Preparation and Characterization of Films from Pea Protein

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The conditions for protein film preparation from an alkaline dispersion of a pea protein isolate were investigated in the presence of polyols as plasticizers. Mechanical and barrier properties of resulting films were studied as a function of protein dispersion conditions, protein and plasticizer concentrations and ratios, chain length of the plasticizer, and pH and composition of the alkaline medium. Neither the mode of protein hydration nor the pea isolate origin had a significant effect on the mechanical properties of pea protein films. However, increasing the plasticizer chain length induced slightly higher surface hydrophobicity but poor mechanical properties. Addition of monoglycerides to film-forming solution allowed a significant improvement of the films during aging. Both tensile strength and surface hydrophobicity increased when ammonium hydroxide was used as protein dispersing agent instead of sodium hydroxide.

Keywords: *Pea protein; film; mechanical properties; barrier properties*

INTRODUCTION

Coatings and films have been used for many decades to protect food from microbial attack and to prevent water loss during storage. The recent renewal of consumer interest with regard to food quality has intensified research in this area. Studies have focused on partial substitution of synthetic packaging materials by biopolymers produced from renewable resources, which may contribute to a reduction in environmental pollution (Gennadios and Weller, 1990). Several biological materials, including polysaccharides, proteins, and lipids, either alone or in mixtures, have been proposed for preparing edible films or coatings (Kester and Fennema,1986). Protein or polypeptides from vegetable origin have been investigated a long time ago as film-forming agents (zein, Cosler, 1957; wheat gluten, Anker et al., 1972). A few recent studies have attempted to optimize film-forming conditions to improve mechanical and barrier properties (Gontard et al., 1992; Gennadios et al., 1993a,b,d). For coating or packaging applications, film permeability is an important characteristic that can markedly influence the storage stability of foods by controlling both water and gas (oxygen and carbon dioxide) transfer (Gennadios et al., 1993c, 1994; Gontard et al., 1994).

Limited information is available on the use of protein legume seeds for packaging applications, although soy milk preparations have been utilized for centuries in traditional Far Eastern cooking for the production of edible films (yuba). The formation conditions for such protein films have been described by several authors (Okamoto, 1978; Kester and Fennema, 1986; Gennadios and Weller, 1990, 1991). It has been postulated that the globular protein structure is first unfolded by heating and then forms an interacting polypeptide network that is strengthened by disulfide and hydrophobic bonds upon subsequent drying of the product. As the resulting films were very brittle, Chuah et al. (1983) proposed plasticization with glycerol. Protein unfolding may also occur by dispersion in an alkaline medium (pH ≈10). Properties of soy protein films obtained in such manner were studied by Brandenburg et al. (1993). These authors used glycerol as a plasticizing agent to reduce intermolecular forces between polypeptide chains, softening the rigidity of the resulting films and therefore improving their mechanical properties.

The film-forming potential of pea proteins has also been demonstrated recently (Viroben et al., 1994; Gueguen et al., 1995,1998). Pea seed production is rather high in the Western European Community, especially in France. However, it may be developed still further to reduce the problem of inadequate exploitation of fallow lands. In this respect, research is in progress to find new outlets for pea production by focusing on the utilization of seed components (starch and protein) for nonfood applications. In our paper, the film-forming properties of pea proteins were studied as a function of protein/plasticizer concentrations and ratios and as a function of protein dispersion conditions, duration, and temperature of drying. In the second part of the study, the film properties were investigated depending on the plasticizer type, selected additives, and protein modifications.

MATERIALS AND METHODS

Preparation of the Pea Protein Films. *Basic Conditions.* Most trials were undertaken with a special isolate referenced in the study as Spepro [N × 5.6 = 70.6% dry matter (dm)] from Provital (Belgium), produced by alkaline extraction followed by isoelectric precipitation. For comparison purposes, some experiments were also conducted either with a commercial ultrafiltrated product from the same origin referred to as Pisane (N × 5.6 = 77% dm) or with an isolate prepared in our pilot plant from another pea batch by an extraction process similar to that used for Spepro; it was referred to as PMW 170 (N × 5.6 = 72.2% dm).

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The film-forming dispersion was generally prepared by mixing the pea protein isolate in an alkaline medium. For comparison, the dispersion was, in some cases, performed either with a Mixograph (National Manufacturing Division, Lincoln, NE) (80 rpm; 1 h) or with a screw-fitted helical device (300 rpm; 24 h). However, most further experiments were carried out using a Polytron homogenizer (Kinematica, Littau, Switzerland) (24000 rpm; 1 min). A plasticizer was added to the protein dispersion. Different plasticizers (Sigma-Aldrich Chimie, St. Quentin, France) were tested, either from the glycol series (ethylene glycol, EG; diethylene glycol, DEG; triethylene glycol, TEG; tetraethylene glycol, TeEG) or from the diol series (1,2-propanediol, PRG; 1,3-propanediol, PRD). In some cases, these plasticizers were compared with glycerol (GLY), a plasticizer commonly used with protein films (Gontard et al., 1992; Gennadios et al., 1993a,b). After plasticizer addition and a second stirring step (same speed but half the duration), the mixture was centrifuged (115*g*; 30 min) to remove foam and air bubbles. The deaerated dispersion (FFS) was then spread on a glass plate (casting technique) covered with a polyester film of Gel Bond PAG (FMC Bioproducts, Rockland, ME). The wet film, the thickness of which was controlled to 500 μm using a calibrated Conway bar, was dried in an air-circulating oven heated at 70 °C for 1 h, according to preliminary studies carried out with DEG. These drying conditions were applied to the film series whatever the plasticizer. After drying and cooling, the film was carefully peeled from the plate, laid onto a glass plate, and kept in an environmental chamber, for 3 days, under constant temperature and relative humidity conditions (20 °C; RH = 60 ± 3%), before characterization. The thicknesses of the films were around 100 ± 10 μm.

Modifications of Film Preparation. In a series of experiments, monoglycerides of heptanoic, undecanoic, oleic, linoleic, linolenic, and erucic acid were added to the FFS to investigate the effect on the hydrophobic character of the films. These molecules were dissolved in DEG at a concentration of 10% (w/w) to avoid phase separation. In comparison, a pea isolate made more hydrophobic by lauryl chloride treatment, following the acylation reaction of Schotten−Baumann (Nechesnyuk et al., 1987), was used in partial substitution to the unmodified isolate in the FFS.

Film Characterization. Mechanical properties (strain at rupture and tensile strength) were determined by means of a traction−compression device (DY34 from Adamel-Lhomargy Testing Instruments, Ivry, France) (five replicates) on film specimens of 75 mm length with a dumbbell shape (5A type, ISO 527-2 standard). Film thickness was measured at five points with a hand-held micrometer (Morton-Blet, Prolabo, Paris, France). The surface hydrophobicity of films was estimated by the contact angle formed by a water droplet placed on the film surface, using Digidrop equipment (GBX Scientific Instruments, Romans, France) (Sanchez et al., 1998). For water vapor permeability evaluation, a disk of the film to be studied, with an exposed area of 9 cm^2, was tightly fixed between the mobile ring and the cup of a cell filled with 5 mL of water. This cell was weighed initially after equilibration at 22 °C and 52% RH in a desiccator containing a saturated solution of magnesium nitrate. The weight loss of the cup was determined every 2 h, for 8 h, and the water vapor transmission through the film was calculated by regression and expressed as water vapor permeability (g m^{-1} s^{-1} Pa^{-1}) of the film. For measuring the water solubility of the film constituents, protein and plasticizer, a film sample (400 mg) was cut into small pieces and submitted to two successive water extractions (2 × 20 mL) for 1 h each. After centrifugation of the dispersion, the two supernatants were pooled and analyzed for their respective protein and plasticizer contents. Soluble proteins were estimated according to the Kjeldahl method, whereas plasticizer concentration was determined by chromatography on a 300 × 7.8 mm ion-exchange column (Shandon), held at 60 °C, according to the method of Bonn (1985). The detection was carried out by differential refractometry.

Optimization of FFS Composition. Response surface methodology was used to determine the influence of selected factors on the mechanical properties of pea protein films prepared from Spepro isolate and DEG as a plasticizer. The experimental scheme was a face-centered composite design for four variables at three levels each. The four independent variables were protein and DEG concentrations (w/w of the FFS), pH, and sodium chloride molarity of the FFS. The dependent variables were strain at rupture and tensile strength of films. Preliminary investigations were required to define the range of the variation for the different parameters that allowed suitable FFS viscosity and film-handling conditions. The following limits for the parameters were consequently defined as

$$9 < \text{pH} < 10.6$$

$$10\% < \text{protein concentration (w/w of the FFS)} < 16\%$$

$$8\% < \text{DEG concentration (w/w of the FFS)} < 18\%$$

$$0\ \text{M} < \text{sodium chloride molarity of the FFS} < 0.6\ \text{M}$$

The complete design consisted of 27 experimental points, including 3 replications of the center point, the characteristics of which are as follows: protein concentration, 13%; DEG concentration, 13%, pH, 9.8; sodium chloride molarity, 300 mM. Each dependent variable, strain at rupture or tensile strength of the film, was analyzed to fit a second-order equation of the form

$$Y = k_0 + k_1A + k_2B + k_3C + k_4D + k_5AB + k_6AC + k_7AD + k_8BC + k_9BD + k_{10}CD + k_{11}A^2 + k_{12}B^2 + k_{13}C^2 + k_{14}D^2$$

where k_i represented constant regression coefficients and A, B, C, and D were protein concentration, DEG concentration, pH, and the sodium chloride molarity of the FFS, respectively.

RESULTS AND DISCUSSION

Standardization of the Casting Procedure. The film-forming properties of protein depend on numerous factors, which play an important part in the quality of resulting films. In our study, it was required to investigate the effects of some parameters on mechanical properties of pea protein films before the proposal of a standard protocol for their preparation by the casting technique.

Technique of Protein Hydration. As protein dispersion and hydration are energy- and time-dependent phenomena, the conditions for preparing the FFS, especially duration and type of mixing, were systematically studied using Spepro (12% w/w of the FFS) and DEG or EG as plasticizer (mass ratio plasticizer/protein 1:1). Three different devices and mixing times were compared: Polytron homogenizer for 1 min, Mixograph for 1 h, and helical stirring device for 24 h. Surprisingly, no significant differences of film mechanical properties could be detected among the three corresponding series of films (Table 1), considering the magnitude of the standard errors. Therefore, for all further experiments, protein dispersion was achieved by mixing for 1 min with the Polytron homogenizer.

Optimization of FFS Composition. Statistical analysis, using the Statgraphics procedure, was applied to results obtained from the 27 experimental films. This procedure allowed an estimation of the significance of the regression coefficients of the second-order equation corresponding to each dependent variable. Significant effects ($P < 0.05$) on strain at rupture due to pH of the FFS (positive effect) and protein concentration and ionic

Table 1. Comparison of Different Techniques of Protein Hydration

mode of hydration	plasticizer type[a]	strain at rupture (%)	tensile strength (MPa)
Mixograph	EG	111 ± 24	3.3 ± 0.2
	DEG	128 ± 22	1.8 ± 0.2
mechanical device	EG	151 ± 50	1.5 ± 0.2
	DEG	131 ± 13	1.9 ± 0.2
Polytron	EG	165 ± 27	2.4 ± 0.2
	DEG	136 ± 29	2.1 ± 0.3

[a] EG, ethylene glycol; DEG, diethylene glycol.

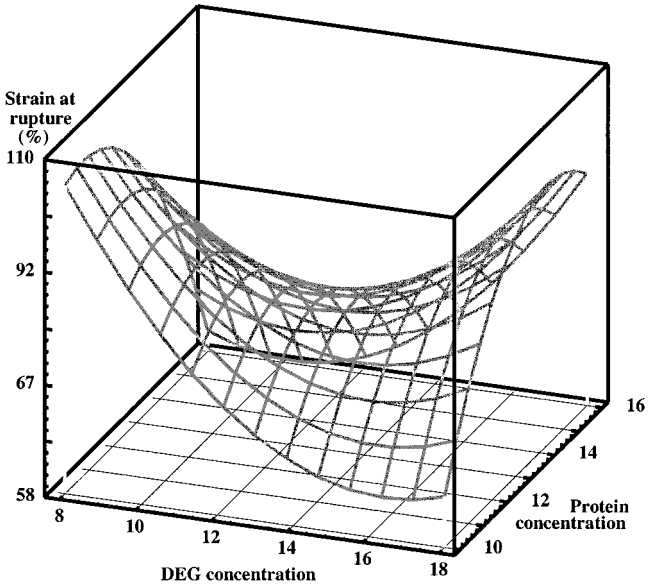

Figure 1. Influence of diethylene glycol (DEG) and protein concentrations in the film-forming solution (% w/w of the FFS) on film strain at rupture.

strength (negative effect) were observed. In addition, a strong relationship between protein and DEG concentrations and, to a lesser extent, between DEG concentration and pH appeared clearly. Data of strain at rupture, presented as response surface, displayed a characteristic "horse-saddled" shape and revealed an optimum protein concentration around 13% (w/w of the FFS) (Figure 1) and an optimum pH around 10 (Figure 2). However, as such a high protein concentration caused a higher viscosity of the FFS and the presence of curdles within the films, it was decided to limit it to 12% maximum (w/w of the FFS). Concerning the tensile strength of the films, the only significant effect ($P < 0.05$) was due to ionic strength of the FFS (negative effect). Ultimately, the following conditions for pea protein film preparation were selected: pH 10; protein concentration, 10−12% (w/w of the FFS), as a function of the protein dispersion viscosity; plasticizer/protein ratio, 0.8−1.0; no salt added.

Influence of the Nature of the Alkaline Medium. To prevent uncontrolled side reactions due to sodium hydroxide concentration during drying, the substitution of this alkaline agent by ammonium hydroxide was tried, following the suggestion of Gennadios et al. (1993a,b). The use of ammonium hydroxide seemed particularly beneficial because it could be removed during the drying step. In our study, this modification of the FFS preparation also allowed the pH to be raised to 12 without gelation of the protein dispersion, in contrast to sodium hydroxide. By comparing mechanical properties of films obtained from the Spepro isolate,

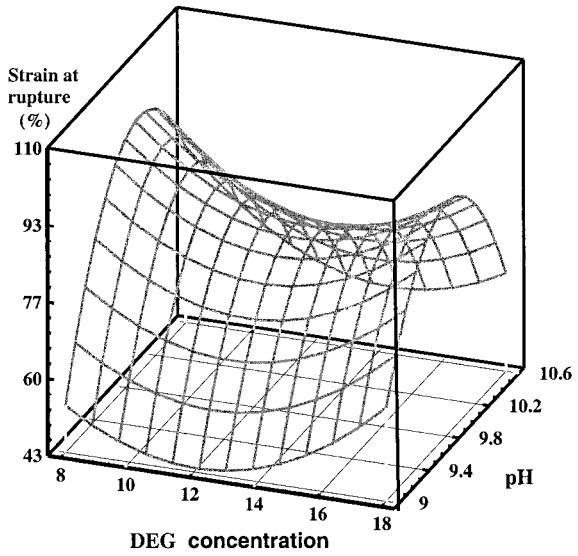

Figure 2. Influence of pH and diethylene glycol concentration (DEG % w/w of the FFS) in the film-forming solution (FFS) on film strain at rupture.

Table 2. Effect of the Alkaline Medium Used in the Film-Forming Solution on Pea Protein Film Properties[a]

composition of alkaline medium	pH	strain at rupture (%)	tensile strength (MPa)	contact angle[b] (deg)
sodium hydroxide	10.2	160 ± 24	1.9 ± 0.1	22
	10.8	124 ± 20	2.8 ± 0.2	28
	11.1[c]	36 ± 10	2.6 ± 0.2	52
ammonium hydroxide	9.8	146 ± 45	1.7 ± 0.2	19
	10.0	144 ± 25	2.4 ± 0.2	41
	10.1	168 ± 38	1.7 ± 0.3	40
	10.4	171 ± 25	2.1 ± 0.3	42
	10.9	77 ± 34	4.3 ± 0.3	41
	11.2	37 ± 9	8.5 ± 0.8	41
	11.9	29 ± 8	9.6 ± 0.1	39

[a] Plasticizer: EG. [b] Mean value of three measurements. [c] Beginning of gelation.

either with ammonium hydroxide or with sodium hydroxide (Table 2), it appears that they are rather independent of both pH and alkali type as the pH remained below 11. Above this critical value, poor mechanical properties were observed in the case of sodium hydroxide related to a beginning of FFS gelation. On the other hand, a drastic increase of tensile strength clearly appeared for ammonium hydroxide. In addition, more transparent films could be obtained. However, high concentrations of ammonium hydroxide (3.5 M) were needed to reach such pH. Consequently, this procedure would probably not be easy to scale-up for obvious reasons of safety, despite its positive effect on the tensile strength and contact angle.

Standard Protocol for Pea Protein Film Preparation. From the mentioned studies, the following protocol of film preparation by the casting technique was proposed. Pea protein was dispersed in 0.07 N sodium hydroxide, to a final concentration of 10−12% (w/w) of the FFS, as a function of the viscosity observed, using a Polytron homogenizer (25000 rpm) for 1 min. The plasticizer was then added, and the mixture was stirred again for 30 s. As the plasticizer is known to enhance the hydrophilic character of the films, it was suggested the plasticizer/protein ratio should be limited to 0.75. Lower ratios introduced difficulties in film recovery from the glass plate. After centrifugation (115*g*; 30 min) of the mixture,

Table 3. Influence of Pea Isolate Origin on Pea Protein Film Properties[a]

pea isolate	strain at rupture (%)	tensile strength (MPa)	contact angle (deg)
Spepro	131 ± 18	1.6 ± 0.1	30 ± 2.5
PMW 170	157 ± 21	1.8 ± 0.1	36 ± 2.0
Pisane	148 ± 16	1.7 ± 0.1	38 ± 2.0

[a] Plasticizer: DEG.

the upper layer was removed and a wet film of 500 μm thickness was spread onto a glass plate. The drying and conditioning of the film were carried out exactly as described under Materials and Methods.

Influence of the Pea Isolate Origin. The mechanical properties and surface hydrophobicity of films prepared from three different protein isolates are presented in Table 3. No significant differences were detected among films if the magnitude of standard errors was taken into account. In particular, the commercial product Pisane did not differ from the others, despite the presence of the pea albumin fraction, due to use of ultrafiltration processing instead of precipitation for isolate production.

Influence of Plasticizer Type on Properties of Pea Protein Films. The carbon chain length has a drastic effect on platicizer content of films after drying. A great proportion of plasticizer is lost during drying, especially for EG, PRD, and PRG. Surprisingly, glycerol, with only three carbons, is more retained in the film, probably due to stronger interactions with polypeptide chains (Table 4).

Despite some differences in plasticizer content, some information about the effect of plasticizer type on film properties could be deduced from these experiments. As a general rule for biopolymers (Banker, 1966; Gontard et al., 1993), an increased plasticizer content led to higher strain and lower tensile strength. In our study, an opposite effect was observed. Lower strain and stress were obtained for the higher molecular weight plasticizers, which was rather surprising because they were recovered in larger concentration in films. This might signify that increasing the carbon chain length would induce a decrease of tensile strength and strain at rupture for pea protein films. By comparing PRG, PRD, and GLY, the number and position of hydroxyl groups seemed also to be an important factor.

The secondary hydroxyl group in PRG slightly enhanced tensile strength. GLY exhibited a rather different plasticizing effect from the other plasticizers having three carbon atoms.

Considering water vapor permeability, only limited differences were observed depending on the plasticizer used. The contact angle was increased for TeG and GLY but more significantly for PRG. The higher contact angle obtained with PRG compared to PRD could be explained by its lower surface energy (38 mJ m^{-2} for PRG versus 49 mJ m^{-2} for PRD).

Aging of Films. When exposed to air at ambient temperature, films became very brittle more or less rapidly, depending on the plasticizer. The composition and properties of films during storage, under constant temperature and relative humidity conditions (20 °C; RH = 60 ± 3%), were studied using different short carbon chain plasticizers. DEG was selected among the glycol series because it is less volatile than EG and compared with PRD representing the diol series and with PRG, which allowed estimation of the influence of a hydroxyl group in secondary position. Results concerning films tested after 3, 10, 17, or 24 days are shown in Table 5. Although the plasticizer level and the mechanical properties exhibited only slight variations with DEG films, they were strongly affected by aging in the case of PRG. The loss of plasticizer content was especially severe between 10 and 17 days of storage and was accompanied by a significant increase of the tensile strength of the films. It was noticeable that the behavior of films differed markedly according to whether they were prepared using PRG or PRD. Moreover, a new compound, not identified, representing 10−20% (w/w expressed as PRD equivalent) of the film dry matter, was detected in the case of PRD, indicating the possible formation of an oxidation product derived from that plasticizer. Soluble proteins (expressed as percent of total protein) decreased significantly during aging, regardless of the plasticizer used, suggesting the occurrence of a reaction between plasticizer and protein matrix or a strengthening of protein−protein interactions due to the progressive elimination of the plasticizer. Concerning the other properties, water vapor permeability did not change, whereas contact angle showed a tendency to decrease during aging.

Effects of Lipophilic Compounds Added or Grafted to the Proteins. Monoglycerides were added to the FFS to make the pea protein films more hydrophobic. An increase of film heterogeneity was observed without significant effect either on their surface hydrophobicity and water vapor permeability (data not shown) or on their mechanical properties (Table 6). The only advantage of using these monoglycerides was to allow for the preparation of very soft films, which did not dry after exposure to air at ambient temperature for as long as 1 month, significantly improving their aging behaviors. As the stability of these monoglycerides in the alkaline medium of the FFS was questionable, another series of experiments was performed at lower pH (≅7.8). These pH conditions represented the limit beneath

Table 4. Effect of Plasticizer Type on Mechanical and Barrier Properties of Pea Protein Films

plasticizer type[a]	no. of C atoms	film plasticizer content[b] (%)	strain at rupture[c] (%)	tensile strength[c] (MPa)	contact angle[d] (deg)	water vapor permeability[e]
EG[f]	2	9.2	152 ± 32	2.2 ± 0.2	21	19
PRD	3	25.0	150 ± 18	1.9 ± 0.1	32	47
DEG[f]	4	35.4	137 ± 30	2.1 ± 0.3	13	21
TEG[f]	6	38.3	121 ± 9	1.0 ± 0.07	21	28
TeEG[f]	8	40.4	100 ± 18	0.7 ± 0.03	40	28
PRG	3	23.8	100 ± 17	3.0 ± 0.4	67	37
GLY[f]	3	33.9	75 ± 10	0.5 ± 0.07	40	29

[a] EG, ethylene glycol; DEG, diethylene glycol; TEG, triethylene glycol; TeEG, tetraethylene glycol; PRD, 1,3-propanediol; PRG, 1,2-propanediol; GLY, glycerol. [b] Mean value of duplicates. [c] Mean values and standard deviations calculated from five measurements. [d] Mean value of three measurements. [e] Mean value of duplicates expressed as [g m^{-1} s^{-1} $Pa^{1-}(10^{11})$]. [f] Data from Gueguen et al. (1998) included here for comparison with PRD and PRG.

Table 5. Influence of Aging on Pea Protein Film Properties

plasticizer type[a]:	DEG				PRG				PRD			
duration of storage (days):	3	10	17	24	3	10	17	24	3	10	17	24
Film Properties												
strain at rupture[b] (%)	122 ± 10	91 ± 17	130 ± 15	141 ± 17	100 ± 17	102 ± 21	21 ± 6	33 ± 6	150 ± 18	137 ± 23	128 ± 23	88 ± 27
tensile strength[b] (MPa)	1.9 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	2.1 ± 0.2	3.0 ± 0.4	4.5 ± 0.2	9.4 ± 0.7	7.7 ± 0.5	1.9 ± 0.1	1.4 ± 0.2	1.3 ± 0.2	0.9 ± 0.1
contact angle[c] (deg)	21			29	67	57	33	38	32	22	15	18
water vapor permeability[d] ($g\ m^{-1}\ s^{-1}\ Pa^{-1}$) × 10^{11}	34.5			32.2	36.9	37.4	36.0	34.9	47.4	38.7	37.7	35.1
Film Composition[e]												
water content[d]	10.1	10.5	10.6	10.5	11.5	10.0	7.3	9.5	10.5	13.3	14.3	11.9
plasticizer content[d]	29.1	34.4	31.1	26.0	23.8	17.7	8.6	9.5	25.0	19.9	18.9	15.5
protein content[d] (N × 5.6)	37.9			35.6	56.7			63.3	42.0			44.8
soluble protein[d] (% of total protein)	26.0			17.4	22.3			14.3	22.8			12.3

[a] DEG, diethylene glycol; PRG, 1,2-propanediol; PRD, 1,3-propanediol. [b] Mean values and standard deviations calculated from five measurements. [c] Mean value of three measurements. [d] Mean value of duplicates. [e] These data are expressed as percent of the film after equilibration.

Table 6. Effect of Monoglycerides Added to the Film-Forming Solution on Pea Protein Film Properties

fatty acid corresponding to the monoglyceride	strain at rupture[a] (%)	tensile strength[a] (MPa)	contact angle[b] (deg)
heptanoic	114 ± 25	1.1 ± 0.1	15
undecenoic	105 ± 14	1.2 ± 0.1	19
oleic	119 ± 27	1.3 ± 0.2	21
linoleic	133 ± 21	1.2 ± 0.1	19
ricinoleic	116 ± 27	1.0 ± 0.1	18
myristic	112 ± 17	1.1 ± 0.1	29

[a] Mean values and standard deviations calculated from five measurements. [b] Mean value of three measurements.

Table 7. Effect of Partial Substitution of Spepro Isolate by Lauryl Chloride Acylated Isolate on Pea Protein Film Properties

acylated isolate/untreated isolate ratio	strain at rupture[a] (%)	tensile strength[a] (MPa)	contact angle[b] (deg)
1:3	170 ± 44	0.95 ± 0.15	16
1:1	120 ± 10	0.70 ± 0.02	16
3:2	102 ± 26	0.65 ± 0.10	7

[a] Mean values and standard deviations calculated from five measurements. [b] Mean value of three measurements.

which protein precipitation began to occur. The resulting films showed slightly higher tensile strength than the corresponding control film without added monoglyceride (4.7 ± 0.4 versus 3.2 ± 0.2 MPa), but, as previously, no effect on surface hydrophobicity was observed. Consequently, some assays were performed using proteins made more hydrophobic by grafting lauryl chain by acylation with lauryl chloride. They were employed in partial substitution of Spepro in the preparation of the FFS. Regardless of the level of substitution, films exhibited lower strain at rupture and tensile strength but surprisingly also poor contact angle values (<20°). Addition of acylated proteins, which are less soluble, might increase the films' heterogeneity (Table 7).

Conclusion. This study clearly showed the potential of industrially extracted pea proteins for films, the mechanical properties of which could vary over a rather large range, depending on the experimental conditions. The beneficial effect on these properties of ammonium hydroxide compared to sodium hydroxide was observed as well as the positive action of monoglycerides on film aging. The carbon chain length of the added plasticizers and the number and position of the hydroxyl groups also were shown to be important factors, demonstrating the great influence of plasticizer chemical structure on occurring interactions with polypeptide chains. Despite their satisfactory mechanical properties, the main drawback of these biomaterials prepared from pea proteins is, as for other biopolymers, their sensitivity toward water. No positive effect was obtained either by addition of monoglycerides or by protein acylation.

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