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Preparation and characterization of proteinous film from lentil (*Lens culinaris*)

Edible film from lentil (*Lens culinaris*)

Fatemeh Bamdad, Amir Hossein Goli, Mahdi Kadivar *

Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan 84156 83111, Iran

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Abstract

This study was conducted to extract protein from lentil seed and prepare edible film from the protein and to determine mechanical, optical and barrier properties of lentil protein concentrate (LPC) film. The film was prepared from LPC (5 g/100 ml water) and glycerine (50%, w/w of LPC). Hunter color value (*L*, *a* and *b*), tensile strength, percentage elongation at break (*E*), puncture strength, water vapor permeability (WVP), moisture content after conditioning at 50% RH and 25 °C for 48 h and total soluble matter after immersion in water, were measured. In regarding to WVP, in spite of difference in film thickness and relative humidity of experiment in different studies, lentil protein film is comparable with other protein films. Characteristics of the lentil protein-based edible films were comparable with other edible protein films. LPC film had more red and less yellow color; it seems that the film had good mechanical properties and water vapor permeability in concomitant with good solubility.

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Keywords: Lentil (*Lens culinaris*); Lentil protein concentrate; Edible film; Mechanical properties; Water vapor permeability

1. Introduction

Coating and films have been used for many decades to protect food from microbial attack and to prevent water loss during storage. Consumer demands higher quality and longer shelf-life in foods, while reducing disposal packaging material and increasing recycleability (McHugh, Avena-Bustillos, & Krochta, 1993). In this regard, considerable research has been reported on edible films, which have many advantages over synthetic films (Tharanathan, 2003).

Several biopolymers, including polysaccharides, proteins and lipids have been used as a biodegradable film. In general, protein films are effective lipid, oxygen and aroma barriers at low relative humidity (RH) conditions, therefore, proteins are used widely to form edible films. Proteins such as gelatin (Arvanitoyannis, Psomiadou, Nakayama, Aiba, & Yamamoto, 1997), whey protein (Fang, Tung, Britt, Yada, & Dalgleish, 2002), wheat and corn proteins (Gennadios & Weller, 1990) and soy protein (Pol, Dawson, Acton, & Ogale, 2002) have been extensively studied.

Limited information is available on the use of legume seeds protein for packaging applications; although, pea (Choi & Han, 2001) and peanut (Jangchud & Chinnan, 1999) proteins along with soy protein have been studied for use as edible films.

Like most synthetic polymers, edible film materials require property modifiers to improve the physical and mechanical properties of the film. As with synthetic plastics, plasticizers are incorporated into the edible coating/film materials which overcomes the brittleness caused by extensive intermolecular forces. The most common edible plasticizers are polyols, mono/di or oligosaccharides, lipids and derivatives (Choi & Han, 2001).

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* Corresponding author. Tel.: +98 311 3913343; fax: +98 311 3912254.
E-mail address: mak120@mail.usask.ca (M. Kadivar).

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Lentil (*Lens culinaris*) belongs to the family leguminosae (Hoover & Ratnayake, 2002) and there are many studies on the composition of lentil protein and its potential value in human diet (Bhatty, 1986; Bhatty, Slinkard, & Sosulski, 1976; Bora, 2002).

On average, the percentage of protein in lentil seed is 20–25% (Monsoor & Yusuf, 2002), and on our best knowledge, there is no work on using lentil protein as an edible film for packaging applications.

One of the most useful functions of edible films is their ability to act as water, gas and oil barriers. Water vapor permeability is one of the most important and widely studied property of edible films. Mechanical properties are as important to edible films as barrier properties are. Adequate mechanical strength ensures the integrity of a film and its freedom from minor defects, such as a pin hole, which ruin the barrier property (Kaya & Kaya, 2000).

The objectives of this work were to: (1) extract protein from lentil seed; (2) produce edible protein film for food packaging from lentil protein and glycerol as plasticizer; (3) determine mechanical, optical and barrier properties of the film.

2. Materials and methods

Lentil was obtained from a local market in Isfahan, Iran. All chemicals and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.1. Preparation of lentil protein concentrates (LPC)

Protein was extracted from lentil seeds according to Monsoor and Yusuf (2002). Lentils were milled to a fine powder (40 mesh), the powder was soaked in alkaline distilled water (pH 11:0) in a ratio of 1:10 (w/v), and the pH was adjusted with 0.1 N NaOH. The powder-solvent mixture was stirred for an hour and kept in a cold room at 4 °C overnight to sediment non-protein constituents. After centrifugation at 2700*g* for 10 min (Sigma 2-16, Germany), the supernatant was collected and pH was adjusted to 5.4 with 0.1 N HCl and allowed to settle in a cold room overnight at 4 °C. Precipitate was collected, centrifuged and vacuum oven dried at 50 °C.

2.2. Determination of protein content

Protein content of lentil seed and LPC was determined by Kjeldahl nitrogen determination (AOAC, 1990) and multiplying the nitrogen values by 6.25.

2.3. Film preparation

Film-forming solution was prepared by dissolving LPC under constant stirring in distilled water (5 g/100 ml water). Glycerin, as a plastisizer, was added 50% (w/w) of LPC. After adjusting the pH value of the solution to 11.0 ± 0.1 with 1 N NaOH, the solution was heated in a water bath at 70 °C for 20 min, strained through cheese cloth and cast on Teflon-coated glass plate (30×30 cm). The film was dried at 60 °C for about 7 h and peeled off the plate for testing.

2.4. Thickness

Film thickness was measured with a Dial Caliper (1618, Japan) at five random positions.

2.5. Conditioning

All film specimens were conditioned prior to testing according to the standard method (ASTM, 1995).

The films were conditioned at 50% RH and 25 °C by placing them in a desicator over a saturated solution of $\mathrm{Mg\,(NO_3)_2 \cdot 6H_2O}$ for 48 h.

2.6. Color

Hunter color parameters (*L*, *a*, *b*), were measured by a Texflash (Data Color, US). Color values was recorded as *L* (lightness, 0 = black, 100 = white), *a* ($-a$ = greenness $+a$ = redness), and *b* ($-b$ = blueness $+b$ = yellowness). Color measurement of film was replicated five times.

2.7. Tensile strength and percentage elongation at break (*E*)

Film tensile strength and elongation at break was determined (Instron Universal testing instrument, 1140, England). Tensile strength was calculated by dividing the maximum force at break by the initial cross-sectional area of specimen.

E was calculated as follows:

$$E = 100 \times (d_{\text{after}} - d_{\text{before}})/d_{\text{before}},$$

where *d* was the distance between grips holding the specimen before or after the break of the specimen.

TS and *E* measurements were replicated three times.

2.8. Puncture strength

Puncture strength was also determined with Instron Universal testing instrument. A cylindrical probe (0.99-cm diameter) was moved perpendicularly at the film surface at constant speed (0.33 cm/s) until it passed through the film.

2.9. Water vapor permeability

Water vapor permeability of film was measured using the method of Ou, Kwok, and Kang (2004). Circular

Fig. 1. The cup used in measurement of WVP.

steel cup with diameter of 5.1 cm and depth of 5.4 cm was used (Fig. 1). After placing 3 g of $CaCl_2$ in the cap, it was covered with the protein film in three replicates. Film was cut circularly with diameter of 6.1 cm and sealed with melted paraffin.

The cups were weighed with their contents and placed in desicator kept at 25 °C. One thousand milliliter of pure water was placed at the bottom of desicator for providing RH of 100% at 25 °C.

Cups were weighed every 24 h for one week. The water vapor transferred through films was determined from the weight gain of the cups. Water gain velocity (slopes) (correlation coefficient was larger than 0.997), water vapor transmission rate (WVTR) and water vapor permeability (WVP) were calculated according to Kaya and Kaya (2000).

2.10. Moisture content

Film sample was weighed (±0.0001 g) into aluminum dishes and dried in an oven at 105 °C for one day.

Moisture content was determined as percentage of initial film weight lost during drying and reported on wet basis.

2.11. Total soluble matter (TSM)

In order to determine TSM, three film samples were weighed (±0.0001 g) and then directly immersed in water (25 °C for 24 h), Undissolved film materials were removed from water, gently rinsed with distilled water and subsequently oven dried (105 °C for 24 h) to determine solubilized dry matter. Initial dry matter values were obtained from MC measurements for the same film.

2.12. Statistical analysis

Statistical data were analyzed using Microsoft Excel.

3. Results and discussion

3.1. Protein content

Edible legume seeds contain relatively large amounts of protein that vary considerably in nutritional value. In this research, protein content of ground lentil and LPC were 23.32 ± 0.4% and 68.86 ± 0.33% (wb), respectively, that is similar to results obtained by Monsoor and Yusuf (2002). According to Aparna, Khatoon, and Prakash (2000), the mean protein content for legumes is 21–25%. Solanki, Kapoor, and Singh (1999) also reported the nutritional value and the total protein content of different legumes grown in India which is similar to present findings. Monsoor and Yusuf (2002) also calculated percent extractability and purity of lentil protein that were higher than that of lathyrus pea and chick pea. This may be because of higher solubility of lentil protein in the solvent system used. The quality of lentil protein was only half to one third that of egg protein. The EAAI (Essential Amino Acid Index) of 63 also suggests that the quality of lentil protein is low compared to an EAAI of 100 for egg protein, 91 for casein and 67 for whole wheat (Osler, 1970). However, protein digestibility of raw pulses varies from 34 in lima bean to 88 in lentil (Leiner, 1976).

3.2. Film formation

Peeled films were slightly red to brown in color with semitransparency and their thickness was 0.155 ± 0.04 mm (155 ± 40 μm). They were strong and flexible enough to be handled.

Because the physical and mechanical properties of protein based films are related to the storage humidity, the specimens were stored under constant RH before examinations.

3.3. Color

LPC films had L, a, and b color values of 25.74, 6.98 and 3.52, respectively. L value for LPC films, as shown in Table 1, is lower than that of peanut protein films reported by Jangchud and Chinnan (1999). They prepared peanut protein films at different pHs (6.0, 7.5 and 9.0) and found that the color of the film is affected by both pH and temperature. Films formed at pH 6.0 were lighter in yellow color and appeared to be more

Table 1
L, a and b Hunter color values of lentil, soy and peanut protein films

Color values	Film type		
	Lentil protein[a]	Peanut protein[b]	Soy protein[c]
L	25.74	38.38	93.37
a	6.98	0.98	−2.11
b	3.52	4.15	12.18

[a] pH 11, 5% LPC, 2/1 (LPC/Gly).
[b] pH 9, 3% PPC, 3/5 (PPC/Gly); Jangchud and Chinnan (1999).
[c] pH 10, 5% SPI, 2/1 (SPI/Gly); Rhim et al. (1998).

opaque and dull than films formed at pH 9.0 where the films were dark yellow. This is mainly due to alkalinity and heat reaction (Jangchud & Chinnan, 1999). Alkaline solvents can extract pigments more than other solvents. Lentil protein concentrate films had greater a value and lower b value than soy protein isolate and pea protein films (Choi & Han, 2001; Rhim, Gennadios, Weller, Cazeirat, & Hanna, 1998). Redness of the film might be due to hull pigments leaching, entered in the solution, during protein extraction.

3.4. Mechanical properties

3.4.1. Tensile strength and elongation

Tensile strength of LPC film is 4.24 ± 1.26 MPa, which is consistent with that of native pea protein concentrate film (4.11 MPa, 70/30 PPC/Gly) as reported by Choi and Han (2001). Heat denaturation of pea protein films (90 °C, 25 min) made it stiffer and an increase in TS value up to 7.3 MPa was observed (Table 2). This suggests that covalent cross-linking caused by heat denaturation of protein is responsible for higher tensile properties. On the other hand, percent elongation at break (%E) for LPC films (58%) was higher than that of heat denatured pea protein concentrate films at the almost same protein/plasticizer ratio and almost 7.5-fold more than %E value of native pea protein film. It can be resulted from slight heat denaturation of LPC films during formation (70 °C, 20 min) and a little more glycerol content. There are evidences that the intensity of heat treatments of proteins at alkaline pH has a significant effect on physical properties of films because it promoted formation of intra- and inter-molecular cross-links (Kim, Weller, Hanna, & Gennadios, 2002). Heating soy protein solution at 80 or 90 °C for various periods of time has led to form films with increased TS, darker color and lowered E and WVP values (Gennadios, Ghorpade, Weller, & Hanna, 1996). Tensile strength and percentage elongation of LPC films were almost similar to that of single coat soy film laminated with corn zein (3.25 ± 0.34 MPa and 42.87 ± 24.75%, respectively) (Pol et al., 2002). Moreover, tensile strength of LPC is comparable to that of conventional polyolefin films (3–10 MPa), as reported by Briston (1988). Lentil protein concentrate films show higher %E value than that for cellophane (about 20%). However, the percent E values for LPC films were considerably lower than those of most commercial synthetic polymer films, like LDPE (about 500%) or HDPE (≈300%) (Pol et al., 2002). Considering these results, it seems that LPC films fell in acceptable range of quality for use as a packaging material under moderate mechanical applications, such as individual wrappers in a large box or carton.

3.4.2. Puncture strength

Puncture strength of LPC films was 1.552 ± 0.2 N for a thickness of 150 μm. It shows that in LPC films, protein network is strong enough and films have suitable cohesiveness. In cottonseed flour films there was a reverse relationship between puncture strength and solubility (Murquie, Aymard, Cuq, & Guilbert, 1995). Glanded cottonseed flour films whose solubility (24.5 ± 0.2%) was almost similar to that of LPC had lower puncture strength (0.77 ± 0.8 N for a thickness of 100 μm) than LPC films. It seems that LPC films have good puncture strength (cohesiveness) in concomitant with good solubility.

3.4.3. Water vapor permeability

Edible films often contain hydrophilic components, such as protein or polysaccharides so besides mechanical properties, water vapor permeability is another impor-

Table 2
Physical and mechanical properties of lentil protein concentrate, soy whey and pea protein isolate films

Properties	Film type			
	Lentil protein[a]	Soy protein[b]	Whey protein[c]	Pea protein[d]
Tensile strength (MPa)	4.24 ± 1.26	8.5 ± 0.5	6.9	7.3 ± 0.4
Elongation (%)	58.22 ± 12.88	31.9 ± 2.4	41	46.8 ± 5.8
Total soluble matter (%)	38.75 ± 3.2	35.1 ± 1.0	≈30	38.7 ± 4.0
Moisture content	23.15 ± 1.6	25.2 ± 1.0[e]	–	–

[a] pH 11, 5% LPC, 2/1 (LPC/Gly).
[b] pH 10, 5% SPI, 10/3 (SPI/Gly); Kunte et al. (1997).
[c] pH 7, 5% WPI, 70/30 (WPI/Gly); Perez-Gago et al. (1999).
[d] pH 9, 10% PPC, 70/30 (PPC/Gly), heat denatured (90 °C for 25 min); Choi and Han (2001).
[e] pH 10, 5% SPI, 2/1 (SPI/Gly); Rhim et al. (1998).

Table 3
Water vapor permeabilities of films

Film	Thickness (mm)	Permeability[a]
Edible films		
Lentil protein concentrate[b]	0.15	0.3095 ± 0.002
Wheat gluten[c]	0.05	0.136
Corn zein[d]	0.12–0.33	0.116 ± 0.019
Other films[e]		
Polyethylene, low density	–	0.00055
Polyvinyl chloride	–	0.00071

[a] Unit of permeability is in ng/m/m^2/s/Pa; *n* is an abbreviation for nano (10^{-9}).

[b] pH 11, 5% LPC, 0.5 (Gly/LPC).

[c] Data for 25 °C and 100% RH gradient (0.3 Gly/protein); Gontard et al. (1993).

[d] Data for 21 °C and 85% RH gradient (0.26 Gly/protein); Park and Chinnan (1995).

[e] Data for 25 °C and 100–90% RH gradient; Kester and Fennema (1989).

tant and widely studied property of them (McHugh et al., 1993). The major protein fraction of lentils, like that of other legumes, is salt soluble or water-insoluble fraction (globulins) which formed an average of 47% of the total seed proteins (Bhatty et al., 1976). Lentils also contain 3.8% water soluble proteins (albumins). The major amino acids of lentils are glutamic acid, aspartic acid, arginine, leucine and lysine. Aspartic and glutamic acids form about 48% of the total amino acids in three cultivars of *L. culinaris* (Bhatty, 1986). Table 3 shows that water vapor permeabilities of edible films were much higher than those of plastic films. Water vapor permeability of gluten film (0.136 ng/m/m^2/s/Pa with 0.05-mm thickness) of Gontard, Guilbert, and Cuq (1993) was lower than that of Lentil protein concentrate film (0.3095 ± 0.002 ng/m/m^2/s/Pa with 0.15-mm thickness) in this study. This difference is probably due to the difference in glycerin/protein (w/w) ratios; these ratios were 0.3 and 0.5, respectively. As reported by Park and Chinnan (1995), corn zein film has lower WVP (0.116 ± 0.019 ng/m/m^2/s/Pa with 0.12–0.33-mm thickness) than that of LPC film, which could be due to higher content of hydrophobic amino acids in corn zein. The presence of hydrophobic amino acids (leucine, proline and alanine at approximately 35 wt%) in corn-zein protein provides fairly good moisture barrier properties (Padgett, Han, & Dawson, 1998). These amino acids account for 13.6 wt% of lentil protein (Bhatty et al., 1976).

3.4.4. Moisture content and film solubility

Protein and moisture content of LPC films were 45.93 ± 0.28 (%db) and 23.15 ± 1.6%, respectively, and the film did not dissolve or break apart after 24 h of incubation.

This confirmed that the protein polymer network was highly stable and that only small molecules (small peptides, monomers and non protein materials) were soluble. Solubility of LPC films is lower than that of peanut protein films at pH 7.5 and 9.0 (Jangchud & Chinnan, 1999); therefore, it shows that LPC films are more stable. Total soluble matter of LPC films (38.75 ± 3.2%db) was similar to that of pea protein concentrate at almost the same protein/glycerol ratio and ≈20% higher than those of whey protein isolate films, when compared to the data of Perez-Gago, Nadaud, and Krochta (1999), it is probably because of a little more glycerol content in LPC films. This indicates that the LPC film has weaker intra-molecular interactions in the aqueous condition compared to those of whey protein (mainly β-lactoglobulin) molecules in heat denatured whey protein isolate films. Therefore, the lentil protein concentrate may be utilized as a replacement for soy protein and whey protein in forming food coatings or edible films. The relatively high solubility of lentil protein films in water could possibly make the films appropriate for hot water soluble pouches, like cellulose ether-based soluble pouch which is commercially available currently.

4. Conclusion

Lentil protein not only has a medium nutritional value but also the protein is a good source for edible film formation and, therefore, application of the film can be suggested. Lentil protein concentrate film is strong and elastic and has a good moisture barrier property along with acceptable physical integrity, as its TS value is comparable to that of other edible films as well as its %*E* value is higher than that of cellophane. Good puncture strength of LPC film indicates its suitable cohesiveness, whereas its WVP value is higher than that of corn and wheat protein films. However, this shortcoming can be improved by further works to find the best protein/plastisizer ratio along with cross-linking treatments of lentil protein films. The characteristics of the lentil protein-based edible films were comparable with other edible protein films, such as soy, pea and whey protein in terms of tensile strength, elongation, moisture barrier property and water solubility. The new film products may exploit the utilization of lentil protein. Regarding color, the film was red to brown with semitransparency, so can be used for packaging of foods which are sensitive to light.

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