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FUNCTIONAL PROPERTIES AND ENVIRONMENTAL ASSESSMENT OF SOY PROTEIN MATERIALS

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ABSTRACT

Soy protein based films plasticized with sorbitol were prepared by casting at pH 7 and 10. The best mechanical properties, both tensile strength and elongation at break, were obtained at pH 10. Poly(ethylene glycol) was also employed in order to analyze the effect of a higher molecular weight plasticizer on mechanical properties. Results were related to changes in physical and chemical properties. The changes observed by infrared spectroscopy and in total soluble matter values indicated different degree of interaction between protein and plasticizer, depending on the type of plasticizer employed. The effect of ageing on mechanical properties was also investigated after 3 months under storage conditions. Furthermore, the environmental assessment of the new biofilms based on soy protein was carried out and compared with conventional PP films, which are commercial food packages nowadays.

Keywords: *food packaging, soy protein, environmental impact.*

INTRODUCTION

The use of renewable resources to produce biodegradable materials with good properties is an area of work that is reaching big interest in the last years [1-4]. These materials could substitute the ones derived from petroleum and contribute to reduce the environmental problems that plastics cause when their useful life is finished. The materials based on proteins are provided with the additional value of being abundant, having relatively low costs and interesting functional properties [5-7]. In the case of biodegradable materials based on proteins used in food industry, proteins might be even edible [8-10], adding nutritional value to food.

Although proteins have the ecological advantage of biodegradability [11, 12], they have some disadvantages like brittleness, therefore addition of plasticizers is necessary to obtain materials with improved mechanical properties. Glycerol has been the most used plasticizer for polysaccharides like starch [13-15], as well as for proteins like whey protein [16] and corn protein [17], but larger molecules can also be employed [18]. Moreover, the processing methods [19, 20] and conditions, such as solution pH, can produce materials with different properties due to the conformational changes that take place in the protein [21-23]. The exhibition of the polar groups in the protein can allow the interaction with the plasticizers employed and the desired improvement of mechanical properties.

Apart from mechanical properties, it is extremely important to consider environmental issues in the product design. The function of food packaging product consists of protecting food against external environment in order to maintain food quality during storage, extending its shelf life, as well as to make food transport and distribution easier. However, as food packaging has short lifetime due to the fact that it is usually employed as short-term or one-time use, it is necessary to assess the environmental impact of food packaging systems in order to be able to design green packaging based on renewable and biodegradable sources [24].

The aim of this work is to analyze the effect of two different plasticizers, sorbitol and poly(ethylene glycol), in order to improve mechanical properties of soy protein-based films. Results were related to physical and chemical changes depending on the film preparation conditions and on the type of plasticizer employed. The effect of ageing under storage conditions on the mechanical properties was also analyzed. Moreover, the environmental impact of these new biodegradable films was compared with non-biodegradable materials

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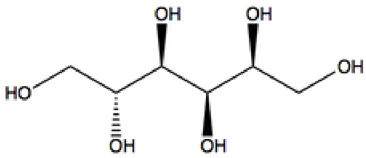
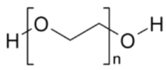
currently used. The study of the environmental burden during the material life cycle was carried out identifying the significant stages and their major impacts.

MATERIALS AND METHODS

Materials

Soy protein isolate (SPI), PROFAM 974, with 90% protein on a dry basis was supplied by Lactotecnia S.L (Barcelona, Spain). SPI has 5% of moisture, 4% of fat and 5% of ash. It has acid character and the isoelectric point is 4.6 due to the high content of glutamic acid (Glu, 19.2%) and aspartic acid (Asp, 11.5%). Sorbitol (SOR) and poly(ethylene glycol) (PEG) with molecular weight of 400 were food grade reagents obtained from Panreac. Molecular structures can be seen in Table 1.

Table 1: Chemical structure of plasticizers used in this study

Name	Molecular structure
Sorbitol	
PEG	

Film preparation

5 g of SPI was dispersed in 100 mL distilled water. Dispersions were heated at 80 ºC for 30 min and stirred at 150 rpm. Then, plasticizer was added and dispersions were maintained at 80 ºC for other 30 min under stirring at 150 rpm. The pH of the solutions was appropriately adjusted with NaOH (0.1M) to the desired value. The notation employed to name the films is SPI30SOR07 for soy protein films plasticized with 30% wt sorbitol and prepared at pH 7, SPI30SOR10 for soy protein films plasticized with 30% wt sorbitol and prepared at pH 10, and SPI30PEG010 for soy protein films plasticized with 30% wt PEG and prepared at pH 10.

Film thickness

Film thickness was measured to the nearest 0.001 mm with a hand-held digimatic micrometer (QuantuMike Mitutoyo). The values obtained for each sample at five different locations were averaged.

Contact angle determination

A contact angle meter (model Oca20, dataphysics instruments) was used to perform contact angle measurements on the surface of the films. A film sample (20 mm x 80 mm) was put on a movable sample stage and levelled horizontally; then a drop of about 3 µL of distilled water was placed on the surface of the film using a microsyringe. The contact angle was measured in a conditioned room by recording contact angle values. Image analyses were carried out using SCA20 software.

Fourier Transform Infrared (FTIR) spectroscopy

FTIR spectra of the films were carried out on a Nicolet Nexus FTIR spectrometer using Attenuated Total Reflectance (ATR) Golden Gate (Specac). A total of 32 scans were performed at 4 cm^{-1} resolution. The measurements were recorded between 4000 and 700 cm^{-1}.

Moisture content (MC) and total soluble matter (TSM)

TSM was expressed as the percentage of film dry matter solubilised after 24 h immersion in distilled water. Three specimens of each film were weighed (m_i) and subsequently dried in an air-circulating oven at 105

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ºC for 24 h. After this time, the films were reweighed (m_d) to determine MC values. Afterwards, samples were immersed in 30 mL distilled water in the presence of sodium azide (0.02%) in order to prevent the microbial growth. The flaks were stored in environmental chamber at 25 ºC for 24 h with occasional gentle stirring. After this time, specimens were dried in an air-circulating oven at 105 ºC for 24 h and weighed (m_f).

MC and TSM values were calculated as:

$$MC\ (\%) = \frac{m_i - m_d}{m_i} 100 \quad (1)$$

$$TSM\ (\%) = \frac{m_d - m_f}{m_d} 100 \quad (2)$$

Mechanical properties

An electromechanical testing system (MTS Insight 10) was used to determine mechanical properties. Tensile strength and elongation at break were determined according to ASTM D1708-93. Films were conditioned in a controlled environment chamber (Dycometal CCK81) at 25 ºC and 50% RH for 48 h before testing. Five replicates were tested for each composition.

Environmental impact

The software used in this study to make the environmental assessment was SimaPro 7.1 (Preconsultants, The Netherlands). On one hand, the data relating to PP packaging films were obtained from Ecoinvent v2.0 database, developed by Swiss Centre of Life Cycle Inventories, available in SimaPro 7.1. On the other hand, the inventory analysis of the biodegradable soy protein film was carried out taking into account the materials used in the laboratory and the energy consumption regarding the manufacture step, although the database of the software was also used for biofilms relating to the raw materials and their conversion processes. The functional unit considered in this study was 1 m^2 of packaging film.

RESULTS AND DISCUSSIONS

Appearance of the films

Films prepared with plasticizer content lower than 30% wt were extremely brittle, as it can be seen in Fig. 1a, so it was no possible to cut samples for mechanical analysis. Nevertheless, all the films prepared with 30% plasticizer were homogeneous, as it is shown in Fig. 1b. At macroscopic scale, films had uniform appearance and there was no pore on the surface. All films were transparent with a yellowish hue, typical for SPI-based films, and had similar thickness, around 150 μm.

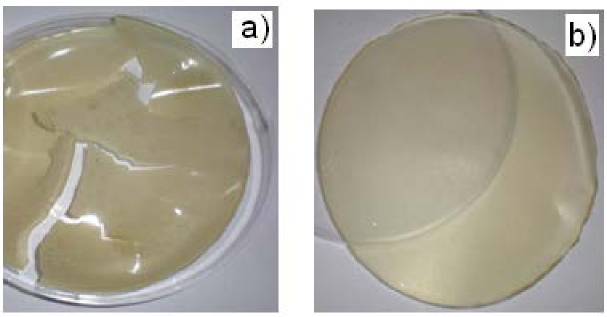

Fig. 1: Visual aspect of a) SPI20SOR10 and b) SPI30SOR10 films.

Water contact angle images are good indicators of the degree of the hydrophobic character of films. The contact angle is higher when hydrophilic character is lower, thus the final state of the water drop on the film surface can be taken as an indication of surface wet ability. The images of contact angles were taken for the SPI-based films plasticized with sorbitol and PEG and are shown in Fig. 2a and b, respectively. It is worth to note that films plasticized with sorbitol are more hydrophilic than the ones plasticized with PEG, as it was expected due to the six hydroxyl groups of the sorbitol molecule (see Table 1).

Fig. 2: Contact angle images for a) SPI30SOR10 and b) SPI30PEG10 films.

FTIR spectroscopy

The FTIR spectra of the SPI-based films prepared in this study are shown in Fig. 3. The main absorption peaks for SPI are related to C=O stretching at 1633 cm^{-1} (amide I), N–H bending at 1514 cm^{-1} (amide II), and C–N stretching (amide III) at 1232 cm^{-1}. The broad band observed at 3271 cm^{-1} is attributable to O–H and N–H groups. The bands in the range from 1200 to 800 cm^{-1} correspond to C-C and C-O bonds in the plasticizers.

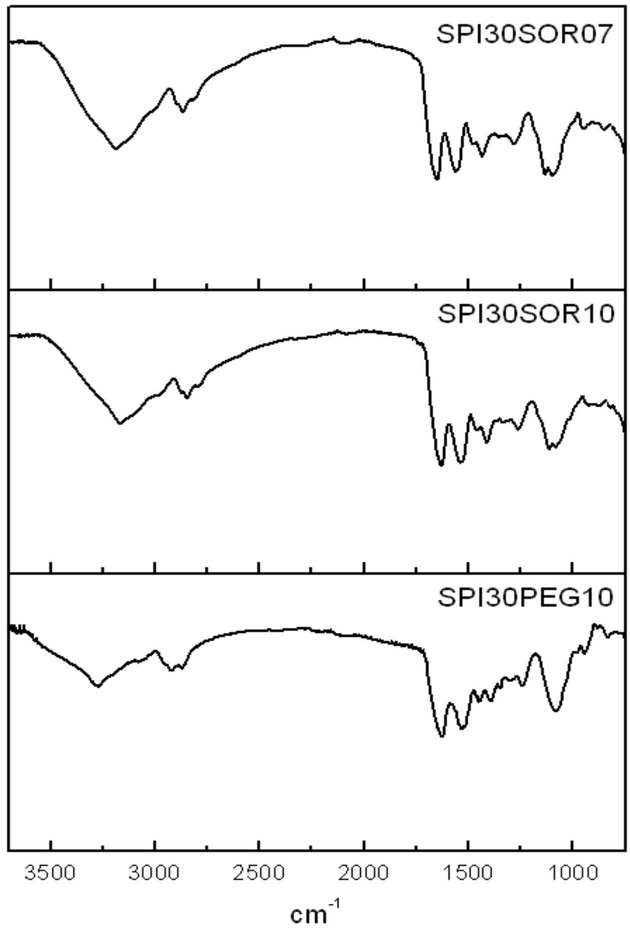

Fig. 3: FTIR spectra of the SPI-based films prepared in this study.

For SPI/sorbitol films prepared at different pHs, it can be seen that the difference in the relative intensity between the bands corresponding to the amide I and II become smaller when the pH was increased, which could indicate that N-H groups in SPI and O-H groups in sorbitol were certainly able to form hydrogen bonding at basic pHs when the protein unfolding is optimal. On the other hand, the intensity of the band corresponding to amide I is higher than the one related to amide II for SPI/PEG films, which could indicate lower interaction between polar groups of protein and PEG, probably due to the higher size of the plasticizer molecule which would not be able to get into the protein chains.

MC and TSM values

Packaging films should maintain moisture levels within the packaged product. Therefore, the knowledge of water solubility and moisture content of the film is very important for food packaging applications. Table 2 shows MC and TSM values for the films prepared with the two plasticizers used in this study. MC values were lower for the films plasticized with PEG. These results are in good agreement with contact angle measurements that shown that SPI/PEG films are more hydrophobic than SPI/sorbitol films.

On the other hand, TSM values decreased for SPI/sorbitol films when the pH was increased, indicating a higher degree of interaction between SPI and sorbitol at pH 10, as it was shown by FTIR results. In the case of SPI/PEG films prepared at pH 10, TSM values were higher than the ones measured for SPI/sorbitol at the same pH. These results could be related to the higher size of the plasticizer, which prevent the plasticizer from getting into the protein chains to interact with polar groups. Nevertheless, in all the cases analyzed in this study, TSM values were high, which would indicate that the plasticizers used did not form covalent linkages with SPI but hydrogen bonds.

Table 2: Thickness, MC and TSM values for the SPI-based films prepared in this study

Film	Plasticizer	pH	Thickness [mm]	MC [%]	TSM [%]
SPI30SOR07	30% wt sorbitol	7	0.147±0.006	6.26±0.18	92.70±5.56
SPI30SOR10	30% wt sorbitol	10	0.149±0.012	6.95±0.33	83.67±2.61
SPI30PEG10	30% wt PEG	10	0.152±0.009	4.72±0.42	86.60±5.50

Mechanical properties

Fig. 4 and 5 show mechanical behaviour for the SPI-based films. It can be seen that both elongation at break and tensile strength were higher when the pH of the initial dispersion was 10 due to a higher unfolding of the protein, which allowed the exposition of the polar groups to be able to interact with the plasticizer molecules, as it was shown in previous works [25].

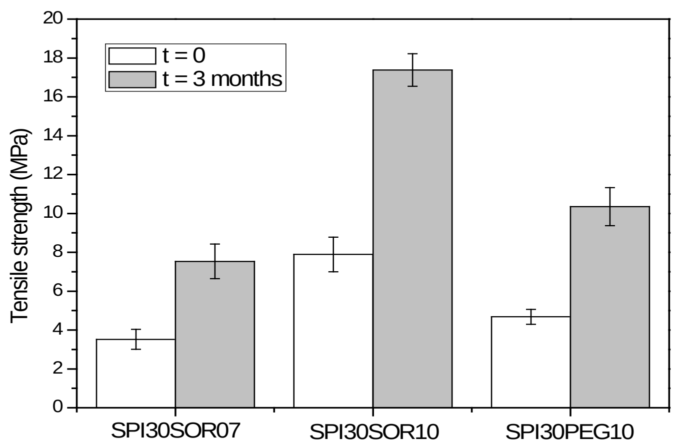

Fig. 4: Tensile strength of the SPI-based films prepared in this study.

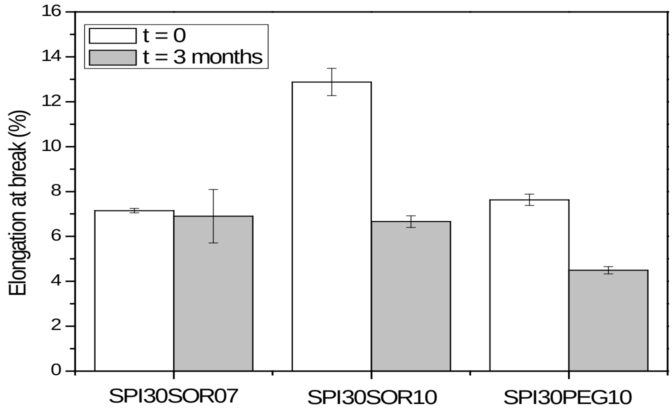

Fig. 5: Elongation at break of the SPI-based films prepared in this study.

Both sorbitol and PEG were compatible with soy protein and able to get into the protein chains to establish hydrogen bonds with the polar groups. As a result, the protein–protein interactions decreased owing to the increased plasticizer–protein interactions. However, the degree of interaction is higher with the small molecules of sorbitol, as it was also shown by TSM results. The hydroxyl groups of plasticizers are expected to be bonded by hydrogen bridges with protein, increasing the free volume, thus increasing the chain mobility and improving mechanical properties.

Finally, the changes observed after 3 months of storage are more significant for the films prepared at pH 10 due to the nature of the new interactions formed between the protein and the plasticizer, which are not covalent, as it was shown above by FTIR and TSM results, and did not prevent the migration of the plasticizer from the film. In the case of sorbitol, the changes on mechanical properties were sharper due to the small size of the molecule.

Environmental impact

The Eco-indicator 99 was the method used to make an environmental assessment of the new biofilms based on soy protein and compare their environmental load with conventional PP films, which are used nowadays for food packaging purposes. The impact categories considered by this model are carcinogens, respiratory organics, respiratory inorganics, climate change, radiation, ozone layer, ecotoxicity, acidification-eutrophication, land use, minerals, and fossil fuels. In order to simplify the system and delimit the research boundary, four main stages are considered: resource extraction, film manufacture, usage and waste disposal. The food packaging film based on soy protein obtained in this study was biodegradable, so that the waste disposal chosen was composting. Different waste scenarios were studied for conventional PP films: incineration, recycling, and landfill. In order to consider the relative importance of the studied impact categories, normalised impact values are represented in Table 3.

Table 3: Normalised environmental impacts of PP films with different disposal scenarios and soy protein biofilms

Impact Category	PP landfill	PP recycling	PP incineration	SPI composting
Carcinogens	$4.50 \cdot 10^{-6}$	$1.25 \cdot 10^{-7}$	$3.81 \cdot 10^{-7}$	$3.68 \cdot 10^{-6}$
Respiratory organics	$1.12 \cdot 10^{-8}$	$1.92 \cdot 10^{-9}$	$1.12 \cdot 10^{-8}$	$2.87 \cdot 10^{-8}$
Respiratory inorganics	$2.08 \cdot 10^{-6}$	$1.34 \cdot 10^{-6}$	$2.14 \cdot 10^{-6}$	$1.92 \cdot 10^{-5}$
Climate change	$1.10 \cdot 10^{-6}$	$4.36 \cdot 10^{-7}$	$2.17 \cdot 10^{-6}$	$2.03 \cdot 10^{-6}$
Radiation	$1.32 \cdot 10^{-8}$	$2.12 \cdot 10^{-8}$	$1.32 \cdot 10^{-8}$	$1.09 \cdot 10^{-7}$
Ozone layer	$7.32 \cdot 10^{-11}$	$1.02 \cdot 10^{-10}$	$6.87 \cdot 10^{-11}$	$6.14 \cdot 10^{-10}$
Ecotoxicity	$2.19 \cdot 10^{-7}$	$8.34 \cdot 10^{-8}$	$1.05 \cdot 10^{-7}$	$3.09 \cdot 10^{-7}$
Acidification/Eutrophication	$1.94 \cdot 10^{-7}$	$1.13 \cdot 10^{-7}$	$2.05 \cdot 10^{-7}$	$9.95 \cdot 10^{-7}$
Land use	$1.69 \cdot 10^{-7}$	$1.73 \cdot 10^{-7}$	$1.59 \cdot 10^{-7}$	$7.30 \cdot 10^{-5}$
Minerals	$5.61 \cdot 10^{-8}$	$6.34 \cdot 10^{-8}$	$5.71 \cdot 10^{-8}$	$1.94 \cdot 10^{-7}$
Fossil fuels	$3.84 \cdot 10^{-5}$	$7.33 \cdot 10^{-6}$	$3.84 \cdot 10^{-5}$	$1.65 \cdot 10^{-5}$

PP films caused the main environmental damages in respiratory inorganics, climate change and fossil fuels. The main process that contributed to the high value of these impact categories was the raw material extraction until its delivery at plant. In the case of recycling as waste scenario, the values obtained in the majority of the impact categories were lower. On the other hand, the most critical process for soy protein films was cultivation of soybeans, which takes into account the use of diesel, machines, fertilizers, and pesticides. In addition, the provision of the land, its transformation, and the emissions from the machinery used represent the main source of impact.

CONCLUSIONS

Mechanical properties of SPI-based films have been improved by the addition of sorbitol at pH 10, showing an increase in both tensile strength and elongation at break in comparison with the films prepared at pH 7. The differences in the protein-plasticizer interactions were analyzed by FTIR that showed changes in the intensity of bands related to amide I and II. Protein-plasticizer interactions were also related to the decrease of total soluble matter values carried out by immersion in water testing. However, it must be said that mechanical properties changed after 3 months of storage, which is a factor to take into account for packaging purposes. According to the environmental assessment, the improvement of agricultural practices for soy production would be convenient in order to be able to reduce the environmental burden associated to the films based on soy protein.

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