Effect of pH on physical properties of edible films from faba bean protein

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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 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 x10^-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.

Key-Words:-faba bean, legume, edible films, protein concentrate, physical properties, alkaline conditions

1 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. Several biopolymers, including polysaccharides, proteins and lipids have been used to form biodegradable films. It is because of this that interest in the study of plant protein films has recently increased, and research on the properties of these films have been reported in several studies [1,2,3,4].

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 [5,6,7]. 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 [3].

Tang et al. [8] 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 [6,9]. Faba bean is a legume that has been poorly studied as a raw material for forming edible film [7]; 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 [10].

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 [11].
Beneficial effects of physical and chemical treatments in proteins based films have been reported by several researchers [3,4,5,9]. 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 and optical properties of faba bean protein films.

2 Materials and Methods
2.1 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).

2.2 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 isolates, following the technique reported by Bamdad et al. [12]. 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).

2.3 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 1 min 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 [12]. 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 [13]. All films were stored in desiccators at 50% RH for 48 h, prior to testing [12].

2.4 Physical properties
2.4.1 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 [10]. Moisture content was determined by measuring weight loss after drying the films at 102 ± 2 °C for 24 h [14].

2.4.2 Total soluble matter
Total soluble matter (TSM) was calculated as the percentage of film dissolved during immersion in distilled water [15]. Film specimens (2x2 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:

\[
\%\text{TSM} = \left( \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \right) \times 100\% \tag{1}
\]

2.4.3 Film protein solubility
An aliquot of the supernatant obtained in the film solubility test was analyzed for protein content according to the technique described by Lowry et al. [16]. Protein concentrations were calculated from a standard curve obtained using bovine serum albumin. The percentage of soluble protein of the film was calculated as reported by Sothornvit and Krochta [17].

2.4.4 Mechanical properties
The mechanical properties of the films were evaluated by puncture test as described in Soradech et al. [18]. 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.

\[
Punctue\ strength = \frac{F_{\text{max}}}{A_{cs}} \tag{2}
\]

Where \(F_{\text{max}}\) is the maximum applied force, \(A_{cs}\) is the cross sectional area of the edge of the film located in the
\[ \text{path of the gap, with } Acs = 2r\delta, \text{ where } r \text{ is the radius of the gap and } \delta \text{ is the thickness of the film.} \]

\[ \% \text{ Elongation} = \left( \frac{\sqrt{r^2 + d^2} - r}{r} \right) \times 100 \quad (3) \]

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.

2.4.5 Water vapor permeability (WVP)

The WVP of the films was measured according to the method described by Andreuccetti et al. [19].

Glass cells (1.42 cm of internal diameter and 7.0 cm in height), with an exposed area of 1.6 cm², 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. 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 (VWTR) and WVP were calculated in accordance with the following expressions:

\[ \text{VWTR} = \frac{m_l}{A} \quad (4) \]

\[ \text{WVP} = \frac{L \cdot \text{VWTR}}{(p_1 - p_2)} \quad (5) \]

Where \( m_l \) is the slope of the weight loss versus time (g/s), \( A \) is the exposed area (m²), \( L \) 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.

2.4.6 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 [12].

2.5 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 \( P<0.05 \).

3 Results and Discussion

The obtained films were strong and flexible enough to be peeled and handled. Table 1 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>
<thead>
<tr>
<th>Treatment</th>
<th>Thickness (mm)</th>
<th>Moisture content (%)</th>
<th>TSM^2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB-7.0</td>
<td>0.073 ± 0.001a</td>
<td>29.65 ± 0.30a</td>
<td>19.21 ± 1.45b</td>
</tr>
<tr>
<td>FB-8.5</td>
<td>0.074 ± 0.008a</td>
<td>28.75 ± 0.28a</td>
<td>22.75 ± 0.94a</td>
</tr>
<tr>
<td>FB-10.0</td>
<td>0.074 ± 0.003a</td>
<td>30.45 ± 2.13a</td>
<td>25.40 ± 0.57a</td>
</tr>
</tbody>
</table>

\(^1\) 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.

3.1 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.

The thickness values were approximately of 0.074 mm, this value is higher than thickness for soy protein films obtained by González et al. [20] with range values between 0.058-0.065 mm. Additionally, moisture content is not very different from the values obtained by Zahedi et al. [14] for edible films from pistachio globulin (34-37%) or by Kowalczyk and Baraniak [3] for pea protein films with moisture content of 18%.

3.2 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 1).

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 [21]. This leads to a dished interaction between the molecules, which resulted in a higher solubility of the resulting films.
Saremnezhad et al. [7] obtained the same behavior in faba bean protein films formed within pH 7-12. On the other hand, total soluble matter obtained is lower than the solubility of lentil protein film (38.75%) and soy protein film (33.94%) studied by Bamdad et al. [12].

### 3.3 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](image1.png)

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

Shiku et al. [22] obtained the same behavior in myofibrillar protein films formed within pH 7-12; while in peanut protein films, protein solubility was higher when pH increases from 6.0 to 7.5 [1].

Adebiyi et al. [23] 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, Cuq [24] mentions that 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.

These results suggest that the mechanism of film formation could be different with the pH range of the film forming solutions.

### 3.4 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/kPa h m², 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](image2.png)

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

Similar behavior was observed by Saremnezhad et al. [7] 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 reported by Choi and Han [15] who obtained 7.42±0.69 g mm/kPa h m² of WVP for pea protein films.

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 [6]. This may contribute to the fact that free passage of water molecules occurs with difficulty.

### 3.5 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).
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. These results are agreement with those obtained by Adebigy et al. [25]. They found a similar effect 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. This is also reported to peanut protein film, where an increase in pH values result in the highest elongation for the resulting films [1].

### 3.6 Color measurements

The color of faba bean protein film was affected by pH of film solutions (Table 2). 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 by Jangchud and Chinnan [1] who prepared edible films from peanut protein that appeared more opaque and dull when they used pH 6.0.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB-7.0</td>
<td>15.08 ± 1.54a</td>
<td>-1.95 ± 0.12b</td>
<td>2.81 ± 0.52a</td>
</tr>
<tr>
<td>FB-8.5</td>
<td>13.20 ± 2.74ab</td>
<td>-1.56 ± 0.20ab</td>
<td>2.54 ± 0.73ab</td>
</tr>
<tr>
<td>FB-10.0</td>
<td>10.65 ± 1.21b</td>
<td>-1.34 ± 0.12a</td>
<td>1.97 ± 0.52b</td>
</tr>
</tbody>
</table>

Note: 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. 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.

### 4 Conclusions

The value of pH used during film forming solution, could significantly affect the physical 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.

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References:


