



# Effects of plasticizers, pH and heating of film-forming solution on the properties of pea protein isolate films

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## ABSTRACT

Effects of glycerol (3–7% w/w) and sorbitol (4–8% w/w) concentration, pH (7.0, 9.0, 11.0) and heating (90 °C, 20 min) of film-forming solution (FFS) on the water vapor permeability (WVP), moisture content (MC), solubility, light transmission and transparency of pea protein isolate (PPI) films were investigated. Films plasticized with sorbitol exhibited significantly lower WVP, lower MC and higher solubility, in comparison with glycerol-plasticized films. Increasing glycerol content of the films led to increases in WVP and MC but did not affect film solubility. In contrast, increase in sorbitol content had no effect on permeability and MC but resulted in increased film solubility. Moisture sorption isotherms of PPI films suggested that the difference in WVP observed among films plasticized with glycerol and sorbitol might be due to the different hygroscopicity of these plasticizers. The pH of FFS did not have a significant effect on WVP and MC. Solubility of PPI films formed from non-heated FFS was not affected by pH, whereas solubility of films formed from heat-treated FFS generally increased when pH was increased from 7.0 to 11.0. Heating of FFS resulted in improved film transparency. All tested films were characterized by excellent ability to absorb UV radiation. Microstructural observation by scanning electron microscopy did not show differences between sorbitol- and glycerol-plasticized films.

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## 1. Introduction

The constantly growing demand for disposable packaging contributes to rapid increase in the quantity of packaging waste in landfills. Individual packaging of products, which is impossible to avoid especially for perishable food-stuffs, turned packaging industry into one of the major sources of municipal waste. The global aspiration to reduce an adverse impact of synthetic packaging on the natural environment is manifested in the growth of interest in use of bio-based packaging. Materials derived from renewable resources do not burden the ecosystems and provide an alternative to the petroleum-derived polymers.

Bio-based packaging can be biodegradable and/or edible, depending on formulation, formation method, and modification treatments. The application of edible films and coatings is one of the innovations of packaging technology aimed to improve quality and extend shelf life of food products by acting as barriers of gases, moisture, aroma, and oils. Edible films are defined as structures being formed separate of any eventual intended use, whereas edible coatings are thin layers of material formed directly on the surface of the food product, thereby become an integral part of food and can be eaten together with. Films can be used as covers, wraps, or separation layers; and they can be formed into casings, capsules,

pouches, and bags. These stand-alone films also are used as testing structures for determination of barrier, mechanical, solubility, and other properties provided by certain bio-based packaging materials.

The main substances applied in the production of edible films and coatings are proteins (collagen, casein, whey protein, soy protein, gluten, zein), polysaccharides (cellulose, starch, chitosan), and lipids. For coating or packaging applications, film permeability is an important characteristic that can markedly influence the storage stability of foods by controlling mass exchange. Protein films provide excellent oxygen barrier properties but show high WVP due to their hydrophilic nature (Krochta, 2002). The poor moisture barrier properties, however, can be improved by introduction of lipids (Anker et al., 2002; Chick and Hernandez, 2002; Fabra et al., 2008; Kim and Ustunol, 2001). Protein film-formers, such as collagen and gelatin have been used for a long time in the manufacture of sausage casings and drug capsules, respectively. Plant proteins also have a great usable potential. Legume seeds are cheap sources of protein with a relatively high nutritional value, which make them a very good raw material for the production of protein prepartes. At the present time, the worldwide market is dominated by soy protein, commercially available as soy flour, soy concentrate, and soy isolate. Low price, quality, and versatile applications make them difficult to compete with. Consequently, a large part of the literature is focused on soy protein-based films. Only a few studies deal with properties of films from pea protein (Choi and Han, 2001,

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2002; Gueguen et al., 1995, 1998; Viroben et al., 2000), and other legume proteins (Bamdad et al., 2006; Bourtoom, 2008; Jangchud and Chinnan, 1999a, 1999b; Liu et al., 2004). Studies by Choi and Han (2001, 2002) showed that pea proteins can be utilized to prepare edible films with water vapor permeability (WVP) and physical characteristics similar to those obtained from soy proteins, whey proteins, or zein. Price comparison made by these authors on whey protein isolate (WPI) (\$13.5–27/kg), soy protein isolate (SPI) (\$3–3.8/kg), corn zein (\$23–35/kg) (Krochta and De Mulder-Johnston, 1997), and pea protein isolate (PPI) (\$2.5–2.8/kg) (Choi and Han, 2001), indicates that utilization of pea protein in food production, including manufacture of the edible films and coatings could contribute to economic benefits. The lack of genetic modification in commercially available pea species (Directive, 2001/18/EC) makes pea protein a great alternative to soy protein preparates, which are mostly obtained from transgenic plants. In addition, pea, unlike soyabean, as well as cereals containing gluten, eggs, fish, peanuts, milk, lupin and products thereof, is not on the list of products that are the cause of allergies and feeding intolerance (Commission Directive, 2007/68/EC).

Climatic conditions of many European countries are conducive to pea growth, therefore pea is the main protein crop cultivated in the European Union (FAOSTAT, 2010). Manufacture of biodegradable and/or edible packaging from renewable raw materials might be a great opportunity for farmers to get a new output market for their goods. However, all up to date researches show that use of protein films and coatings on an industrial-scale depends on progress in the improvement of their properties. Edible films and coatings with satisfactory properties should have sufficient structural integrity, low WVP, resistance towards water, grease, UV light, etc. In this respect various film-forming variables need to be examined to determine their effect on protein-based film properties. In previous studies, only alkaline (pH 9.0–12.5) casting process has been used to prepare the pea protein-based films (Choi and Han, 2001, 2002; Gueguen et al., 1995, 1998; Viroben et al., 2000). Such high film alkalinity is undesirable when films are intended for use in edible packaging. In the majority of literature concerning the use of edible protein-based coatings to improve quality and prolong shelf life of food products, no pH adjustment during coating formulation is applied. For this reason in our study we considered the possibility of PPI film formation at pH 7.0 (neutral).

The aim of this work was to determine the influence of type and concentration of plasticizer, pH and heating of film-forming solution (FFS) on the water vapor barrier properties, moisture content, solubility, light transmission and transparency of PPI films. The effect of plasticizer type on the moisture sorption and film microstructure was also investigated. However, all factors studied in this work have been intensively investigated and published by other authors, these fundamental factors were never simultaneously combined to optimize the protein film formation.

## 2. Materials and methods

### 2.1. Materials

Pea protein isolate Propulse (ProFlo)<sup>TM</sup> (82.0 ± 2.0% protein) was kindly provided by Parrheim Foods Co. currently Nutri-Pea Limited (Portage la Prairie, MB, Canada). Glycerol and sorbitol (Sigma Chemical Co., St. Louis, MO, USA) were used as plasticizers.

### 2.2. Film preparation

Films were obtained from 10% (w/w) aqueous PPI solutions containing various amounts of plasticizers, 3–7% (w/w) glycerol or 4–8% (w/w) sorbitol. After stirring (14,000 rpm, 2 min), the pH values

of FFS were adjusted to pH 7.0, 9.0 and 11.0 with concentrated NaOH solution. Next mixtures were degassed under vacuum to remove any dissolved air. Films were formed by casting 11 g of solution on leveled polystyrene Petri dish (Nunc, Roskilde, Denmark) with an area of 145 cm<sup>2</sup>. Following solutions were drying at room temperature (25 ± 1 °C) for about 12 h. In the case of thermally modified films the FFS (100 ml) were heated in water bath at 90 °C for 20 min, and after cooling to 25 °C were mixed again (14,000 rpm, 1 min) to obtain homogeneity.

### 2.3. Film thickness and conditioning

Film thickness was measured to the nearest 2.54 μm with a hand-held micrometer (Mitotuyo No. 7327, Tokyo, Japan). Three to five thickness measurements were taken on each testing specimen, depending on its dimensions. All film specimens were conditioned for 48 h in a versatile environmental test chamber (MLR-350, Sanyo Electric Biomedical Co. Ltd., Japan) at 50% relative humidity (RH) and 25 °C before testing.

### 2.4. Water vapor permeability

The WVP (g mm/m<sup>2</sup> d kPa) was calculated as:

$$\text{WVP} = (\text{WVTR } L) / \Delta p \quad (1)$$

where WVTR (g/m<sup>2</sup> d) is the water vapor transmission rate of films measured at 25 °C and 50% RH gradient,  $L$  (mm) is the thickness of film specimens, and  $\Delta p$  (kPa) was the difference in partial water vapor pressure between the two sides of film specimens. WVTR was determined gravimetrically using a modification of the (PN-ISO 2528, 2000), also known as the cup method.

The permeation cell (poly(methyl methacrylate) cups) had an internal diameter of 7.98 cm (exposed film area: 50 cm<sup>2</sup>) and an internal depth of 2 cm. Film specimens (10 cm diameter disks) were mounted onto the open circular mouths of cups filled with 10 g of anhydrous calcium chloride (0% RH). The lid was fixed by six screws, a rubber O-ring gaskets helped to assure a good seal. The cups were placed in an environmental chamber set at 25 °C and 50% RH. Weights of the cups were recorded every 2 h for a period of 10 h. The slopes of the steady state (linear) portion of weight grow versus time curves were used to calculate WVTR.

### 2.5. Moisture sorption isotherms

Film pieces (1.5 × 1.5 cm) were dried at 40 °C in a vacuum chamber containing P<sub>2</sub>O<sub>5</sub> to constant weight (for 1 week). Afterwards, film specimens were sealed in chambers with constant RH atmospheres. The equilibration chambers were set up according to ASTM E104-02 (ASTM E 104-02, 2003) and consisted of small, hermetic boxes containing saturated salt solutions and a perforated plastic plate to suspend the sample above the salt solution. The solutions of LiCl, CH<sub>3</sub>COOK, MgCl<sub>2</sub>, K<sub>2</sub>CO<sub>3</sub>, NaBr, NaCl, and KCl stored at 25 °C (± 1 °C) equilibrate with the headspace air resulting in a RH of 11.3%, 22.5%, 32.8%, 43.2%, 57.6%, 75.3% and 84.2%, respectively. The samples were removed from the chambers and weighed after incubating for 2 weeks (preliminary experiments determined the samples reached a constant mass after approximately 7–10 days). Equilibrium moisture content was calculated from the increase in mass of the dried sample after equilibration at a given RH. The water activity of each of the salt solutions was calculated as %RH/100. All tests were performed in triplicate. The experimental moisture sorption isotherm values were averaged and fitted by the Guggenheim–Anderson–de Boer (GAB) model (Eq. (2)) as follows:

$$M = (m_0 C k a_w) / [(1 - k a_w)(1 - k a_w + C k a_w)] \quad (2)$$

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