EFFECT OF PH ON PROPERTIES OF MUNG BEAN (*VIGNA RADIATA*) PROTEIN ISOLATE FILMS.

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Abstract

Mung bean protein isolate was prepared from mung bean (*Vigna radiata*) flour by extraction with 1N NaOH and precipitated at pH 4 with 1N HCl. Neutralization of the dispersed precipitation at pH 7 was carried out with subsequent freeze-drying. Glycerol (3 %) was added to the mung bean protein isolate (5 %) solution to form a transparent edible film. The films were then adjusted to pH 8, 10 and 12 with 2N NaOH and dried at 60 °C for 12 hours. Colour measurements were conducted on the mung beans flour, its isolate and protein films. The water vapour permeability of the films was evaluated at 30 °C using the ASTM (1981) Method E96-80, with some modifications. The surface structures of the films were determined using scanning electron microscopy (SEM). At pH 8, 10 and 12 the films were noted to differ significantly ($p<0.05$) in L, a and b values respectively. The water vapour permeability of film at three different pH did not differ significantly ($p<0.05$). At pH 10 the surface of film is homogenous exhibiting a rigid and dense morphology. No crack-line is apparent with the presence of small pores observed on the condensed surface compared to others films at pH 8 and pH 12.

Keywords: mung bean protein isolates; edible-film; SEM; color; water vapor permeability.

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1.0 Introduction

Edible films can be generally defined as a thin, continuous layer of edible coating material or a film placed between food components by wrapping, immersing, brushing or spraying to provide a barrier to mass transfer of moisture, oxygen, lipids and solutes (Lent, 1998; Gennadios and Weller, 1990). Edible film could function as selective barriers to movements of gasses, vapors and solutes (Gennadios *et al.*, 1993). Edible film has a potential to reduce package complexity, waste and cost of packaging systems. In additions to the biodegradability, the films can be consumed along with food, provides additional nutrients, enhanced sensory characteristics and may include antimicrobials (Ryu, 2002).

Vigna radiata is native to northeastern India-Myanmar region of Asia. In Egypt, mung bean has been recently introduced by the Ministry of Agriculture (El-Adawy, 2000). It is primarily grown in Asia, Africa, South and North America and Australia principally for its protein-rich edible seeds.

In this study, mung bean protein isolates were used as a basic material in edible film due to its excellent source protein (20-27 %), and its essential amino acid composition which is comparable favourably to soybean, kidney bean and FAO/WHO reference (Thompson, 1977; Coffmann and Garcia, 1977, El-Adawy, 2000). Mung beans are inexpensive and are readily available in the market.

The objectives of this research are: (i) to develop a protein-based edible film from mung bean protein isolates at three different pH, (ii) to determine the color of the mung bean flour, mung bean protein isolates and films, (iii) to determine the thickness and water vapor permeability of the films and (iv) to conduct a detailed surface structural examination of such films using scanning electron microscopy (SEM).

2.0 Material & Method

2.1 Preparation of mung bean flour

Mung bean (*Vigna radiata*) was purchased from a local store. Good quality mung bean seeds were selected and later washed with water for 4 times. The seeds were soaked in water for 3 hours at room temperature (30 °C). The soaked beans were then washed with distilled water (4 times) and then dried in a hot air dryer (Afos) at 50-60 °C for 12 hours. The dried mung beans were then ground into flour using a grinder (Retsch GmbH & Co. KG). The mung bean flour was then kept in a plastic container and stored at 4 °C (Prabhavat, 1990).

2.2 Preparation of mung bean protein isolate

Mung bean flour was mixed with 1 N NaOH to pH 9 in a ratio of 15: 1 (water: flour, v/w). The mixture was mixed moderately using an incubator shaker (Controlled Environment Incubator Shaker, Brand – New Brunswick Science New Brunswick, N.J., USA) at 25 °C for 20 minutes. Insoluble components were removed by centrifugation (Centrifuge – 5100 Kubota) at 1000 X G for 20 minutes. 1 N HCl was added to the liquid supernatant to precipitate the mixture. Precipitation was considered complete at pH 4 by using a pH meter (model Orion 420A). The precipitated protein was separated by centrifugation at 1000 X G for 20 minutes and washed once with distilled water. The

pH of the recovered precipitated protein isolates was adjusted to pH 7 with 1 N NaOH. The recovered precipitated protein isolates were freeze-dried using a Freeze Dryer at –50 °C for 2 days (Labconco, Germany) (Thompson, 1977).

2.3 Formation of film

Protein-based edible films were developed according to Gennadios et al., (1996b) with some modification. Films were prepared by dispersing mung bean protein isolates (5 % w/v) and potassium sorbate (0.3 % w/v) in distilled water. Followed with the addition of glucose (5 % w/v) and glycerol (3 % w/v). The protein isolate solution was homogenized using a homogenizer (model Labor Technic Ultra-Turax T25) at 8000 – 9500 rpm for 3 minutes. The pH was adjusted with 2 N NaOH and the solution was homogenized for an additional 1 minute. The solution was heated in the water bath at 75-80 °C for 20 minutes and stirred by using the overhead stirrer (model Lab-Egg). The solution was then cooled at 37 ± 0.30 °C for 3-5 minute. The films were then casted on a Teflon-coated glass plate (26.0 cm x 16.5 cm) to form a thin film and later dried at ≈ 23 °C for 65 hours before being dried in an oven (model Memmert, U.S.A.) at 60 °C for another 12 hours. The dried films were then peeled off from the Teflon-coated glass plate.

2.4 Thickness

Thicknesses of the films were measured randomly with a caliper micrometer (Mitutoyo, Japan). Thickness values were represented by means of five readings.

2.5 Color measurement

Color of sample films were measured by using a color difference meter (Minolta Spectrophotometer CM-3500d Osaka, Japan). The color of the mung bean flour and mung bean protein isolates were measured by placing the samples in petri dish. A 'Target Mask' (CM-126) was used to limit the illumination area of the area.

The instrument was calibrated with a calibration zero box (CM-A120) followed by a white dish (CM-A120).

L, a and b-value will be displayed on the computer which is installed with the Spectromagic Version 2.11, 1998 (Minolta) program.

2.6 Water vapour permeability (WVP)

WVP was evaluated using a modified ASTM (1981) Method E96-80, with some modifications. The test film was sealed onto modified cups containing silica gel (Relative Vapour Pressure (RVP) = 0). Cups were measuring 6.0 cm (i.d.) by 7.3 cm (o.d.) by 4.5 cm deep with an exposed film area of 28.3 cm^2. The cups were then placed in a desiccators maintained at 30 °C and RVP = 0.22 using saturated salt solution of potassium acetate. The cups were weighed daily over a 6 days period. Changes in weight of the cups were plotted as a function of time. The constant rate of weight gain was obtained by linear regression with $r^2 \geq 0.99$. WVP (kg Pa^{-1} s^{-1} m^{-1}) was calculated as $WVP = [WVTR / S (R_1 - R_2)] \times d$, where S = saturation vapour pressure (Pa) of water at test temperature, R_1 = RVP in the desiccator, R_2 = RVP in the permeation cell, and d = film thickness (m).

2.7 Water vapour transmission rate (WVTR)

The water vapour transmission rate (WVTR) was calculated from the slope of the straight line divided by the test area. WVTR (kg s^{-1} m^{-2}) for each type of film was determined with two individually prepared films as the replicated experimental units.

2.8 Scanning electron microscopy

The films from each treatment were viewed under the scanning electron microscopy (SEM). Films were mounted onto aluminum stubs with double-sided tape. Prior to SEM, films were dried using a Freeze Dryer (Labconco, Germany). The films were then coated with gold using a Sputter Coater (Polaron SC 515) to a thickness of 30 nm. SEM was conducted using a Scanning Electron Microscope (model Leica Cambrige S-360).

2.9 Statistical analysis

Thickness, water vapor permeability and L, a and b-values were analyzed by ANOVA using the Minitab program version 13.0 (Minitab Inc. 1994). Significant differences were determined by least significant difference (LSD). All comparisons were made at 5 % level of significance.

3.0 Results & Discussion

The thicknesses of the protein-based edible films were in the range of 190 – 270 μm.

TABLE I: Thickness of the Films.

Sample	Thickness, (μm)
5 % protein isolate + 3 % glycerol – pH 8	**261.2 ± 0.25^{a}**
5 % protein isolate + 3 % glycerol – pH 10	**197.4 ± 0.22^{b}**
5 % protein isolate + 3 % glycerol – pH 12	**214.4 ± 0.24^{b}**

Mean within a colomn with the same letter are not different significantly at the 5 % probability level.

From Table II, the L values were found to differ significantly ($p<0.05$) between the mung bean flour and its isolate. L value was affected by the extraction process of protein isolate due to the modification of pH solution with sodium hydroxide and hydrochloric acid. The addition of sodium hydroxide was found to darken the solution as observed by the reduction in L values.

a and hue (θ) value for mung bean flour and protein isolate was observed to differ significantly ($P<0.05$). Mung bean protein isolate was found to be greener than flour. Mung bean protein isolates were in the yellow-green quadrant as compared to the mung bean flour. The b value of flour and protein isolate was not different significantly ($P<0.05$).

TABLE II: Colour of Mung Bean Flour and Protein Isolates.

Value	Mung bean flour	Mung bean protein isolates
L	89.77 ± 0.16^{a}	74.17 ± 0.34^{b}
a	0.76 ± 0.02^{a}	-0.12 ± 0.01^{b}
b	15.57 ± 0.26^{a}	15.06 ± 0.24^{a}
θ	87.23 ± 0.03^{b}	131.14 ± 0.60^{a}

Means within a column with the same letter are not significantly different at the 5 % probability level.

Table III shows the L, a, b and hue (θ) values of protein (5 %) film. L values for films were found to differ significantly ($p<0.05$). Protein (5 %) film at pH 12 had lower L value due to the denaturation of the protein isolate by the modification of pH solution. a values were found the differ significantly ($p<0.05$) among films. Protein (5 %) film was found to greener than its isolate. Higher pH resulted a decreased in green color significantly ($p<0.05$) in the films.

Higher pH significantly ($P<0.05$) affected the b values of protein (5 %) film prepared from pH 8 and pH 12. The hue (θ) value of the protein films was not different significantly ($P<0.05$). The films were in the more towards yellow quadrant.

TABLE III: Colour of Protein Films.

Value	Film – pH 8	Film – pH 10	Film – pH 12
L	93.04 ± 0.04^{a}	92.13 ± 0.02^{b}	86.74 ± 0.01^{c}
a	-3.31 ± 0.01^{a}	-2.87 ± 0.01^{b}	-0.39 ± 0.01^{c}
b	22.28 ± 0.01^{c}	23.31 ± 0.03^{b}	42.08 ± 0.03^{a}
θ	81.56 ± 0.02^{a}	82.98 ± 0.03^{a}	82.98 ± 0.03^{a}

Means within a column with the same letter are not significantly different at the 5 % probability level.

From the results in Table IV, protein (5 %) films at pH 8 have higher water vapour permeability than those made at pH 10 and pH 12. At pH 10 and pH 12, water vapour permeability was not different significantly ($p<0.05$). Water vapour transfer through these films depends on the additional formation of cross-linking due to the Maillard reaction, which occurred in the film, resulted in the number of site for water sorption (Beng, 2002). Furthermore, formations of a higher degree of cross-links reduce water vapor transmission and thus improve barrier properties of the films (Robertson, 1992).

TABLE IV: Water Vapour Permeability of Protein Films.

Film type	WVTR x 10^{13} (Kg s^{-1} m^{-2})	WVP x 10^{6} (Kg Pa^{-1} s^{-1} m^{-1})
pH 8	1.59 ± 0.25^{a}	5.05 ± 0.15^{a}
pH 10	1.41 ± 0.50^{a}	3.70 ± 0.10^{a}
pH 12	1.59 ± 0.25^{a}	4.48 ± 0.27^{a}

Means within a row with the same letter are not significantly different at the 5 % probability level.

Figure I and II shows the surface structure for protein (5 %) film at pH 8 and pH 12 respectively. The film structure was not smooth on the surface area. Presence of cracks and pores was found at 2000X magnification respectively.

FIGURE I – Protein (5 %) film at pH 8, viewed at a magnification of 2000x.

FIGURE II – Protein (5 %) film at pH 12, viewed at a magnification of 2000x.

Figure III and IV show the surface structure for protein (5 %) film at pH 10. The surface of film was found homogenous exhibiting a rigid and dense morphology. No crack-lines were apparent with the presence of small pores observed on the condensed surface. The surface has more coagulated protein spots, which was tiny and compact, compared to the film at pH 12. This may have attributed from the formation of additional cross-linking that makes the film denser resulting in a more compact surface microstructure (Beng, 2002). Presence of pores and water vapour observed at 1000X magnification.

FIGURE V – Protein film at pH 10, viewed at a magnification of 1000x.

FIGURE VI – Protein film at pH 10, viewed at a magnification of 2000x.

4.0 Conclusions

pH film had an effect on properties of the film. Film at pH 10 has lower water vapor permeability than those made at pH 8 and pH 12 was noted to have a homogenous exhibiting a rigid and smooth surface area.

5.0 Acknowledgement

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