Physical and chemical properties of edible films from faba bean protein

C. Montalvo-Paquini, M. Rangel-Marrón, E. Palou and A. López-Malo

***Abstract*—** Natural polymers derived from natural sources like proteins of plants, offer great opportunities for the food industry due their biodegradability and ability to supplement nutritional value of foods. In this study, the effect of different values of pH (7.0, 8.5 and 10.0) of film forming solution on physical and chemical properties of faba bean edible films was investigated. The edible films were prepared by the solution casting method with 3% w/w of faba bean protein concentrate (FPC) and glycerol (50%, w/w of FPC) as plasticizer. Films were evaluated for thickness, water content, soluble matter, protein solubility, puncture strength, elongation, water vapor permeability (WVP) and color. The pH value did not have significant influence on moisture content and thickness. The total soluble matter and protein solubility showed a significant increase as pH forming solution increased from 7.0 to 10.0. However, edible films obtained had a good stability since polymer did not exceed 26% of total solubility film, while the protein solubility was not greater than 3%. At alkaline conditions the edible films showed the lowest WVP (0.96×10^{-10}g mm/kPa h m^2) and the highest values of puncture strength (17.92 MPa) and elongation (44.43 %). Edible protein films from faba bean had a lightly yellow color. The effect of pH on chemical properties has been explained using Fourier transform infrared (FTIR) spectroscopy and thermogravimetric analysis.

***Keywords*—** edible films, faba bean, legume, physical properties, protein concentrate.

I. Introduction

THE consumer´s growing demand to purchase nature friendly products has caused the food industry to develop biodegradable packaging materials to protect and extend food shelf life. In this sense, different kinds of new polymer composites had been developed like protein-based polymeric (metallocene linear low density polyethylene-hydrolyzed protein) or hydrogel (polyvinylpyrrolidone-carboxymethyl cellulose) [1]-[2].

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C. Montalvo-Paquini is student in the Doctoral program of Food Science in Universidad de las Américas Puebla, México.
(e-mail: claudia.montalvopi@udlap. mx).

Rangel-Marrón M. is student in the Doctoral program of Food Science in Universidad de las Américas Puebla, México.
(e-mail: marcela.rangelmn@udlap. mx).

E. Palou is Professor in the Department of Chemical, Food, and Environmental Engineering at Universidad de las Américas Puebla. Phone: +52(222) 2292000; fax +52(222)2292727 (e-mail: enrique.palou@udlap.mx).

A. López-Malo is Professor in the Department of Chemical, Food, and Environmental Engineering at Universidad de las Américas Puebla. Phone: +52(222)2292000; fax +52(222)2292727 (e-mail: aurelio.lopezm@udlap.mx).

However, in the nature exists several biopolymers, including polysaccharides, proteins and lipids that can be been used to form biodegradable films due to its abundance and biodegradability. The major drawbacks of such films are their relatively low water resistance and poor vapor barrier properties resulting from their hydrophilic nature [3]. However, protein films have a relatively good oxygen barrier and mechanical properties due to their large number of polar groups and extensive polymer interchain interactions forming a hydrogen-bonded network structure which is very tightly packed ordered [4]. It is because of this that interest in the study of protein films has recently increased, and research on the properties of these films have been reported in several studies [5]-[8].

Legume seeds are cheap sources of protein with a relatively high nutritional value, which make them a very good raw material to form protein films and appear to be an interesting alternative to synthetic plastics for food packing. However, few studies have been dedicated to the film forming ability of these proteins [9]-[11]. In particular, soy bean edible films have received considerable attention because their ability to produce more flexible, smooth, and clear films compared with other plant sources [7]. Tang *et al*. [12] showed that bean proteins have a good potential to form cast films with mechanical strength similar to those obtained from soy proteins. But there are other potential legumes as a source of edible protein films. Additionally, casting process has been used to obtain legumes protein based films like peanut, mung bean and soy [10], [13]. Faba bean (*Vicia faba L.*) is a legume that has been poorly studied as a raw material for forming edible film [11]; however, it has a high potential as source of high quality protein content since it may contain up 30% of protein, depending on the cultivation conditions and variety [14]. The faba bean is very important in the Mexican highlands due to its use as a fresh vegetable, as dry seed and as fodder.

On the other hand, the functional properties of proteins are highly dependent on structure heterogeneity, thermal sensibility and hydrophilic behavior. Proteins can be subjected to the action of acid, alkali, solvents or heat to yield partially denatured polypeptide chains that are the basis for the extended structures required for film formation. Protein films are formed when these extended structures associate through hydrogen, ionic, hydrophobic and covalent bonding to form protein matrix [15].

Beneficial effects of physical and chemical treatments in proteins based films have been reported by several researchers [7]-[9], [13], [16]. Properties of protein-based films depend

on various factors such as the source of protein, pH of protein solution, plasticizers, the preparation conditions and substances incorporated into the film-forming solutions. In this respect various films forming variables need to be examined to determine their effect on protein based film properties.

The objective of this study was to investigate the effect of different pH values (7.0, 8.5 and 10.0) of film forming solution on water vapor permeability, moisture content, solubility, mechanical, optical properties, chemical structure and thermal stability of faba bean protein films.

II. Metohology

A. Materials

Faba bean was purchased from a local market in San Pedro Cholula, Puebla (Mexico). Glycerol, used as plasticizer, was from Merck (Inc., Whitehouse Station, NJ, USA).

B. Faba bean protein concentrate

Faba bean was ground in an industrial blender and passed through a sieve (40 mesh) to obtain a fine powder. Alkaline-acid extraction was performed to obtain protein concentrates, following the next method: faba bean powder was stirred in distilled water by adjusting the pH value to 11.0 with 1N NaOH to increase solubility of the protein. The mixture was then centrifuged at 8,000 rpm for 30 min and the supernatant obtained was acidified to pH 5.4 with 1N HCl. The proteins were separated by centrifugation at 8000 rpm for 20 min and the freeze-dried to obtain faba bean protein concentrate (FPC) [17].

C. Film formation

Film forming solutions were prepared by dissolving FPC (3% w/v) in distilled water under constant stirring, the pH value was adjusted to 7.0, 8.5 and 10.0 using 1N NaOH, glycerol was added (1.5% w/v), as a plasticizer. The solutions obtained were homogenized using a homogenizer (Silverson, Model L4R, England) at 9000 rpm for 1min and were degassed under vacuum and then, heat-denatured at 80 °C for 20 min in a water bath and cooled to 37 °C for 3-4 minutes [17]. Measured volumes (4 ml) of film forming solution were poured onto horizontal flat silica on tray (5 cm diameter) and then dried at 40 °C for 18 h [18]. All films were stored in desiccators at 50% RH for 48 h, prior to testing [17].

D. Physical properties

Thickness and moisture content

Thickness of the films was measured with a micrometer (Mitutoyo, model MDC-1, Japan) at 10 random positions of the film [14]. Moisture content was determined by measuring weight loss after drying the films at 102 ± 2 °C for 24 h [19].

Total soluble matter

Total soluble matter (TSM) was calculated as the percentage of film dissolved during immersion in distilled water [20]. Film specimens (2 cm x 2 cm) were placed in 10 ml distilled water with potassium sorbate (0.01% w/v) to prevent microbial growth. The samples were soaked in a water bath at 20 °C for 24 h with gentle stirring. The films were removed from the solution and dried (102 ± 2 °C) for 24 h to determine the weight of dry matter that did not dissolve in water. This determination was performed in triplicate for each film type obtained. The percentage of total soluble matter of the films was calculated using the formula below:

$$\%\text{TSM} = \left(\frac{Initial\ dry\ weight - Final\ dry\ weight}{Initial\ dry\ weight}\right) * 100\% \qquad (1)$$

Film protein solubility

An aliquot of the supernatant obtained in the film solubility test was analyzed for protein content according to the Lowry´s technique [21].

Protein concentrations were calculated from a standard curve obtained using bovine serum albumin and the percentage of soluble protein of the film was obtained [22].

Mechanical properties

The mechanical properties of the films were evaluated by puncture test [23]. A texture analyzer (Texture Technologies Corp., NY, EE.UU.) with a spherical puncturing probe (diameter 1.4 cm) was employed. The film was placed in a holder with a circular gap (r = 0.8 cm). The probe was driven through the film with a speed of 1 mm/s and force displacement curves were recorded through a 25 N load cell. The puncture strength and percentage of elongation were calculated using Eqs. (2) and (3), respectively. The average of six measurements was performed.

$$\text{Puncture strength} = \frac{\text{F}max}{Acs} \qquad (2)$$

Where F*max* is the maximum applied force, A*cs* is the cross sectional area of the edge of the film located in the path of the gap, with Acs=2rδ, where r is the radius of the gap and δ is the thickness of the film.

$$\%\ \text{Elongation} = \left(\frac{\sqrt{r^2+d^2}-r}{r}\right) * 100 \qquad (3)$$

Where *r* is the radius of the film exposed in the circular gap of the film holder and *d* represents the displacement of the probe from the point of contact to the point of puncture.

Water vapor permeability (WVP)

The WVP of the films was measured according to the following method: glass cells (1.42 cm of internal diameter and 7.0 cm in height), with an exposed area of 1.6 cm^2, were filled with silica gel to a level of 1 cm below the top. The films were cut into circles and placed on top of cells sealing hermetically with parafilm and then placed in a desiccator containing saturated NaCl solution (75% HR). Weights were measured every 8 h over a period of 3 days at 25 °C [24]. When the relationship between the weight gained and time

was linear, the slope of the plots was calculated by linear regression.

The vapor transfer rate (WVTR) and WVP were calculated in accordance with the following expressions:

$$WVTR = \frac{ml}{A} \quad (4)$$

$$WVP = \frac{L \cdot WVTR}{(p1-p2)} \quad (5)$$

Where *ml* is the slope of the weight loss versus time (g/s), *A* is the exposed area (m^2). *L* is the average film thickness (mm), while p_1 is the partial pressure inside the desiccator, and p_2 is the partial pressure inside the cells.

Color measurements

Color values of the films were measured with a colorimeter (Colorgard System/05) calibrated with a white standard plate (*L**= 92.89, *a**= -1.06 and *b**= 0.82). Film color was measured by the *L**, *a** and **b* color scale. *L** values range from 0 (black) to 100 (white) and chromaticity parameters *a** (green= -60 to red= +60) and *b** (blue= -60 to yellow= +60). Five measurements were made for each sample and for each type of film 5 replicates were performed [17].

E. Chemical properties

Infrared spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) analysis was carried out at room temperature to obtain information about the main functional groups of the films matrix. Spectra were obtained using a Shimadzu FT-IR spectrometer (IRAffinity-1, Europe) and were run at least ten scans in the range of 4000 and 500 cm^{-1} for each of the analyzed samples [24].

Thermal properties

Thermal stability of films was analyzed by thermo-gravimetric analysis (TGA). Non-isothermal degradation measurements were performed in a Perkin Elmer TGA 7. Tests were run from room temperature up to 600 °C at a heating rate of 10 °C/min under nitrogen atmosphere (10 ml/min) to avoid thermo-oxidative reactions.

F. Statistical Analysis

The Minitab 16.0 program (Minitab, Inc., State College, PA, EE.UU) was used to perform analysis of variance (ANOVA). The statistical differences between mean values were established at P<0.05.

III. Results

The obtained films were strong and flexible enough to be peeled and handled. Table I summarizes the thickness, moisture content and total soluble matter (TSM) of faba bean protein films made by casting film-forming solutions adjusted at different pH values.

Table I Properties of protein edible films obtained from faba bean[1].

Treatment	Thickness (mm)	Moisture content (%)	TSM[2] (%)
FB- 7.0	0.073 ± 0.001a	29.65 ± 0.30a	19.21 ± 1.45b
FB- 8.5	0.074 ± 0.008a	28.75 ± 0.28a	22.75 ± 0.94a
FB-10.0	0.074 ± 0.003a	30.45 ± 2.13a	25.40 ± 0.57a

[1] Two means followed by the same letter in the same column are not significantly (P>0.05) different. [2]Total Soluble Matter. FB: Faba Bean.

Thickness and moisture content

The film forming-solution adjusted at different pH values did not significantly affect (P>0.05) their thickness and moisture content (Table I).

The thickness values were approximately of 0.074 mm, this value is higher than thickness for soy protein films with range values between 0.058-0.065 mm [25]. Additionally, moisture content is not very different from the values obtained for edible films from pistachio globulin (34-37%) [19]. On the other hand, pea protein films were obtained with lower moisture content (18-22%), probably due to the fact that films had been prepared with a higher concentration of pea protein isolate (10% w/w) [7].

Total soluble matter

This is an important property since potential applications of edible films, may require water insolubility to enhance product integrity and water resistance. The pH values of film forming-solution had a significant effect (P<0.05) in total soluble matter at alkaline pH (Table I).

The faba bean protein films tested did not dissolve or break after 24 h of incubation; this confirms that protein polymer was stable and that only small molecules of peptides were soluble. The films obtained with alkaline solubilizing process might have a lower level of cross linking with the weaker bonding, which would be associated with the shorter chain length of protein molecules [26]. This leads to a dished interaction between the molecules, which resulted in a higher solubility of the resulting films.

S. Saremnezhad, M. H. Azizi, M. Barzegar, S. Abbasi, E. Ahmadi [11], obtained the same behavior in faba bean protein films formed within pH 7-12, but in alkaline treatment (pH 12.0) their solubility increased up to 40 %. The values obtained in this work were lower, probably due to the fact that the film forming solution was heat treated and this could have an effect in the polymer formation. Furthermore, total soluble matter obtained is lower than the solubility of other legume protein films such as lentil protein film (38.75%) and soy protein film (33.94%) [17]. The lower solubility of faba bean films can be an indication of the high stability of protein network.

Film protein solubility

Edible films obtained with the most alkaline film forming-solution (pH 10.0) showed protein solubility significantly (P<0.05) higher than those films obtained at pH 7.0 or 8.5 (Fig. 1).

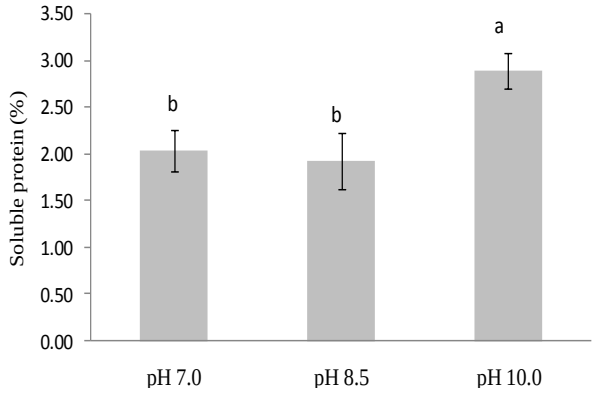

Fig. 1 Protein solubility of faba bean edible films. Columns with the same letter are not significantly (P>0.05) different.

Y. Shiku, P. Y. Hamaguchi, M. Tanaka [27] obtained the same behavior in myofibrillar protein films formed within pH 7-12 and the same case was for peanut protein films, where protein solubility was higher when pH increases from 6.0 to 7.5 [5].

A. P. Adebiyi, R. E. Aluko, [28] found that pea protein isolate and pea protein fractions were more soluble in the alkaline pH than acidic or neutral, which may be due to an increase in net protein charge as pH was increased. Moreover, the low molecular weight protein chains (i.e., monomer and small peptides) formed during the conditioning of the film-forming solution and immobilized in the film network could constitute the water soluble protein components of the films [29]. These results suggest that the mechanism of film formation could be different with the pH range of the film forming solutions.

Water vapor permeability (WVP)

Edible films from faba bean obtained at pH 8.5 and 10.0 significantly (P<0.05) reduced the WVP (0.96±0.08 and 1.08±0.03 g mm/kPah m^2, respectively), in contrast with the films obtained at lower pH (Fig. 2). The adjustment of acid or alkaline pH to obtain edible films, might affect the WVP property of the film by modifying the charge of protein molecule, resulting in the differences in WVP.

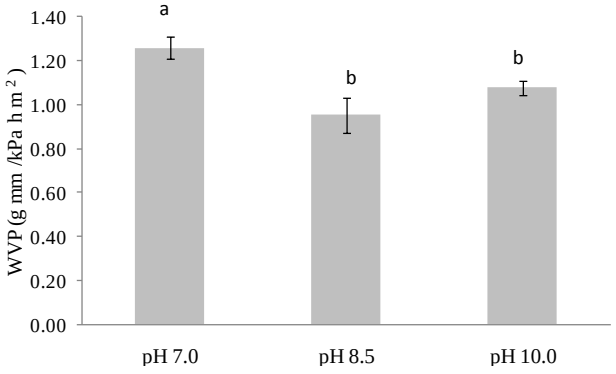

Fig. 2 Water vapor permeability of faba bean edible films. Columns with the same letter are not significantly (P>0.05) different.

S. Saremnezhad, M. H. Azizi, M. Barzegar, S. Abbasi, E. Ahmadi [11] observed similar results on faba bean films, where the lowest values of WVP were obtained in films prepared in alkaline pH (12.0). Additionally, the values of WVP obtained in this work were lower than the values reported for pea protein films (7.42±0.69 g mm/kPa h m^2) [20].

The pH is the major parameter that affects the peptide charge. At alkaline conditions, a pH away from the isoelectric point, promotes protein denaturation, unfolding and solubilization. The same charged groups repelled each other and produced a stretching of the polymer chain when functional groups on a linear polymer are ionized during dissolution facilitating a fine stranded network [10]. This may contribute to the fact that free passage of water molecules occurs with difficulty.

Mechanical properties

Mechanical properties of edible protein films provide an indication of film integrity under stress conditions and during processing, handling and storage of foods, it could be an important factor to increase shelf life. Puncture strength was evaluated as the hardness of protein films, which was the maximum force exhibited by the films under test conditions. The puncture strength of edible films from faba bean increased as pH increased from 7.0 to 10.0 (Fig. 3).

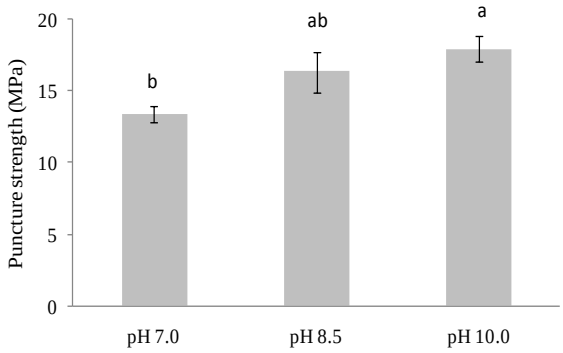

Fig. 3 Puncture strength of faba bean edible films. Columns with the same letter are not significantly (P>0.05) different.

Alkaline pH values increase the solubility of the protein concentrate into the film-forming solution to form a thinner polymer network. This is also reflected in the percentage elongation obtained, as it increases as the pH values become more alkaline (Fig. 4).

Elongation is the maximum change in length of the test specimen before breaking and higher percent elongation indicates that the film was more flexible. At alkaline pH far from their isoelectric point, the protein is completely solubilized and interacts with the glycerol in a greater proportion. The glycerol acts as a lubricant to facilitate the movement to reduce frictional forces between the polymer chain and this can increase flexibility in the film.

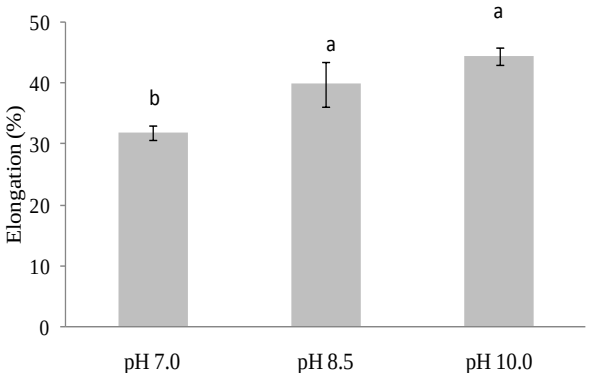

Fig. 4 Puncture strength of faba bean edible films. Columns with the same letter are not significantly (P>0.05) different.

These results are agreement with those obtained on rice bran protein based edible films prepared at different pH (6, 8, 9, 10, 11) where the puncture strength of films increased up to pH 8.0 [30]. This is also reported to peanut protein film, where an increase in pH values result in the highest elongation for the resulting films [5].

Color measurements

The color of faba bean protein film was affected by pH of film solutions (Table II). In general, edible films from faba bean presented amber color and differences were hardly observed between the treatments. Additionally, all films had a transparent appearance, unlike the results obtained on edible films from peanut protein that appeared more opaque and dull at pH 6.0 [5].

Table II Parameters of color of edible films from faba bean[1].

Treatment	L*	a*	b*
FB-7.0	15.08 ± 1.54a	-1.95 ± 0.12b	2.81 ± 0.52a
FB-8.5	13.20 ± 2.74ab	-1.56 ± 0.20ab	2.54 ± 0.73ab
FB-10.0	10.65 ± 1.21b	-1.34 ± 0.12a	1.97 ± 0.52b

[1] Two means followed by the same letter in the same column are not significantly (P>0.05) different. FB: Faba bean.

The results of the measurements performed on the films accord with the CIE system showed that lightness (L^*) values decreased significantly (P<0.05) when pH increased to 12 and the films showed a slightly dark color. The value a^* and b^* showed the same behavior, resulting in a lighter greenish yellow film. At alkaline pH, value b^* decreased and this made the films appear a little less yellowish.

These results are similar to the data of mung bean films prepared in alkaline conditions and faba bean films prepared at different pH values where darker films were obtained at higher pH (12.0) [10]-[11]. This could be due to the fact that alkaline solvents than can extract pigments more than other solvents [5].

FTIR analysis

The chemical structure of the edible films made at different pH values was investigated by Fourier transformed infrared (FTIR) spectroscopy. The spectra of edible films from faba bean protein obtained are displayed in Fig. 5.

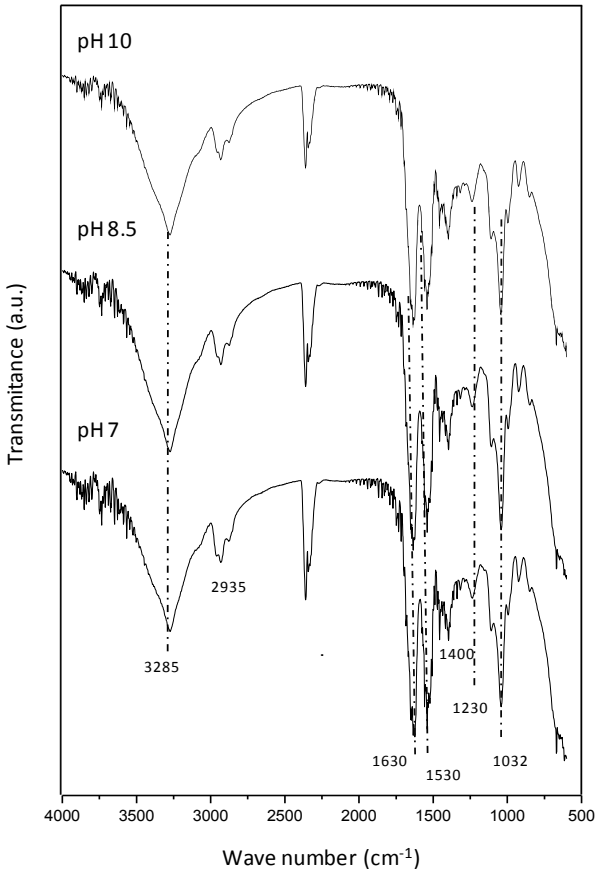

Fig. 5 FTIR spectra of FPC edible films processed at different pH values of film forming solution.

The FTIR spectrum of edible films shows that no change take place in the peaks characteristic of protein and glycerol, specifically in the 1200-800 cm^{-1}region, which could indicate that glycerol does not react with the protein through covalent bonds. The band at 1032 cm^{-1} is associated with the extension band of C-O in C1 y C3, while the peak at 1109 cm^{-1} is linked to the extension band of C-O in C2. The peak situated around 1032 cm^{-1} might be related to the interactions arising between plasticizer (OH group of glycerol) and film structure [31].

The main absorption band of peptide linkage is related to C=O stretching at 1630 cm^{-1} (amide primary), N-H bending at 1530 cm^{-1} NH (amide secondary) and C-N stretching at 1230 cm^{-1}.

The peak 2935 cm^{-1} is related to the tension band of C-H (saturated aliphatic), while the band at 3285 cm^{-1} is characteristical of the free OH groups, which are able to form hydrogen bonding [25]. On the other hand, the band at 1400 cm^{-1}, corresponding to the vibrations of COO-group, appears due to the amphoteric character of proteins where the carboxyl group could be ionized due to the fact that the film forming

solution was adjusted at alkaline pH. P. Guerrero, K. De la Caba [32] and P. Guerrero, A. Retegi, N. Gabilondo, K. De la Caba [33], obtained similar FTIR spectral data for soy protein edible films with glycerol as plasticizer.

It was observed a slight difference in spectra in the band at 1032 cm^{-1} for edible film obtained at pH 10.0. The decrease in vibrational wavenumber could be indicative of a hydrogen bonding interaction between polymer molecules in the film [31].

Alkaline pH might induce unfolding of protein, which promote intermolecular protein interaction during the film formation that allows the interaction between protein-protein and plasticizers [34]. Similar behavior was observed in the spectra obtained from skin gelatin films prepared at pH 7 and pH 11 in previous work [31].

Thermal properties

TGA thermograms revealed thermal degradation behavior of films prepared at different values of pH are presented in Fig. 6.

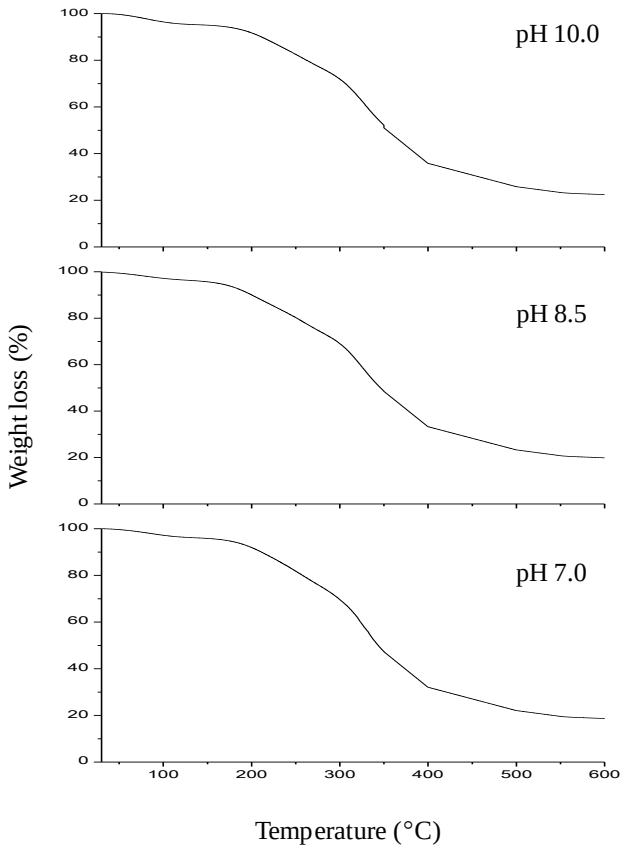

Fig. 6 TGA curves of FPC edible films processed at different pH values of film forming solution.

All films exhibited three main stage of weight loss. The first weight loss stage was observed approximately at temperature from 70 °C to approximately 150 °C, possibly related to the loss of free water absorbed in the film. The second stage weight loss appeared approximately at 190-220 °C, was most likely associated with the loss of glycerol and structurally bound water. This temperature is higher than the boiling point of glycerol (182 °C), which indicates that some kind of interaction (hydrogen bonds) exists between BCP and glycerol. The third weight loss stage, appear approximately at 320 °C, possibly due to decomposition of higher interacted protein fractions of faba bean.

These values are in agreement with the ones obtained in previous works [31]-[33]. All films presented similar behavior, but in the curve of the edible films obtained at pH 7.0 and 8.5 the weight loss observed at 320 °C was 38.06 % and 38.67 %, respectively and these values were higher that the weight loss of the film at pH 10.0, (35.34 %). These results suggest that the different values of pH of film forming solution affected the thermal stability of the edible films and this could be due to an increase in the interaction between the peptides at alkaline pH.

IV. Conclusion

The value of pH used during film forming solution can significantly affect the physical and chemical properties of protein films. Thickness and moisture content did not show significant difference at neutral or alkaline pH values. On the other hand, total soluble matter and soluble protein showed a significant increase ($P<0.05$) when their values increased 30% and 40% respectively, in the most alkaline pH values. However, the films showed a better physical integrity than other reported legumes protein based films.

The main benefit of alkaline treatments (pH 10.0) was a decreased of 20% on water vapor permeability and a similar increase of puncture strength and elongation (38%, approximately). In these conditions relatively strong films could be obtained.

Color properties of the faba bean protein based films were lighter yellow and clear at different pH values, this characteristic could be used on light-sensitive foods.

The interaction between the proteins in alkaline conditions (pH 10.0) promoted the formation of a compact network that had effect on the chemical properties of faba bean films.

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Montalvo-Paquini C. is Professor-Researcher in the Biotechnology Engineering Faculty of the Universidad Politécnica de Puebla since 2005. MC. Montalvo received her Master degree in Biotechnology in 2000 from CINVESTAV-IPN. M.C Montalvo is student in the Doctoral program of Food Science in Universidad de las Américas Puebla.

Rangel-Marrón M. is Professor-Researcher in the Chemistry Faculty of the Autonomous University of Carmen since 2005. MC. Rangel-Marrón received her Master degree in Food Science in 2004 from Universidad de las Américas Puebla. M.C Rangel is student in the Doctoral program of Food Science in Universidad de las Américas Puebla.

Palou E. is Professor in the Department of Chemical, Food, and Environmental Engineering at Universidad de las Américas Puebla. Dr. Palou is co-author of Nonthermal Preservation of Foods edited by Marcel Dekker, editor of two books, authored over 26 book chapters and more than 120 scientific publications in refereed international journals, is member of the Journal of Food Protection Editorial Board. Dr. Palou received his PhD in Engineering Science in 1998 from Washington State University in EE.UU. He has presented over 310 papers in international conferences. He belongs to the National Research System of Mexico as a National Researcher Level III.

López-Malo A. is Professor in the Department of Chemical, Food, and Environmental Engineering at Universidad de las Américas Puebla. Dr. López-Malo is co-author of Minimally Processed Fruits and Vegetables, editor of two books, authored over 30 book chapters and more than 100 scientific publications in refereed international journals, is member of the Journal of Food Protection Editorial Board. Dr. López-Malo received his PhD in Chemistry in 2000 from Universidad de Buenos Aires in Argentina. He has presented over 300 papers in international conferences. He belongs to the National Research System of Mexico as a National Researcher Level III.