

Properties of a New Edible Film Made of Faba Bean Protein Isolate

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ABSTRACT

There has been a renewed interest in edible films made of renewable and natural polymers such as protein, polysaccharide and lipids. Natural polymers derived from natural sources like food protein, offer the greatest opportunities because of their biodegradability and their ability to supplement nutritional value of foods. Faba bean is a valuable source of protein and is cultivated in large quantities in Iran. Therefore, in this study, we investigated the potential of faba bean protein isolate (FPI) as a new protein source for preparation of an edible film and determined the effects of film forming solution pH and plasticizer concentration on the film properties. Results showed that increasing the pH improved mechanical properties (tensile strength and elongation) and solubility of the films, but, caused the water vapor permeability (WVP) and lightness of the films to decrease. Plasticizer concentration did not affect WVP over the studied range. Elongation and solubility of the films increased and the tensile strength decreased by increasing plasticizer content of the film forming solutions. The lowest WVP and the highest tensile strength were observed at pH 12 and 40% (w/w of FPI) glycerol concentration.

Keywords: Edible film, Faba bean, pH, Plasticizer, Protein isolate.

INTRODUCTION

In recent decades, potential barrier properties of edible films against moisture, gases, and solutes, their ability to carry food additives, such as flavours, anti microbial agents, antioxidants and colors (1) and their biodegradability and, consequently, reduction of the environmental pollution caused by the application of traditional plastic packaging, have all attracted interest in studying of different edible films and coatings. Edible films can be prepared from proteins, polysaccharides, lipids or the combination of these components (2). Thereinto, proteinous edible films due to their nutritional value and better mechanical and gas barrier properties compared to those of lipid and polysaccharide films are more

attractive (3). Studying different aspects of proteinous edible films and coatings production for improving their properties and investigation on their usage and potentialities have attracted many researchers, especially in recent years.

In this respect, some topics of interest include cross linking of soy protein isolate and carboxyl methyl cellulose blend films by Maillard reaction and studying the resulting film properties (4), developing a composite film based on whey protein isolate and mesquite gum (5), structural and functional properties of soy protein isolate and cod gelatin blend film (6), effects of drying condition on properties of soy protein films (7), influence of spelt bran on the properties of whey protein isolate composite films (8), improvement of antioxidant properties of squid skin gelatin films by

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addition of hydrolysates from squid gelatin (9), physical properties and internal microstructure of films made of catfish skin gelatin and triacetin mixtures (10), reducing fat uptake in cassava product during deep fat frying (11), physical and antimicrobial properties of grape seed extract, nisin and EDTA incorporated soy protein edible films (12) and molecular mobility and oxygen permeability in amorphous β -lactoglobulin films (13).

As mentioned above, properties of films from different protein sources have been investigated extensively, but, there is still limited information on the use of legume seeds protein in the formulation of edible films, although the characteristics of some leguminous protein-based edible films such as pea (14,15), peanut (16), soy (17,18,19) and lentil (20) have been studied.

Faba bean (*Vicia faba L.*) is a rich source of food quality protein and, depending on its cultivar, may contain up to 35% protein (dry basis). It is a popular legume food in Iran and many other countries and is cultivated in large areas, specially in the northern parts of Iran. It is also used as feed in southern parts of the country.

To the best of our knowledge, use of faba bean protein as an edible film has not yet been studied; therefore, our objective in the present study was to investigate the potential of faba bean protein isolate in preparation of edible films at different pH values (7, 9, and 12) and plasticizer contents (40, 50, and 60% w/w of faba bean protein isolate), by measuring films mechanical properties, water vapor permeability, total soluble matter and color and determine the conditions to obtain the best mechanical strength and the lowest water vapor permeability.

MATERIALS AND METHODS

Materials

Dried faba beans (*Vicia faba L.*) were obtained from Agricultural Research,

Education and Extension Organization of Iran. All chemicals and solvents were of analytical grade made by Merck (Darmstadt, Germany).

Preparation of faba bean protein isolate

Dried faba beans were milled in a grinder (Moulinex 220, Italy) to obtain a fine powder (40 mesh), from which protein isolate (FPI) was prepared using a modified method of alkaline extraction and acid precipitation described by McCurdy and Knipfel (21). The powder was suspended in distilled water in the ratio of 1:10 (w/v) at ambient temperature, adjusting the pH to nine with 1 M NaOH and stirring with a magnetic stirrer (IKA model RH B2, Germany) for 1 h, the suspension was centrifuged (6900 KUBOTA, Japan) at $9400 \times g$ for 4 min. The pH of the supernatant was adjusted to 5.1 with 1 M HCl to form a proteinous precipitate, then, centrifuged at $9600 \times g$ for 6 min. Finally, the collected precipitate was diluted with distilled water (1:1 w/w), neutralized with 1 M NaOH, and freeze-dried.

The protein content of FPI was determined by total Kjeldahl nitrogen method and multiplying the nitrogen value by 6.25 (22). The amino acid composition of FPI was also determined according to the method used by Adebawale *et al.* (23).

FPI film preparation

Film forming solutions were prepared by dissolving 5 g FPI under constant stirring in 100 ml distilled water, the pH was adjusted to the desired levels (7, 9 and 12) with 1 M NaOH and glycerol, as plasticizer, was added at 40%, 50% and 60% w/w of FPI. Solutions were strained through cheesecloth and cast on leveled Teflon coated glass plates. Films were peeled from the plates after drying at 25°C and 50% RH for about 12h. All film specimens were conditioned at 25°C and 50% RH for 48 h in a chamber

with controlled temperature and relative humidity prior to testing (24).

Thickness

Thickness of films was measured with a digital micrometer (Mitutoyo, Japan) to the nearest 0.001 mm at least at seven random positions.

Color

Hunter color parameters (L^* , a^* and b^*), were measured by a Colorflex (4510, USA). Color values were recorded as L^* (lightness, 0 = black, 100 = white), a^* ($-a$ = greenness $+a$ = redness) and b^* ($-b$ = blueness $+b$ = yellowness). The change of color was evaluated by comparing total color difference (ΔE) of the films. ΔE was calculated as:

$$\Delta E = \left[(L^*_{\text{standard}} - L^*_{\text{sample}})^2 + (a^*_{\text{standard}} - a^*_{\text{sample}})^2 + (b^*_{\text{standard}} - b^*_{\text{sample}})^2 \right]^{0.5}$$

Standard values for white calibration plate were $L^* = 92.23$, $a^* = -1.29$ and $b^* = 1.19$

Mechanical properties

Tensile strength (TS) and percent elongation at break (E%) were measured with a texture analyzer (Zwick B2 2.5/ TH 1S, Germany) following the guidelines of ASTM Standard Method D 882-02 (24). Initial grip separation and cross-head speed were set at 50 mm and 500 mm/min, respectively. TS was expressed in MPa and calculated by dividing the maximum load by the original minimum cross-sectional area of the specimen. E% was calculated by dividing the extension at the moment of rupture of the specimen by the initial gage length of the specimen and multiplying by 100.

Water vapor permeability (WVP)

The WVP of films were measured using the ASTM method E96-00 (25). Film

specimens were sealed on glass permeating cups containing 8 ml distilled water (100% RH) with silicagel vacuume grease and a plastic bond. Binder clips (No. 155, Tingting, China) were used to hold the film in place. The cups were placed in a desiccator containing silica gel at its bottom (0% RH) at 25°C. The cups were weighed at 12 h intervals over 3 days and WVP of the film was calculated as:

$$\text{WVP} (\times 10^{-10}) = (\text{WVTR}) L / \Delta P$$

Where WVTR is the measured water vapor transmission rate ($\text{g}/\text{m}^2 \cdot \text{s}$) through a film specimen, L is the mean film thickness (m) and ΔP is the partial water vapor pressure difference (Pa) between the two sides of the film specimen.

Total soluble matter

Total soluble matter (TSM) was calculated as the percentage of film dry matter dissolved during immersion in distilled water for 24h. For measuring TSM, film pieces ($20 \times 20 \text{ mm}^2$) were weighed and dried for 24 h in an air circulating oven at 105°C to determine their moisture content. The dried film pieces were placed in 100 ml beakers containing 50 ml of distilled water. Beakers were covered with parafilm and stored at 25°C for 24 h under constant shaking at 70 rpm. After 24 h, residual film pieces were rinsed gently with distilled water and dried in air circulating oven (105°C) for 24 h. The weigh of dissolved dry matter was calculated by subtracting the weight of insoluble solid matter from the initial weight of the solid matter (26).

Microstructure study

A sample of film (40% glycerol and pH 12) was coated with a fine layer of gold and its surface and cross section examined in a model XL30 scanning electron microscopy (Philips, Netherlands). The samples were



examined using an accelerating beam at a voltage of 20 KV.

RESULTS AND DISCUSSION

Protein content and amino acid analysis

Protein content of dried and ground faba beans and FPI were determined to be $29\% \pm 0.46$ and $84.6\% \pm 4.8$, respectively. Proportion of each amino acid of FPI was also determined and the result is presented in Table 1. These values are similar to those reported by Sosulski and McCurdy (27). According to these researchers, faba bean is comparable to soy bean in lysine content, but has a greater deficiency in methionine and cysteine and, like field pea, does not contain sufficient threonine or tryptophan to supplement a diet deficient in these essential amino acids. However, faba bean ranks among the world's most surviving grain legume crops (28) and it can be compared with high protein soy bean, thus has potential to be used as a rich source of protein in the formulation of edible films.

Mechanical properties

Statistical analysis showed that pH and glycerol concentration affected tensile strength (TS) and elongation at break (E%) of FPI films (Figure 1a,b) significantly ($p < 0.05$). The mean thickness values for

Table 1. Amino acid content of FPI.

Amino acid	Concentration (mg /g)
Asp	84.5
Glu	16.5
Ser	35.8
Gly	31.2
His	21.1
Arg	74.5
Thr	25.5
Ala	29.7
Pro	40.7
Tyr	26.5
Val	40.8
Met	2.3
Ileu	37.8
Leu	65.6
Phe	39.1
Lys	49.8
Cys	3.2

films prepared at pH 7, 9 and 12 were 81.6 ± 4.2 , 77.4 ± 4.8 and 76.4 ± 2.1 μm , respectively. TS showed a significant increase at pH 12 after almost constant low values at pH 7 and 9. Increasing pH also affected elongation at break of FPI films ($p < 0.05$) and caused them to increase, both of which are assumed to be due to increase in protein-protein interactions at more alkaline conditions. According to Hamaguchi *et al.* (29), increasing protein solubilization in acidic and alkaline conditions and interaction of solubilized proteins with each other during drying is the cause of TS improvement in muscle protein films, prepared under acidic and alkaline

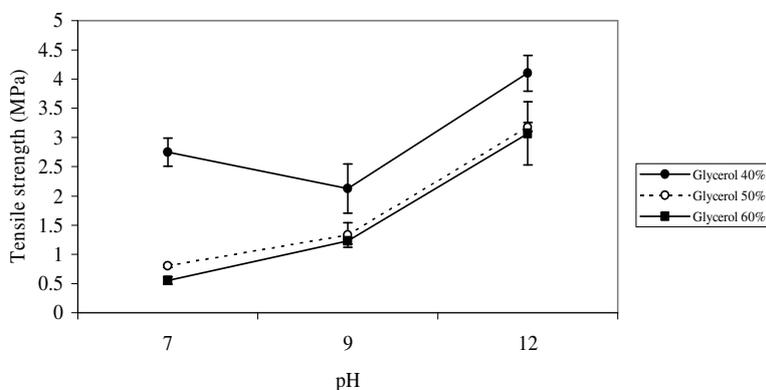


Figure 1a. Effect of pH and plasticizer concentration on tensile strength of FPI films.

conditions. Our results are also in agreement with the results obtained by other researchers. Gennadios *et al.* (30) reported that TS of soy protein isolate film was unaffected by pH from 6 to 9 but was affected by more acidic or alkaline conditions. They also reported that elongation of SPI film increased slightly when pH of film forming solution increased.

As shown in Figure 1a,b increasing the concentration of plasticizer decreased tensile strength, while increasing the elongation at break. Glycerol, as plasticizer, increases film flexibility via reducing hydrogen bonding between protein chains and increasing the spacing (31). The highest elongation (203.84 ± 8.37) was obtained at pH 12 and 60% glycerol content. Fairly *et al.* (32), reported that in all types of edible films, a small increase in glycerol concentration results in a large drop in tensile strength and increase in elongation.

The highest TS value (4.10 ± 0.31 Mpa) was obtained at pH 12 and 40% glycerol content that, as shown in Table 2, is a little bit lower than that of lentil protein concentrate film at almost the same conditions.

Comparison of FPI mechanical properties (Figure1) with soy bean protein isolate films (Table2) showed lower TS of FPI films. The reason can be denaturation of soy proteins

and increasing of cross-links as a result of using heat treatment during SPI film preparation. According to Liu *et al.* (33), heat treatment of peanut protein film forming solution increases the cross linkings of protein chains, resulting in the formation of tighter and more compact protein network and the subsequent increased mechanical strength. Also, higher elongation of SPI film compared to FPI film (at same pH) can be due to using a little bit more glycerol in the formulation of SPI film. Mechanical properties of FPI film are also comparable with that of heat denatured whey protein films. TS of FPI film (pH 7 and 40% glycerol content) was similar to that of whey protein concentrate (WPC) film prepared at pH 8 with the same glycerol content, although it should be noted that whey protein film forming solution was heat-denatured at 80 °C. Thus, the TS value similarity of FPI and WPC films in the absence of heat treatment during FPI film preparation can indicate the tighter protein network of faba bean protein isolate film.

FPI films at pH levels of 9 and 7 showed 3.4 and 2.5 fold more elongation than WPC film, respectively. The lower elongation of WPC film can be attributed to the denaturation of its proteins during heat treatment.

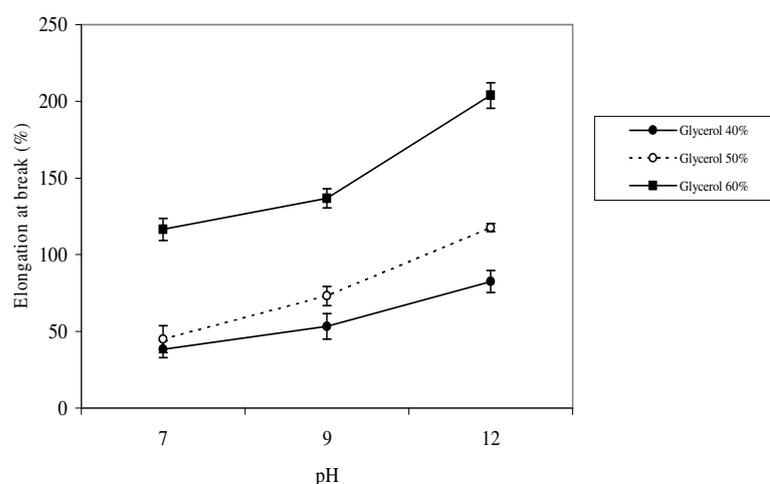


Figure 1b . Effect of pH and plasticizer concentration on elongation at break of FPI films.

**Table 2:** Mechanical and water vapor barrier properties of some protein films.

Film type	Tensile strength (MPa)	Elongation at break (%)	WVP×10 ⁻¹⁰ (g/m.pa.s)	Thickness (mm)
Soy protein ^a	3.72±0.25	152.6±5.6	2.6	0.089
Lentile protein ^b	4.24±1.26	58.22±12.88	3.09	0.150
Whey protein ^c	2.7±0.26	15.4±2.33	-----	0.081
Peanut protein ^d	3.66	147.46	1.3	0.1

^a pH 9, 5% SPI, 1.6/1 (SPI/ Gly), Heat treated (85°C, 30min) ; (3)

^b pH 11, 5% LPC, 2/1 (LPC/Gly), Heat treated (70°C, 20min) ; (20)

^c pH 8, 5% WPC, 2.5/1 (WPC/Gly), Heat treated (80°C, 30min) ; (38)

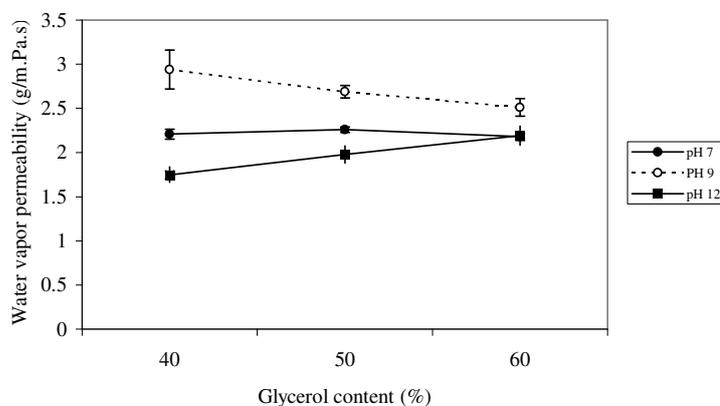
^d pH 7.5, 3% PPC, 0.6/1 (PPC/Gly), Heat treated at 90°C ; (16)

Water vapor permeability

The water vapor permeability of FPI films was not affected by the amount of plasticizer at the studied range, but, it was affected by pH significantly ($p < 0.05$). Independence of WVP with respect to plasticizer content also has been reported previously by Choi *et al.* (14). They concluded that WVP was not influenced by the amount of glycerol in pea protein concentrate film over the range of 20-40%. In FPI film, as observed in micrographs taken by scanning electron microscopy (Figure 5A), it seems that formation of small pouches of glycerol in protein matrix created a pathway for moisture transfer across the film.

Figure 2 shows water vapor permeability of faba bean protein isolate films. The lowest amount of WVP was observed at pH

12 and 40% glycerol content. The mean thickness values of films prepared at pH 7, 9, and 12 for water vapor permeability test were 65.5 ± 13 , 82.3 ± 4.7 and 66 ± 2.2 μm , respectively. Film samples showed higher WVP values at pH 9 and that could be attributed to the higher film thickness at pH 9. Increase in WVP as the result of thickness increase has been reported by many researchers. Bertuzzi *et al.* (34) observed linear increase in WVP with thickness in high amylose corn starch films. Several explanations have been reported for thickness effect on WVP. Mc Hugh *et al.* (35) observed that, as film thickness increased, the film provided an increased resistance to mass transfer across it; consequently, the equilibrium water vapor partial pressure at inner film surface increased. Other researchers attributed the thickness effect to film swelling as a result

**Figure 2.** Effect of pH and plasticizer concentration on water vapour permeability of FPI films

of attractive forces between polymer and water (36).

In general, high hydrophilicity of proteins and using hydrophilic plasticizers in the formulation of protein-based edible films make them poor water vapour barriers (37). As it is seen in Figure 3 and Table 2, WVPs of FPI films at pH levels of 7 and 9 and 60% (weight of protein) glycerol content are lower than that of SPI film at pH 8 and the same glycerol concentration. FPI films also showed lower WVP than that of lentil protein concentrate film. This difference can be due to the presence of higher amounts of hydrophobic amino acids (leucine, proline and alanin) in faba bean protein isolate in comparison with those of lentil protein concentrate. Hydrophobic amino acids (leucine, proline and alanin) account for 17% wt of faba bean protein isolate (27), but 13.6% wt of lentil protein (20). Comparison of the WVPs of FPI and peanut protein concentrate (PPC) films (Table 2) indicated the lower WVP of PPC film at pH 7.5, although the reason could be due to applying heat treatment during PPC film forming solution preparation. According to Jangchud *et al.* (16), WVP reduction as a result of using heat treatment is due to greater cross-linking and formation of a more compact protein network and structure.

Color

The results showed that only pH had significant effect on films color, while plasticizer content did not affect color significantly ($p > 0.05$). Total color differences are shown in figure 3. As it is depicted, films color became darker when pH increased to 12. As it is depicted, films color became darker and more yellow when pH increased to 12. By increasing pH, films color shifted from light amber to dark amber. These results are in agreement with those obtained by Jangchud *et al.* (16). They found that the color of peanut protein films, prepared at different pH levels (6, 7.5, and 9) were affected by both pH and temperature. Darkening of films by increasing pH can be due to alkalinity of the solutions. Alkaline solvents extract pigments more than other solvents (20).

Total soluble matter (TSM)

Immersion of film pieces in water for 24 h did not cause the films to break apart, indicating the high stability of protein network. Results showed significant effect ($p < 0.05$) of pH on films solubility. Total soluble matter of films increased by increasing pH from 7 to 12. Also, Jangchud

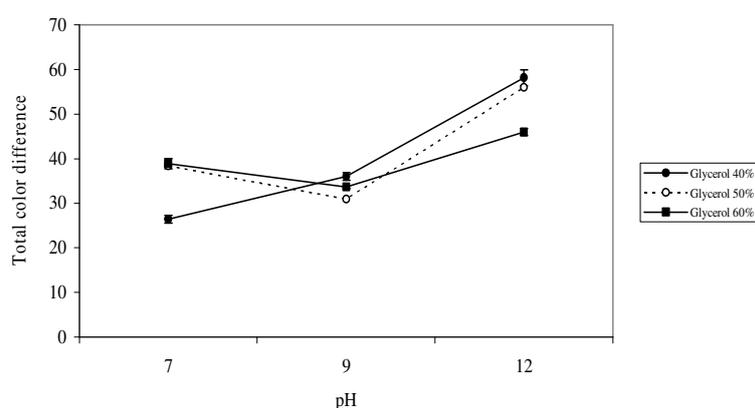


Figure 3. Effect of pH and plasticizer concentration on the total color difference of FPI films.

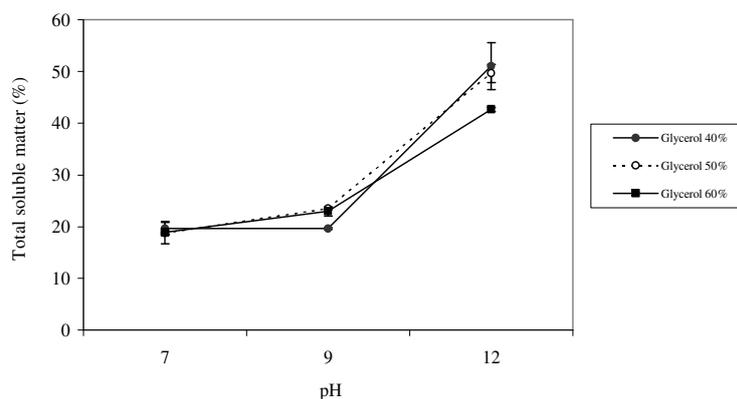


Figure 4. Effect of pH and plasticizer concentration on total soluble matter of FPI films.

et al. (16), showed the significant increase of peanut protein films solubility as pH of film forming solution increased from 6 to 9.

Comparison of the solubility of FPI films (Figure 4) with other leguminous protein-based edible films, showed that the TSM of FPI films were, respectively, 18%, 14%, and 11% lower than that of pea protein concentrate (14), soy protein isolate (31) and lentil protein concentrate (20) films with almost the same pH and protein / glycerol ratio. This indicates that FPI film has relatively stronger intra molecular interactions in the aqueous condition compared to those between the mentioned proteins. It should be noted that pea, soy bean, and lentil proteins were heat treated and their films solubility was influenced by heat, but, we did not use any heat treatment during FPI films preparation. Therefore, the lower solubility of FPI films can be an indication of the high stability of protein network. However, the solubility of films increased by increasing pH and the maximum TSM was observed at pH 12 (46.9%), showing the susceptibility of FPI films to both high and low solubility film applications.

Microstructure study

A film sample (pH 12 and containing 40% glycerol) was chosen for microstructure

study due to its better results regarding its mechanical and water vapor barrier properties.

As shown in Figure 5A, it seems that small pouches of glycerol has been formed in protein network that can be responsible for moisture transfer across the film. Film cross section micrograph (Figure 5B) also shows rough microstructure that is characteristic of proteinous films.

CONCLUSION

Flexible films were successfully prepared from faba bean protein isolate at different pH levels and plasticizer concentrations. pH and plasticizer concentration affected both mechanical properties and solubility of the films. Water vapor permeabilities of the films were only affected by pH, but not by glycerol concentration. Films color varied from pale amber to dark amber depending on pH. The characteristics of faba bean protein isolate film were also comparable with other edible protein films such as soy bean, pea, peanut, and lentil protein. The relatively high water solubility of FPI films prepared under alkaline conditions could make such films appropriate for high solubility film applications such as manufacture of water soluble pouches. By applying different modification treatments such as heat treatment or other protein

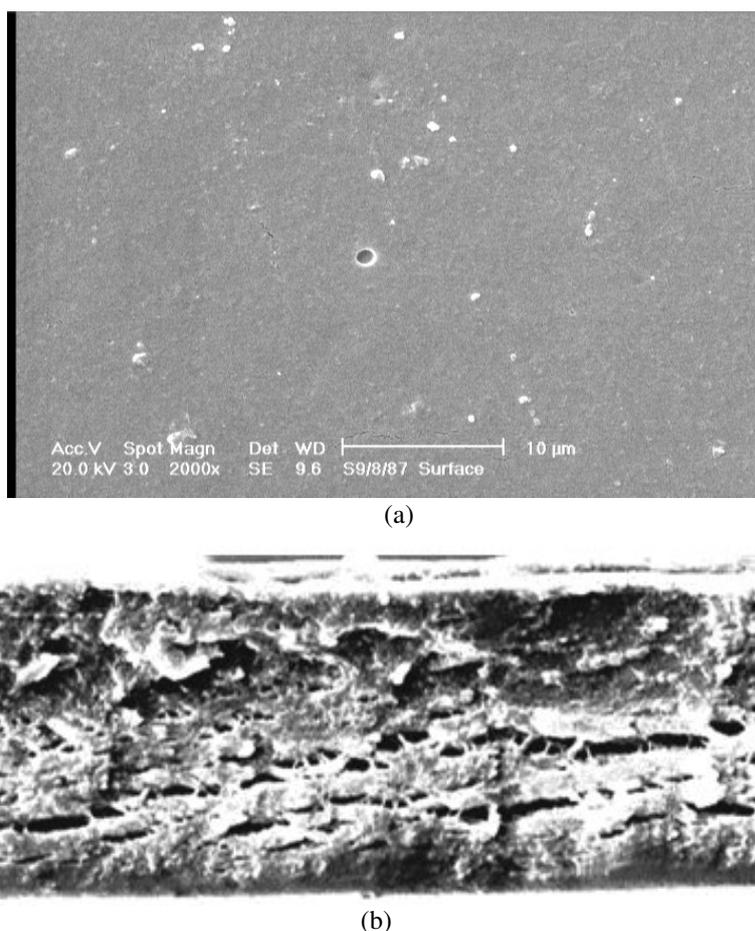


Figure 5. Scanning electron microscopy picture of film prepared at pH 12 with 40% glycerol. a) film surface
b) film cross section.

denaturing techniques, FPI films also can be a good candidate for using as low solubility edible films. Also, considering semitransparency of the films, their use for packaging of light sensitive foodstuffs can be recommended.

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ویژگی‌های یک فیلم جدید خوراکی تهیه شده از ایزوله پروتئین باقلا

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چکیده

علاقه به تولید فیلم های خوراکی از منابع تجدید پذیر و پلیمر های طبیعی مانند پروتئین ها، پلی ساکارید ها و چربی ها افزایش یافته است. پلیمرهای طبیعی از قبیل پروتئین های غذایی بدلیل زیست تخریب پذیر بودن و داشتن خواص تغذیه ای دارای پتانسیل بالایی برای استفاده در فیلم های خوراکی می باشند. باقلا یک منبع غنی از پروتئین بوده و به مقدار زیادی در ایران کشت می شود. بنابراین در این تحقیق توان بالقوه ایزوله پروتئین باقلا بعنوان یک منبع جدید پروتئینی برای تولید فیلم خوراکی، مورد بررسی قرار گرفته و اثرات pH محلول تشکیل دهنده فیلم و غلظت پلاستی سایزر روی ویژگی های فیلم حاصله مطالعه شده است. بدین منظور از ایزوله پروتئینی باقلای تهیه شده در سه pH ۷، ۹ و ۱۲ و سه غلظت پلاستی سایزر ۴۰، ۵۰ و ۶۰ درصد وزن ایزوله پروتئین باقلا فیلم تهیه گردید. بر اساس نتایج بدست آمده افزایش pH سبب بهبود خواص مکانیکی (مقاومت در برابر پارگی و کشش پذیری) و میزان حلالیت، کاهش نفوذ پذیری به بخار آب و تیره تر شدن فیلم ها شد. غلظت پلاستی سایزر در محدوده مورد مطالعه اثر معنی داری روی نفوذ پذیری به بخار آب نشان نداد. افزایش غلظت پلاستی سایزر سبب افزایش میزان کشش پذیری و انحلال فیلم ها و کاهش مقاومت آنها در برابر پاره شدن گردید. کمترین نفوذ پذیری به بخار آب و بیشترین مقاومت در برابر پارگی در فرمول تهیه شده در pH ۱۲ در حضور ۴۰ درصد گلیسرول مشاهده شد.