ ,  $b$  and  $\Delta E^*$  values of tested samples are shown in Table 4.

Higher  $a$  and  $b$  and slightly lower  $L$  values were observed for films prepared under alkaline conditions, in comparison with those prepared under acidic conditions ( $p < 0.05$ ). It was estimated that the films prepared at alkaline pH were more likely yellowish than those prepared at acidic pH, as evidenced by greater  $b$  value. The result indicated that alkaline conditions might induce the formation of yellowish pigment, especially via Maillard reaction. Alkaline conditions probably induced the hydrolysis of proteins and sugars, leading to the availability of amino group from amino acids and carbonyl group from reducing sugar. In the alkaline medium, amino groups are deprotonated and, hence, have an increased nucleophilicity. As a consequence, the Maillard reaction was favored, particularly during drying of the film (27).

**Table 4.** Color for A and B samples, with different content of glycerol

Sample	A35%	A50%	A65%	B35%	B50%	B65%
$L$	64.18 $\pm 0.08^*$	64.54 $\pm 0.28$	65.43 $\pm 0.37$	64.75 $\pm 0.26$	65.21 $\pm 0.19$	64.91 $\pm 0.17$
$a$	-1.21 $\pm 0.07$	-1.19 $\pm 0.06$	-1.25 $\pm 0.20$	-1.49 $\pm 0.09$	-1.73 $\pm 0.05$	-1.47 $\pm 0.08$
$b$	1.96 $\pm 0.19$	1.96 $\pm 0.16$	2.11 $\pm 0.38$	3.59 $\pm 0.32$	5.04 $\pm 0.33$	3.54 $\pm 0.21$
$\Delta E^*$	2.18 $\pm 0.12$	2.16 $\pm 0.11$	2.30 $\pm 0.18$	3.23 $\pm 0.23$	4.23 $\pm 0.21$	2.55 $\pm 0.09$

(\*) – Mean  $\pm$  SD from 8 measurements

At the pH 3, glycerol did not affect total difference in color ( $p > 0.05$ ), but it showed a significant effect when the pH 11 was used for film preparation ( $p < 0.05$ ). Since glycerol

is a colorless component, this effect of plasticizer was probably related to a dilution effect due to its increasing concentration in the film-forming solution, without any probable association with the plasticizing effect of glycerol (28).

### Light transmittance and transparency

Light transmittance (%T) in the UV-vis range and transparency (T) values of tested samples are presented in Table 5.

**Table 5.** Light transmittance and transparency for A and B samples, with different content of glycerol

Wave length [nm]	%T					
	A35%	A50%	A65%	B35%	B50%	B65%
200	0	0	0	0	0	0
280	0.4	0.5	0.6	0.3	0.2	0.2
350	72.8	74.2	73.5	51.6	46.9	57.8
400	76.6	77.9	77.3	57.7	53.1	63.8
500	80.9	82.1	80.9	63.6	59.6	69.5
600	82.6	84.0	82.3	68.8	61.9	71.4
700	83.6	85.3	83.4	67.4	63.6	72.9
800	84.4	86.2	84.2	68.5	65.2	74.3
600	T					
	2.98 ±0.46	2.55 ±0.22	2.69 ±0.25	6.15 ±0.52	6.78 ±0.53	4.62 ±0.43

All tested samples showed the excellent barrier for light transmission in UV-range (200 nm – 0%), probably owing to the high content of aromatic amino acids in protein-based structure, capable to absorb UV-light (29). In the visible range (350-800 nm), the light transmittance of films prepared at pH 3 ranged from 72.8 % to 86.2 %, but the much lower values were found for the films prepared at pH 11 (46.9% - 74.3%). At the same pH, %T of film slightly increased with increasing glycerol content.

The transparency values of films prepared at pH 3 were lower than that prepared at pH 11, indicating that the former was more transparent than the latter ( $p < 0.05$ ). Glycerol content did not affect the transparency of the films ( $p > 0.05$ ).

### Film solubility

Solubility in water is an important property of edible films, since potential applications may require water insolubility to enhance product integrity and water resistance. However, in some cases water solubility of the film before consumption of the product might be beneficial (30). The FS of tested samples is presented in Table 6.

**Table 6** – Film solubility for A and B samples, with different content of glycerol

Sample	A35%	A50%	A65%	B35%	B50%	B65%
FS [%]	3.4133 ±0.87*	5.5533 ±0.71	9.9900 ±0.77	5.7900 ±0.61	9.3300 ±1.11	12.8933 ±0.29

(\*) – Mean ± SD from 3 measurements

The myofibrillar protein-based films exhibit very low FS, in comparison to the other edible films (lentil, soy, whey and pea protein-based films have FS approximately 38, 35, 30 and 39%, respectively (24)). This result suggested that the myofibrillar protein polymer network was highly stable and that only small molecules (small peptides, monomers and non-protein materials) were soluble.

At the same glycerol content, all films prepared at pH 3 had a lower FS value than those prepared at pH 11 ( $p < 0.05$ ). This result suggests that the films obtained with alkaline solubilizing process had a lower level of cross-linking with the weaker bonding, which was possibly associated with the shorter chain length of protein molecules. This leads to a lowered interaction between the molecules, which resulted in a higher solubility of the resulting films.

At the same pH, all films with greater glycerol content exhibited a higher FS ( $p < 0.05$ ). As reported by Cuq (31), in general, hydrophilic plasticizers, such as glycerol, enhanced water solubility. It is probably because increasing the plasticizer content in the film increased the water-soluble dry content. The relationship between water-soluble dry matter and hydrophilic plasticizer content is linear (32).

## CONCLUSIONS

Considering the results obtained for mechanical properties, it seems that chicken breast protein-based films fall in the acceptable range of quality for use as a packaging material, such as individual wrappers in a large box of carton. Water vapor permeability of tested samples was much higher than those of typical polymeric packaging materials, such as low-density and high-density polyethylene films, which considerably limits the application of films for food packaging. Light transmittance results suggested that the films could retard lipid oxidation by UV-light in a food system, but not by visible light. Considering color and transparency results and significant effect of pHs of the film-forming solution on these parameters, it might be concluded that the films prepared at pH 3 are more transparent and clear enough for packaging food product, in comparison with the films prepared at pH 11.

Almost all studied properties were affected by the glycerol concentration; nevertheless the effect of the pH was not evident in all properties. Protein is a good source for edible film formation and therefore the application of the film could be suggested, but there is still a number of limitations to be overcome. The shortcomings of the films could be minimized by further works to find out the best protein/plasticizer ratio, type of plasticizer, pH of the film-forming solution, etc.



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### **УТИЦАЈ САДРЖАЈА ГЛИЦЕРОЛА И рН ВРЕДНОСТИ РАСТВОРА ЗА ПРИПРЕМАЊЕ ЈЕСТИВИХ ПРОТЕИНСКИХ ФИЛМОВА НА ЊИХОВЕ ФУНКЦИОНАЛНЕ КАРАКТЕРИСТИКЕ**

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У раду је испитан утицај садржаја глицерола и рН вредности раствора за припремање протеинских филмова на њихове функционалне карактеристике. Филмови су произведени од протеина пилећег белог меса, у киселој (рН 3), односно базној (рН 11) средини, уз различит садржај глицерола (35%, 50% и 65% м/м на садржај протеина). Садржај глицерола је значајно утицао на механичке особине, пропустљивост водене паре, боју при рН 3 и растворљивост филмова ( $p < 0.05$ ). Ниво рН је имао значајан утицај на пропустљивост светлости, боју, транспаренцију и растворљивост филмова ( $p < 0.05$ ). Узимајући у обзир резултате механичких особина и растворљивости филмова, закључује се да добијени филмови имају прихватљиве особине за примену у својству амбалажног материјала, али пропустљивост водене паре, боја, пропустљивост светлости и транспаренција морају бити побољшане да би се могли успешно применити.

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