Resistance to moist conditions of whey protein isolate and pea starch biodegradable films and low density polyethylene non-degradable films: a comparative study

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Abstract. Biodegradable packaging materials are degraded under the natural environmental conditions. Therefore using them could alleviate the problem of plastics accumulation in nature. For effective replacement of plastics, with biodegradable materials, biodegradable packages should keep their properties under the high relative humidity (RH) conditions. Therefore the objectives of the study were to develop biodegradable packaging material based on whey protein isolate (WPI) and pea starch (PS). To study their mechanical, oxygen barrier and solubility properties under different RHs compared with those of low density polyethylene (LDPE), the most used plastic in packaging. Films of WPI and PS were prepared separately and conditioned at different RH (30-90%) then their properties were studied. At low RHs (≤50%), WPI films had 2-3 times lower elongation at break (E or stretchability) than PS and LDPE. Increasing RH to 90% significantly (P<0.01) increased the elongation of PS but not WPI and LDPE films. LDPE and WPI films kept significantly (P<0.01) higher tensile strength (TS) than PS films at high RH (90 %). Oxygen permeability (OP) of all films was very low (<0.5 cm³ μm m⁻² d⁻¹ kPa⁻¹) below 40 % RH but increased for PS films and became significantly (P<0.01) different than that of LDPE and WPI at ≥ 40 % RH. Oxygen permeability of WPI and LDPE did not adversely affected by increasing RH to 65 %. Furthermore, WPI and LDPE films had lower degree of hydration at 50 % and 90 % RH and total soluble matter than PS films. These results suggest that WPI could be successfully replacing LDPE in packaging of moist products.

1. Introduction
The interest in biodegradable packaging such as edible films and coatings is increasing because they consist of natural materials, therefore control environmental pollution [1,2]. The problem of accumulation large amounts of synthetic plastics, in particularly LDPE, is increasing. Furthermore, the increase usage of LDPE in packaging accompanied with their slow degradation rate, leaded to ecological problems [3,4]. Biodegradable materials may be applied on a food or between heterogeneous food components to prevent mass transfer phenomena within foods that could deteriorate the quality [5]. For successive replacement of LDPE in moist products such as foods and cosmetics, biodegradable materials should retain their properties in moist conditions [1,5,6].
Biodegradable materials should also be tasteless, free of toxic substances, barrier to water, aroma, and gases in particularly oxygen; the most stimulating gas for spoilage reactions. They could also be used as a carrier or encapsulating material for functional ingredients such as antioxidants, antimicrobial agents, pigments or nutritional substances contributing to microbial and chemical stability [7,8,9].

Protein based biodegradable films and coatings such as those based on pea protein, soy protein and whey protein have received increased interest due to their high barrier properties, superior mechanical properties and nutritional quality. This allows them to either substitute the synthetic packaging materials or simplify the total packaging requirements [6,10]. Being amphophilic in nature, proteins in films and coatings showed enhanced functionality in food preservation when being mixed with variety of hydrophobic and hydrophilic additives such as antimicrobial and antioxidant compounds [11,12]. Strong peptide and covalent bonds in protein polypeptide structure could enhance superior mechanical properties of proteins. Among proteins, WPI could be the most appropriate for large scale production of biodegradable packages because it is a by-product of cheese industry, and readily available at reasonable price.

Starch is complex carbohydrate consists of repeated glucose molecules and represents the major constituent of legumes and cereals. It contains of about 25% amylose (regions of linear compact arrangement) and 75% amylopectin (regions of branched arrangement) [13,14]. Pea starch is attractive raw material for biodegradable packaging because of its low cost, renewability, biodegradability, high-amylose content (40%) and has low gelation temperature [14,15]. Films made of high-amylose starches, showed excellent oxygen barrier properties, lower water solubility and retrogradation temperature and more stable mechanical properties at high RHs compared to those made of normal starches [16,17,18]. High-amylose starch is a very useful film-forming material due to its strong gelation properties and helical linear polymer structure [14]. For appropriate application of these packaging materials in moist products, they should keep their properties when exposed to high humidity conditions (RH > 50%). Although biodegradable materials appeared useful alternative to the conventional synthetic packaging materials, the amount of studies on resistance WPI and PS to high RH comparing to LDEP is still at limited level nevertheless the potential of huge market [19]. The objective of the current study is to test mechanical, oxygen barrier and solubility properties of WPI and PS based biodegradable film material at different RH levels comparing that with LDPE. Therefore determine the possibility to replace LDPE by these biodegradable materials in particularly in moist products.

2. Methodology

2.1. Materials
High-amylose pea starch (PS; 40% amylose) and whey protein isolate (WPI) were supplied from Parrheim Foods Co. (Portage-la-Prairie, MB, Canada), and Davisco Foods Int., (Mn., USA.), respectively. Glycerol from Sigma Chemical Co. (St. Louis, MO) was used as a plasticizer. LDPE films were donated from Al-Hadaf Int. Co. RZ (Amman, Jordan).

2.2. Film preparation
Aqueous dispersions of 3% and 12 % (w/w) PS and WPI, respectively and separately were prepared then glycerol was added at the ratio of 1:2 (w/w) of glycerol:polymer. The dispersions were heated with continuous mixing until boiling and kept at the boiling temperature for 3 min to allow complete gelatinization of the polymers. The film-forming solutions were cooled down to ~ 60 °C then casted into poly styrene petri-dishes (10 cm dia) by pouring 12 g per dish resting on leveled smooth surface. The petri-dishes (six for each composition) were kept at room temperature for 24 hr to allow the film-forming solution to be dried before the films being peeled off. The thickness of the films was measured with a caliper micrometer (B.C. Ames Co., Waltham, MA) at 5 random positions of the film. The average thickness was used for the proceeding mechanical test and oxygen permeability determination. The films were transferred into large plastic bags (LDPE sandwich bags) and arranged side by side into one layer thickness. The filled bags were then gently pressed on the both sides of the
films which allowed both sides of the films to stick onto the bag to minimize the access of air or moisture to the films. Then the bags were sealed and stored at room temperature maximum for one week, till the testing day.

2.3. Tensile test
Film specimens (1 cm × 8 cm) were cut and conditioned in a desiccator for 3 days at 30, 50 or 90% RH over the super-saturated salt solutions of magnesium chloride, magnesium nitrate and potassium nitrate, respectively and separately at room temperature. Tensile strength (TS) and elongation-at-break (E) were determined from strain-stress curve using a texture analyzing instrument (MuliTest, 1-D, Meemisin, West Sussex, UK) following the procedure outlined in ASTM method D882-91 (ASTM 1991). Initial grip distance and crosshead speed were 5 cm and 100 mm/min, respectively. Tensile strength was calculated by dividing the peak load by the cross-section area (thickness × 1 cm) of the initial specimen. E was expressed as the percentage of change in the length of the specimen to the original length between the grips (5 cm). TS and E measurements were done from three replications.

2.4. Oxygen permeability (OP)
The oxygen transmission rate (OTR) was measured using OX-TRAN 2/61 (Mocon, Minneapolis, MN). The measurements (in triplicates for each film) were done at 25 ºC and the films were equilibrated into RH of 30, 40, 50 and 65% during the experiment using appropriate saturated salt solutions. The OP was calculated by the following equation:

\[ \text{OP} = \frac{\text{OTR}}{\Delta P_{O2}} \times \text{Thickness} \]

The ΔP_{O2} is the partial pressure difference of oxygen between the two sides of the film, which was 1 atm during the experiment.

2.5. Soluble matter (SM) and degree of hydration (DH)
Film specimens (1.5 cm × 1.5 cm, in triplicates) were dried for 3 days in a desiccator over anhydrous calcium sulphate. The specimens were accurately weighed to obtain the initial dry weight (W_i). The specimens were immersed in test tubes containing 5 mL distilled water. The tubes were covered with aluminum foil and incubated at ambient temperature for 24 hr with occasional gentle agitation. The films then were dried in the oven at 70 ºC for 24 hr and weighed to obtain the final dry weight (W_f). The percentage of soluble matter (% SM) of the films was calculated using the following equation:

\[ \% \text{SM of the films} = \left( \frac{W_i - W_f}{W_i} \right) \times 100 \]

The degree of hydration was calculated by conditioning the prepared films at 50% and 90% RH. The films were weighted before (W_b), after (W_a) conditioning and after drying (W_f) in an oven at 70 ºC for 24 hr.

The amount of water absorbed by the films was determined using the following equations:

Water absorbed by conditioning (W_a - W_b)
Total water content of conditioned films (W_a - W_f)
Proportion of water in the conditioned films (W_a - W_f / W_a)

3. Results and discussion
3.1. Films appearance
WPI and PS films were easily handled, and flexible (figure 1). WPI films were transparent and had mild yellowish color whereas PS films were opaque to translucent and have off-white color.
3.2. Mechanical properties

Figure 2 shows representative strain-stress curves for LDPE, WPI and PS films at different RHs. Tensile strength and E of LDPE were not affected as RH increases from 30% to 90% (Table 1). Their values are comparable to those reported by Salih et al., [18] under similar conditions. Amin et al., [21] reported that LDPE films at high RH conditions (75%) underwent dielectric relaxation and increased molecular movement that reduced TS and increased E of these films. In the current results, no such effect of high RH on LDPE films was noticed, which could be due to shorter conditioning period (3hr) applied on films compared to that (12hr) by Amin et al., [21]. This longer conditioning period may allow better plasticization effect of water on polymer molecules than the shorter period. Tensile strength of WPI significantly reduced by increasing RH to 90% whereas E was not significantly (P≥0.01) affected (Figure 2 and Table 1). Furthermore, WPI films had higher TS and lower E than PS films at 90% RH indicating that WPI films are more resistant to high RH conditions than PS films. Pea starch films were the most affected ones because TS significantly reduced and E significantly increased (Table1) at 90% RH. Films made of whey protein concentrate (WPC) was reported by Tulamandi and Rizvi [22] to have lower TS and higher E at high water activity (0.8-1) of films. Bertuzzi et al [17] found out that stress at break decreases and strain at break increased for high amyllose corn starch films plasticized with glycerol (30% of starch weight) by increasing water activity of films from 0.1 to 0.9. The microstructure of these films changes from dense and homogeneous to a more open heterogeneous and more motile structure by increasing water activity [21].

Figure 1. Photographic images of: (a) LPDE film, (b) prepared WPI film and (c) prepared PS films.

Figure 2. Representative strain-stress curves for LDPE, WPI and PS films at 50% and 90% RH.
Table 1. Tensile strength (TS) and elongation at break (E) of the starch films at different relative humidity

| Film | RH (%) | TS (MPa)         | E (%)          |
|------|--------|------------------|----------------|
| LDPE | 30     | 9.903 ± 0.014   | 436.3 ± 1.2    |
|      | 50     | 9.443 ± 0.224a  | 421.0 ± 9.2b   |
|      | 90     | 9.645 ± 0.153a  | 419.2 ± 2.6b   |
| WPI  | 30     | 8.561 ± 0.014b  | 181.3 ± 1.2c   |
|      | 50     | 8.493 ± 0.224b  | 182.9 ± 9.2c   |
|      | 90     | 6.932 ± 0.153c  | 271.4 ± 10.6c  |
| PS   | 30     | 5.773 ± 0.061c  | 491.3 ± 32.6b  |
|      | 50     | 5.261 ± 0.454c  | 483.7 ± 12.0b  |
|      | 90     | 4.326 ± 0.370d  | 765.6 ± 13.6a  |

Means within the same column with different letters are significantly (p<0.01) different.

Although TS and E of PS films were the most affected by high RHs conditions compared to LDPE and WPI films (Figure 2 and table 1). Our previous study [24] showed that PS films are more resistant to high RHs than rice starch (RS) films. This was related to the higher amylose content in PS than RS films; 40% and 30%, respectively. Amylose is the helical linear polymer of the films and responsible for keeping the tensile strength properties of high-amylose starch films. In another study [18], it was reported that stress at break of cassava-starch films increased with amylose content and decreased with glycerol and water contents. The high-amylose films were stiffer, more resistant to fracture and less stretchable than high amylopectin films and have more stable microstructure at higher RHs (70%) with strong molecular orientation, crystalinity and heat resistance [25, 26].

3.3. Oxygen permeability (OP)

LDPE, WPI and PS films are excellent oxygen barriers (OP < 0.5 cm³ µm² d⁻¹ KPa⁻¹) at RH below 40% (figure 3). This indicates good oxygen barriers properties for all films at low RH conditions. Increasing RH from 40% to 65% increases OP of all films in particularly in PS films; OP was 1.7 cm³ µm² d⁻¹ KPa⁻¹ at 65% RH. At intermediate humidity (~50% RH), LDPE and WPI films kept low OP (≤0.6 cm³ µm² d⁻¹ KPa⁻¹), whereas PS films lost their oxygen barrier properties (OP was 1.45 cm³ µm² d⁻¹ KPa⁻¹) (figure 3). It was reported that LDPE films needs longer exposure period (≥12h) to high RH to lose its barrier properties [21]. Oxygen permeability of WPI and PS films was significantly different (p < 0.01) from each other at high RH (50%-60%) whereas no significant differences in OP between WPI and LDPE films at the same RHs. Therefore WPI had oxygen barrier properties similar to LDPE than PS films at high RH condition. Forssell and others [27] found out that amylose films plasticized with glycerol have good oxygen barrier at RHs below 50% and glycerol content up to 30% but this barrier properties lost at higher RHs (50-90%). In another study [26], it was reported that increasing the surrounding RH from 50 to 90% resulted in increasing of oxygen permeability of both amylose and amylopectin films. Increasing the RH above 50% produced more loosen structure in more hydrophilic than hydrophobic polymers that increased mobility of oxygen molecules through these films [22, 24]. WPI films kept their properties at high RHs could be due to their higher hydrophobicity than PS films [12].
3.4. Degree of hydration (DH) and soluble matter (SM)

Table 2 shows degree of hydration of LDPE, WPI and PS films when conditioned at 50% and 90%. LDPE absorbed very low amounts of moisture; this could be due to the high hydrophobicity of this polymer or due to the short conditioning period [21]. At any of the tested RHs, the amount of moisture absorbed by WPI is lower than that by PS films. This is expected as WPI is more hydrophobic therefore could have lower capability to bind water [12]. The higher degree of hydration in PS films is likely to be responsible for inferior mechanical and barrier properties of this film at high RH (≥50%) conditions than the less hydrated films (e.g. LDPE and WPI). WPI and PS films have similar soluble matter (SM) of 32% and 34.3%, respectively (Table 3). This may be caused by the incorporated soluble component that is glycerol. The glycerol:polymer ratio in these films is 1:2. Therefore, total loss of glycerol from the films may result in 33% of solubility that is very close to glycerol content. Although PS has higher hydrophilicity than WPI, both polymers form gelatinized network via electrostatic interactions in WPI and starch granular swelling in PS by heating [28]. These more integrated microstructure could be more resistance to dissolution by water, therefore no statistical difference in SM% between these two films was detected.

Table 2. Degree of dehydration (DH) as affected by RH and total soluble matter (SM) of LDPE, WPI and PS films.

| Weight of film (g) at RH | LDPE  | WPI  | PS   |
|------------------------|-------|------|------|
| Weight before conditioning \((W_b)\) | 1.290 | 1.330 | 1.160 |
| Weight after conditioning \((W_a)\) | 1.295 | 1.331 | 1.580 |
| Weight after drying \((W_f)\) | 1.281 | 1.329 | 0.860 |
| Water absorbed by conditioning \((W_a - W_b)\) | 0.005 | 0.001 | 0.420 |
| Total water content of conditioned films \((W_a - W_f)\) | 0.014 | 0.002 | 0.520 |
| DH \((W_a - W_f) / W_f\) | 0.011 | 0.004 | 0.380 |
| SM (%) | 0.001 | 32.0 | 34.3 |

5. Conclusion
LDPE films showed no detectable differences in mechanical properties, oxygen barrier and degree of hydration at different RH levels. Tensile strength of WPI films was reduced by increasing RH to 90 %, but it was significantly higher than that of PS films. WPI and LDPE films kept reasonable oxygen permeability.
barrier up to 65% RH whereas PS film quickly lost its oxygen barrier. LDPE and WPI films had lower degree of hydration and soluble matter at all the tested RHs than PS films. The more hydrophilic polymer (PS) is likely to lose its mechanical and barrier properties by hydration at higher RH than less the hydrophilic polymers (LDPE and WPI). WPI could better replace LDPE in packaging of moist products such as foods and cosmetics.

6. References
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