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Research Article
Lentil By-products as a Source of Protein for Food Packaging Applications

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Abstract

Background and Objective: There is an increasing need for biodegradable, environmentally friendly and functional food-packaging materials. In this regard, proteins obtained from agri-food industry by-products may become a promising and sustainable (less impact, valorisation) source of such materials. This work investigates the suitability of lentil protein-based films for food packaging applications. **Materials and Methods:** Lentil protein concentrate was extracted from lentil by-products using a procedure patented by SICA. Protein films containing different plasticisers were produced by casting after denaturation and adjusting the pH of the protein solution. The effect of plasticisers on solubility, moisture content and the mechanical and barrier properties was analyzed. Finally, migration tests were carried out. **Results:** Plasticiser was found to have no effect on total soluble matter, although the moisture content increased in the case of glycerol. Films plasticised with sorbitol exhibited significantly lower water vapour and oxygen permeability and were also stronger and less flexible. Migration tests complied with current legislation in the case of isooctane but exceeded the legal limits for 95% ethanol. **Conclusion:** The results of this study confirmed that it is possible to obtain lentil protein films with suitable properties for food packaging applications from lentil by-products. These films may become a promising component of new biodegradable and functional food-packaging systems.

Key words: Lentil by-products, valorization, lentil protein films , barrier properties, migration, food packaging

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Several types of residues, such as straw, hay, husk, leaves, etc. are generated during legume production. Indeed, in 2012 around 3 million tonnes of legume residues were generated in the European Union, mainly from soybeans and peas (estimated data from LEGUVAL project. Deliverable 1.1)[1].

The major components in legume by-products are proteins, starch and dietary fibre and the main current uses of these by-products are for animal feed and agricultural practices. The average chemical composition of these by-products is as follows: 21% dry residue, 6% protein, 3.5% starch, 8% fibre and 2% sugars (data from LEGUVAL project)[1,2]. In response to current legislation and environmental concerns, the industry is increasingly having to find an alternative use for residual matter and in this regard, the recovery of high value compounds is a profitable way of reusing by-product streams.

Like most pulses, lentils are a rich source of protein, containing between 20.6 and 31.4% of these compounds[3]. They are also an excellent source of several nutritional factors and their consumption is associated with several positive effects on human health. Another field of application is the development of edible films based on lentil proteins[3].

Biodegradable/edible films prepared from different renewable biopolymers, such as polysaccharides, proteins, lipids or combinations thereof, have been studied as an innovative proposal for coatings and active food packaging. Of these, proteins have a good film-forming capacity and possess an excellent barrier to aromas, oxygen and lipids at low relative humidity[4]. However, due to their hydrophilic nature, they are poor moisture barriers[5]. Polymers containing groups that can associate via hydrogen or ionic bonding result in films with excellent oxygen barriers but which are susceptible to moisture. Additionally, to decrease brittleness and avoid cracks in the polymeric matrices, hydrophilic plasticisers must be incorporated into protein films, which usually causes a decrease in WVP[6, 7]. In general, for film formation, protein must be denatured by acids, bases, heat and/or solvents in order to form more extended structures. Finally, although protein films have been found to possess satisfactory mechanical properties[4], their mechanical strength in comparison with synthetic polymers limits their application in food packaging[8]. Various methods to enhance the physical properties of protein films have been suggested such as effects of plasticization, pH alteration, lipid addition, cross linking of proteins or irradiation among others[9].

Previous studies have provided promising initial results using lentil proteins. Thus, Bamdad *et al.*[10] prepared an edible film from lentil protein and showed that it had good mechanical properties and water vapour permeability as well as good solubility and Ochoa-Yepes *et al.*[11] developed biodegradable composites with starch, glycerol and lentil flour and showed that the latter can act as a reinforcement for starch films, thus leading to more flexible films.

Other studies related to legume proteins and the effect of different plasticisers have also been published. Kowalczyk and Baraniak[12] concluded that the type of plasticiser had a great influence on water vapour permeability and moisture content of pea protein isolate films plasticized with glycerol or sorbitol, being the hydrophilicity of plasticiser the most important factor.

This study aimed to evaluate lentil by-products as a source of lentil protein concentrates and to determine the effect of different plasticisers on the functional properties of lentil protein films obtained from this protein concentrate. The physical properties of protein films are strongly influenced by the type and amount of plasticiser used[12]. No other study in the literature has reported the valorisation of lentil by-products to produce films with suitable properties to be used in food packaging.

MATERIALS AND METHODS

Study area: The study was carried out in TECNALIA (Álava, Spain) and SSICA (Parma, Italy) from November, 2015 until March, 2017.

Materials: Dehydrated lentil by-products were provided by an Italian processing company from Castelluccio di Norcia. The lentil protein concentrate was obtained at the Stazione Sperimentale per l'Industriadelle Conserve Alimentari (SSICA) in Parma (Italy), where its composition was also determined. Lentil protein concentrate-based films were developed at the Health Division of TECNALIA in Miñano (Alava). The films were characterized jointly by TECNALIA and SSICA as part of the LEGUVAL project.

Reagents (NaOH, sorbitol, glycerol and polyethylene glycol 400) were supplied by Scharlab (Spain) or by Sigma (Italy, ethanol and isooctane).

Methods

Preparation of lentil protein concentrate (LPC): A visual examination of dehydrated lentil by-products was carried out in order to identify their bulk composition (peels, pods, broken grains, grains with different sizes or colours and seeds).The percentage of inert substances, including leaves, soil or small stones, was determined and calculated as a percentage of all by-products by weight. Three replicates were performed and the mean and standard deviation (SD) calculated.

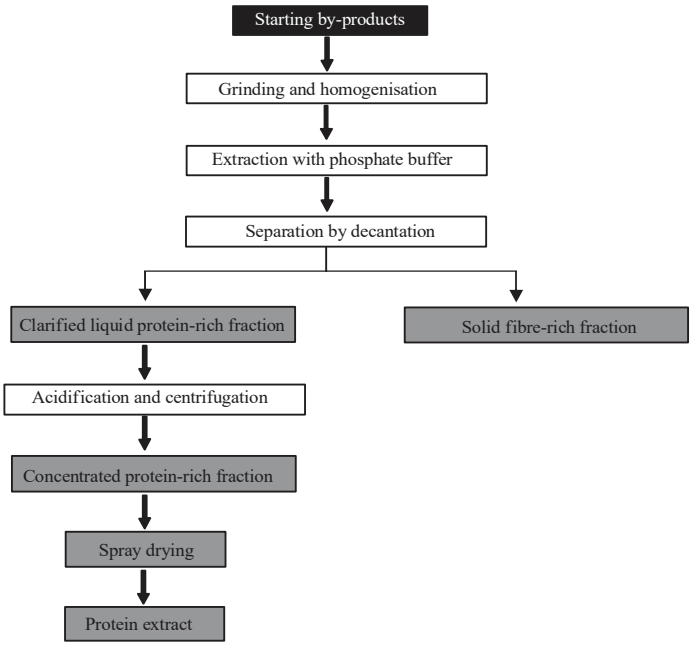

Fig. 1: Flow-sheet of the extraction procedure applied on a pilot scale for LPC production

Chemical characterization of the lentil by-products was also performed to quantify dry matter, protein and fibre content. The dry residue was determined directly by drying the product at 70°C at reduced pressure or, alternatively, at 100°C and atmospheric pressure, for 4 h[13]. The protein content was determined using the Kjeldahl method[14,15]. Fibre content (as total dietary fibre) was determined using an enzymatic assay based on method 985.29 published in the 16th edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC)[16].

Protein extraction was carried out by adapting a procedure previously optimized on a laboratory and pilot scale and patented by SSICA[17] (Fig. 1). After a preliminary phase of sieving to remove inert components, the lentil by-products were ground in a hammer mill to provide a material very similar to commercial lentil flour. This flour was then homogenised through a Fryma colloidal mill and the proteins extracted with phosphate buffer in a mixer. The resulting solid and liquid fractions were separated in a Pieralisi decanter and then using an Alfa-Laval solid-liquid separator. The protein-rich phase was collected and dried in a spray-drying niro atomiser.

The lentil protein concentrate was characterized in terms of dry weight and protein, fibre, starch sugar and ashes content. The methods for determining dry weight, protein content and fibre content were described above. The starch content was determined using a UV method first published by Boehringer Mannheim GmbH, using a commercial kit from R-Biopharm. As for sugars, the sucrose, D-glucose and D-fructose contents were analyzed using a UV method first published by Boehringer Mannheim GmbH, using a commercial kit from R-Biopharm. Ash as determined using a gravimetric method (UNI EN 1135:1997)[18] after calcination of the sample inside a furnace at 525±25°C.

Preparation of LPC-based films: The films were obtained in a similar way to that used by Bamdad *et al.*[10], with some modifications. Thus, a film-forming solution was prepared by dissolving LPC 10 and 3% (w/v) of the plasticiser (sorbitol (SOR), glycerol (GLY) or polyethylene glycol 400 (PEG4 00)) in distilled water. This mixture was then basified to pH 11 (with concentrated NaOH solution) and heated in a water bath at 80°C for 30 min with constant stirring, before being strained through a cheese cloth. The solution was then cast on levelled petri dishes. A constant amount of 2.34 g of total solids was cast onto an area of 144 cm^2 in order to maintain film thickness. Film-forming solutions were dried at 23°C and 45% relative humidity (RH). The free standing films were then peeled off manually.

Film thickness and conditioning: Films were cut into specimens and conditioned in a climatic chamber (Model Climacell 222-Comfort, MMM Group, Germany) at 50% RH and 25°C. Sample thickness was measured at 10 different points using a digital micrometre (Mitutoyo ID-F125, Japan). Ten random locations around each film sample were used for average thickness determination.

Moisture content (MC) and total soluble matter (TSM): The film samples were weighed and dried in an oven at 105°C until constant weight. MC was determined in triplicate for each type of film and calculated as the percentage weight loss relative to the initial weight.

TSM was expressed as the percentage of film dry matter solubilised after immersion in water for 24 h[19]. Dried film pieces were shaken in 50 mL of water at 25±1°C for 24 h, then the undissolved film residues were removed from the water and re-dried to determine solubilised dry matter. Initial dry matter values were obtained from MC measurements for the same film. All analysis were performed in triplicate.

Film solubility represented total soluble matter dissolved in water, mainly including water-soluble proteins and plasticisers.

Water vapour permeability: The WVP measurements were performed in a PERMATRAN-W3/33 (Mocon, Inc., Minneapolis, MN, USA), according to the ASTM E398 standard test method (ASTM)[20]. Sample films were cut into a circle with a diameter of 4 cm and the test area was 5 cm^2. Measurements were carried out in quadruplicate. WVP was calculated[21] as:

$$WVP = \frac{WVTR}{\Delta P}$$

where WVTR is defined as:

$$WVTR = \frac{\text{Change in weight (g)}}{\text{Time (h)} \times \text{test area} (m^2)}$$

and ΔP is the partial pressure difference of the water vapour across the film.

Oxygen transmission rate (OTR): OTR was measured using a PERMEO2 permeabilimeter (Extra Solution, Lucca, Italy) instrument using 50 cm^2 samples of the examined films according to standards ASTM[22] 3985-95 and DIN[23] 53380. Permeability measurements were conducted at 23°C and 5 and 50±3% RH. Three samples were tested for each type of film and average permeability values (expressed as the volume of permeant passing through a film, per unit area and time, normalized for the thickness) are presented.

Mechanical properties: Tensile strength (TS) and elongation at break (E) were determined by performing a tension test using a TAXT2 Texture Analyser (Stable Micro Systems, Surrey, UK) as per the ASTM D882 standard (ASTM)[24]. Film samples with a width of 25.4 mm were obtained using a double guillotine. Prior to the test, the thickness, weight and length of each strip were measured to calculate the section (width×thickness), area (width×length) and gram age (weight×area).

Tension measurements were carried out using a 30 kg load cell at room temperature (23±1°C) and 50% RH. The initial grip separation was set at 50 mm and the cross-head speed was set to 0.8 mm min^{-1}. After strip break-up, the computer recorded a force/distance curve, from which tensile strength (MPa) and relative elongation (%) were calculated. At least 10 replicates were analysed for each film type.

Fourier transform infrared (FTIR) spectroscopy: FTIR spectra of all films were recorded using a Performer Spectra Tech spectrometer with an ATR diamond crystal[21]. A total of 64 scans were performed at 4 cm^{-1} resolution. Measurements were recorded between 400 and 4000 cm^{-1}. The samples were measured at three points to check for film homogeneity and found to yield similar spectra.

Migration tests: Overall migration tests were carried out for LPC-based films as per EC[25] Reg. 1935/2004, for materials and articles intended to come into contact with food and EC[26] Reg. 10/2011, specific for plastic materials, to verify their safety for food contact. Tests were performed under conditions suitable to study contact with fatty foods like cheese as per EU Reg. 10/2011. To that end, the simulants used were 95% ethanol and isooctane as alternatives to oil. The tests in 95% ethanol were performed by dipping the films in simulant at 40°C for 10 days. In the case of isooctane, dipping was carried out at 20°C for 2 days as it is a more aggressive simulant than oil as reported in Reg. 10/2011.

Statistical analysis: Experimental results were expressed as mean±standard deviation and subjected to analysis of variance (One way-ANOVA). Means were compared using Tukey's least significance test at a significance level of $\alpha = 0.05$, using the R programme.

RESULTS

Protein extract characterization: Dehydrated lentil by-products were sampled after the cleaning and selection phases during the lentil transformation process.

All samples were homogeneous, apart from the presence of a few small stones, leaves and above all, sand, all of which contribute to the percentage of inert compounds. Samples mainly consisted of grains of different colours or broken

grains. The results of the determination of percentage inert compounds and the chemical characterization of both the by-products and lentil protein concentrate obtained can be found in Table 1.

The extract was characterised by a fairly high protein content, which was further concentrated with respect to the starting lentil flour (protein content of 25.16%). It can be seen that the water content is around 2-3%, which is typical when using the spray-drying technique[27]. Only traces of fibres and sugars were present, while the starch content was around 20%. Both fibre and starch contents were considerably reduced in comparison to the starting flour, according to the INRAN nutritional composition tables[28] which, for dry lentils, give fibre and starch contents of around 13.8 and 44.8%, respectively.

Film formation: LPC-based films were obtained by solution casting. Three different plasticisers were used at the same concentration in this study in order to determine their influence on film properties. Plasticisers are needed to improve protein process ability and flow ability, with biopolymeric films usually being plasticised using hydroxyl compounds[29].

All films were dark brown and transparent, with a thickness of 85±10 mm (Table 2). They were strong and flexible enough to be handled, except for the film lacking plasticiser, which was very brittle and could not be cut into samples to perform the mechanical analysis and barrier properties. On a macroscopic scale, all films had a uniform appearance and there were no pores on the surface. All these results are summarized in Table 3.

Moisture content and total soluble matter: The use of edible films and coatings as protective layers on food or food packaging requires these materials to be water resistant, especially for high a_w products. As such, solubility is an important property of edible films. The moisture content and total soluble matter values are shown in Table 2. Moisture content ranged between 11 and 25%, being the higher for the film containing glycerol. Total soluble matter values were around 40 % for all films containing plasticiser. Immersion of film samples in water for 24 h only caused small breaks in the films, thus indicating the high stability of the protein network. Only slight differences in solubility were observed when using different plasticisers.

Very similar MC and TSM values were reported by Bamdad *et al.*[10] for lentil, soy and pea protein-based films plasticised with glycerol.

Water vapour permeability: The influence of plasticiser type on the WVP of LPC films is shown in Table 4. The WVP of films prepared with sorbitol was lower than for PEG 400- and glycerol-containing films. Films prepared with glycerol gave the highest WVP values.

Table 1: Chemical characterization of lentil by-products and lentil protein concentrate (g/100 g of dry matter)

Parameters	Inert compounds (%)	Water content (%)	Proteins (%)	Fibres (%)	Starch (%)	Sugars (%)	Ashes (%)
Lentil by-products	3.89±0.09	9.16±0.06	25.16±0.18	8.23±0.41	nd	nd	nd
Lentil protein concentrate	nd	2.90±0.74	62.87±0.36	1.67±0.10	20.12±1.04	traces	8.44±0.14

Mean±SD of 3 replicates, nd: Not determined

Table 2: Thickness, moisture content and total soluble matter in LPC-based films

Samples	Thickness (mm)	Moisture content (%)	Total soluble matter (%)
LPC-0% P	83.3±7.7[a]*	12.2±0.6[a]	7.1±1.8[a]
LPC-30% SOR	81.9±10.6[a]	11.5±0.4[a]	39.8±1.3[b]
LPC-30% GLY	79.6±7.8[a]	25.4±0.3[b]	36.1±1.2[c]
LPC-30% PEG 400	94.1±10.9[a]	11.3±0.5[a]	41.7±0.9[b]

*Means in a column bearing the same letter are not significantly different ($p<0.05$), LPC-0% P: Lentil protein film without plasticiser, LPC-30% SOR: Lentil protein film with 30% sorbitol, LPC-30% GLY: Lentil protein film with 30% glycerol, LPC-30% PEG 400: Lentil protein film with 30% polyethylene glycol 400

Table 3: Visual appreciations of lentin protein concentrated films

Samples	Colour	Transparency	Visual appearance	Handling
LPC-0% P	Dark brown	Yes	Uniform. No pores	Brittle
LPC-30% SOR	Dark brown	Yes	Uniform. No pores	Flexible
LPC-30% GLY	Dark brown	Yes	Uniform. No pores	Flexible
LPC-30% PEG 400	Dark brown	Yes	Uniform. No pores	Flexible

LPC-0% P: Lentil protein film without plasticiser, LPC-30% SOR: Lentil protein film with 30% sorbitol, LPC-30% GLY: Lentil protein film with 30% glycerol, LPC-30% PEG 400: Lentil protein film with 30% polyethylene glycol 400

Table 4: Mechanical and barrier properties of LPC-based films

Films	WVP (g m^{-2} day)	OPR (cm^3 m^{-2} day) 5% RH	OPR (cm^3 m^{-2} day) 50% RH	TS (Mpa)	E (%)
LPC-30% SOR	14.13±2.12[a]**	nd*	0.62±0.43[a]	11.04±0.59[a]	42.22±11.29[a]
LPC-30% GLY	133.68±7.54[b]	0.88±0.16[a]	37.96±16.63[b]	5.59±0.49[b]	145.85±14.69[b]
LPC-30% PEG 400	74.35±5.44[c]	14.39±0.61[b]	58.46±9.63[b]	7.60±0.66[c]	121.92±7.43[c]

*nd: Not determined as films tended to fracture, ** Values for each film are the mean±standard deviation, values with different superscript letters in a given column differ significantly ($p<0.05$) according to the Tukey test, LPC-0% P: Lentil protein film without plasticiser, LPC-30% SOR: Lentil protein film with 30% sorbitol, LPC-30% GLY: Lentil protein film with 30% glycerol, LPC-30% PEG 400: Lentil protein film with 30% polyethylene glycol 400

Table 5: Results of overall migration tests performed on LPC-based films

Simulant	Samples	Overall migration (mg dm^{-2})
95% Ethanol 40°C, 10 days, dipping	LPC-30% SOR	65.80±2.63[a]*
	LPC-30% GLY	20.35±1.57[b]
	LPC-30% PEG 400	61.03±0.71[a]
Isooctane 20°C, 2 days, dipping	LPC-30% SOR	0.10±0.10[a]
	LPC-30% GLY	0.19±0.07[a]
	LPC-30% PEG 400	2.93±1.01[b]

Values were obtained by applying a fat reduction factor of 3 (UE Reg. 10/11), *Means in a column bearing the same letter are not significantly different ($p<0.05$), LPC-0% P: Lentil protein film without plasticiser, LPC-30% SOR: Lentil protein film with 30% sorbitol, LPC-30% GLY: Lentil protein film with 30% glycerol, LPC-30% PEG 400: Lentil protein film with 30% polyethylene glycol 400

Because of the differences in amino acid composition, molecular properties and the film network formed, the barrier behaviour cannot be generalised. WVP values are similar to but lower than, those reported by other authors for LPC/Gly films[10] and soy protein-based films with different plasticisers[30].

Oxygen permeability: As regards the oxygen barrier properties, the differences between the values obtained (Table 4) may also be due to the different composition, shape and size of the plasticisers as also suggested by Sothornvit and Krochta[31]. Films containing sorbitol presented a significantly lower oxygen barrier than films prepared with sorbitol or PEG 400.

Mechanical properties: Mechanical properties were evaluated by measuring the tensile strength (TS) and elongation at break (E) from the stress-strain curves for each film. As can be seen from Table 4, the TS values increased with decreasing plasticiser hydrophilicity, with the E values showing the opposite behaviour. Films containing sorbitol were the least deformable materials.

Fourier transform infrared spectroscopy: The ATR FTIR spectra of films are shown in Fig. 2. Because the storage humidity affects the physical and mechanical properties of protein-based films, specimens were stored at constant temperature and humidity before being examined.

The spectra of the LPC films show relevant peaks at around 1630, 1535 and 1240 cm^{-1}, which are characteristic of amide I C = O stretching), amide II (N-H bending) and amide III vibrations (C-N and N-N stretching), respectively. The absorption at around 3275 cm^{-1} exhibits a very broad band, attributed to the free and associated hydroxyl groups, which are able to form hydrogen bonds with the carbonyl group of the peptide linkage in the protein. The C-H stretching vibration is observed at 2922 cm^{-1}.

Migration tests: The results obtained for overall migration tests performed on LPI-based films in 95% ethanol and isooctane are reported in Table 5.

The calculated standard deviations for isooctane are quite high. The overall migration values determined in isooctane after 2 days at 20°C all comply with the legal limit of 10 mg dm^{-1}. The film containing PEG 400 presented higher migration values in isooctane than those containing glycerol or sorbitol, the values of which were similar. In 95% ethanol, after 10 days at 40°C, all films analyzed exhibited values exceeding the legal limit. In particular, sorbitol and PEG 400 films exhibited significantly higher values than the glycerol film, probably due to the stronger binding of this plasticiser to the protein network. In all cases, after the end of the test, the tendency of the films to crush was observed, with the drying and crushing effects being very strong in 95% ethanol. This phenomenon probably depends on the test conditions adopted to comply with the EU regulation(dipping), which also exposes the edges of the sample to the simulant and the nature of the biomaterials, which are not suitable for contact with this type of simulant.

DISCUSSION

This study shows that protein films can be successfully obtained from lentil by-products with the

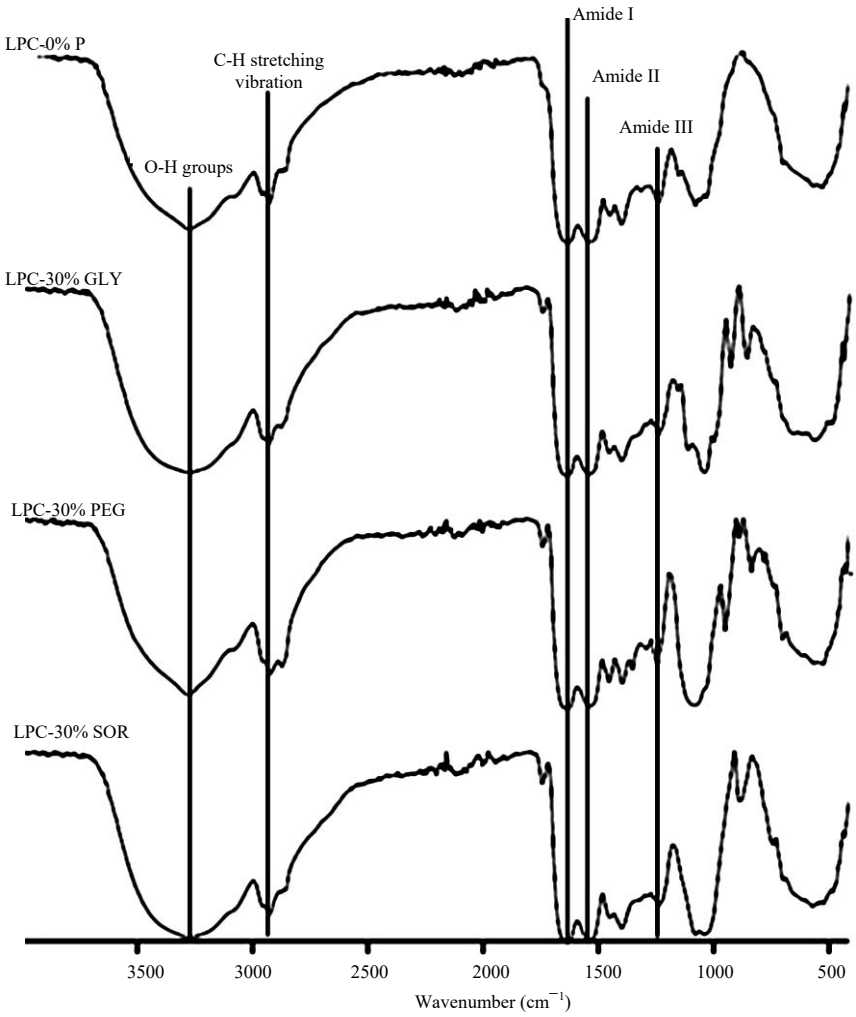

Fig. 2: Infrared spectra of LPC-based films containing different plasticisers

mechanical and barrier properties being significantly affected by the type of plasticiser.

Plasticiser was found to have no effect on total soluble matter, although the moisture content increased in the case of glycerol. Films plasticised with sorbitol exhibited significantly lower water vapour and oxygen permeability and were also stronger and less flexible. Migration tests complied with current legislation in the case of isooctane but exceeded the legal limits for 95% ethanol.

The results presented in this paper show that it is possible to use industrial by-products in the development of environmentally friendly packaging systems. Therefore, the utilization of these products could represent a great opportunity to produce biodegradable packaging systems.

The protein content of dehydrated lentil by-products is very similar to the percentage protein reported for dry lentils in the INRAN nutritional composition tables[28] (22.7%) and for lentil pulse grains in the scientific literature[32,33] (25.80%). In contrast, the fibre percentage, in terms of insoluble components, is very different (8.23 vs. 12.91% for dry lentils and 30.5% for lentil pulse grains).

The differences observed between the composition of dehydrated lentil by-products and that reported in INRAN tables[28] are probably due to the fact that the sample analyzed consists of by-products from the industrial process rather than a product destined for the market.

With regard to the protein extract, in light of the results achieved, it can be seen that application of the pilot extraction method to lentil by-products was successful, with this method proving effectives in terms of protein concentration and fibre and starch removal.

The increased solubility of the films containing plasticiser can be attributed to the weaker protein-protein interactions in the films and to the solubility of the plasticiser itself.

Plasticisation with glycerol allowed the production of films with a slightly lower TSM than plasticisation with

PEG 400 and sorbitol. A similar behaviour has been found for other protein-based films[12,34]. As noted by these authors, low molecular weight plasticisers, such as glycerol, probably incorporate into the protein chains more easily than their larger counterparts. As such, glycerol may be more strongly bound to the macromolecules in the protein network than sorbitol and polyethylene glycol.

In protein films, the dry matter solubilised in water likely comprises small molecules, such as plasticisers and small polypeptides[10].

The difference in hydrophilicity of the plasticisers is reflected in the MC of the films. Indeed as expected, films plasticised with glycerol exhibit a higher MC than those containing polyethylene glycol and sorbitol.

Both the type and amount of plasticiser strongly affect the physical properties of protein films[35] and the polarity of the plasticiser molecule also influences the film properties. Compared with polar plasticisers, plasticisers with a low polarity do not compete for hydrogen bonding sites and, therefore are less effective at disrupting intermolecular interactions in a protein matrix. Thus, sorbitol, which is less polar than PEG400 and glycerol, could induce lower oxygen and water vapour permeability.

The better water vapour barrier properties of edible films containing sorbitol as plasticiser compared with those containing glycerol have been reported by several authors. Thus, Wan *et al*.[34] observed that soy protein films plasticised with sorbitol exhibited an approximately four times lower WVP than glycerol-plasticised films. Kowalczyk and Baraniak[12] also reported the same behaviour for pea protein isolate films. These differences in WVP values could be due to the different protein type and concentration and to the different plasticiser content in the films.

Oxygen permeability is also affected by the plasticiser. In this regard, PEG 400 contains O atoms alternating with 2 carbon atoms along the molecule backbone, which could be more accessible to interact with protein chains via H-bonds than the OH groups found on adjacent C atoms in both sorbitol and glycerol. This may result in a higher free volume in the PEG 400 film matrix that could allow for easier oxygen transmission. Saremnezhad *et al*.[36] reported similar values for soy protein/GLY and pea protein/GLY films. The TS value reported previously for LPC[10] was similar to that obtained in this study, although elongation at break (E) was significantly lower, which can be attributed to the lower protein content in the film and to the different type and concentration of plasticiser used.

It is well known that plasticisers modify the functional properties of films by increasing barrier properties, extensibility and flexibility and by decreasing cohesion, elasticity and rigidity[37]. Similarly, in addition to barrier properties, the polarity and hydrophilicity of plasticisers also affect the mechanical properties. The hydroxyl groups of glycerol may interact more readily with amino and acid groups in the protein, thereby decreasing inter-and intramolecular interactions between protein chains and improving the ability of protein macromolecules to move, thus resulting in a higher flexibility of these films compared with those containing PEG 400 and sorbitol[38]. In addition, the higher moisture content of glycerol films could also influence their mechanical properties as water also acts as a plasticiser.

The spectroscopic data are in agreement with those reported previously by other authors for other legume-based protein films[27,39] and show that no changes occur in the characteristic peaks of protein and plasticisers, thus indicating that plasticisers do not react with the protein via covalent linkages.

The migration results suggest that these protein films could be suitable for non-aqueous food, although more research is necessary in order to reduce the migration in polar simulants and increase the applicability of these films.

The global concern about the impact of synthetic packaging on the natural environment is manifested in the growing interest in use of bio-based packaging. The application of edible films and coatings is one of the innovations aimed to solve this issue, although the results of many studies indicate that properties of protein films are still not comparable to those of synthetic films. Thus, more research is needed to improve the mechanical and water barrier properties of these films. The scaling up of the manufacturing process of these films to a commercial level is also a challenge.

CONCLUSION

The results of this study show that it is possible to prepare homogeneous and flexible films, containing different plasticisers, from lentil proteins extracted from by-products and that both mechanical and barrier properties are significantly affected by the type of plasticiser used. Films containing sorbitol are more resistant and present better barrier properties (lower WVP and OPR).The application of these protein-based coatings or films to plastic- or cellulose-based packaging materials may allow better control of the barrier and mechanical properties of the packaging system as a whole. Finally, although migration in isooctane is below the legal limits, more research is necessary in order to reduce the migration in other simulants and increase the applicability of these films.

SIGNIFICANCE STATEMENT

This study shows that it is possible to develop environmentally friendly packaging films based on proteins extracted from industrial by-products. Two benefits are likely to result from this development: Economic (waste management usually represents a cost for companies) and ecological (resource saving will allow the environmental impact to be reduced).

There is great potential for the effective utilisation of food processing by-products in the field of food packaging applications that would be more cost-effective, efficient and sustainable.

In the perspective of achieving a compostable or biodegradable option for future packaging, more research is needed to improve the insufficient water resistance and inherent colour of these films as well as work on scaling the processes up.

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