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New Thermo-Molded Biodegradable Films Based on Sunflower Protein Isolate: Aging and Physical Properties

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Summary : Smooth black films are obtained. Films plasticized with glycerol have the least stress at break, but show the highest strain at break (glycerol 33%: 6.7 MPa, 150%). The ethylene glycol family shows an increase in stress at break when the aliphatic chain length increases, while strain at break decreases. Lastly, a rigidification phenomenon of films occurs with the two plasticizers 1,2-propanediol and ethylene glycol, which can be attributed to a loss of plasticizer (followed by HPLC). The triethylene glycol produces the most resistant films, with a great elasticity (TEG 33%: 9.5 Mpa, 100%). Moreover, the films produced with this plasticizer keep their properties during time, as good as films produced with glycerol. The water vapor permeabilities of all those films are low (in the region of 10^{-12} $g.m^{-1}.s^{-1}.Pa^{-1}$); even if contact angle measurements carried out with water show the hydrophilic character of the films surface. This last result is in agreement with the water uptake tests which show a loss of plasticizer and an absorption of water (in the region of 100% of the dry matter of film) ; without considering the plasticizer nature, and this in spite of a nearly total insolubility of the crosslinked proteic network.

Keywords: aging; film; polyhydric alcohols; sunflower protein; thermo-moulding

Introduction

For about the last ten years, research interest into the use of renewable materials as petroleum alternative for plastic packaging has increased a lot. There is also a need to develop ecological polymers in order to make a better use of our natural environment. Because of their complex chemical structure which allows for the formation of low solubility plastics contrary to those obtained with polysaccharides, proteins are interesting candidates for the formation of biodegradable films. Furthermore, proteins are abundant, renewable, and inexpensive natural raw materials.

Investigations on the film-forming potential of different proteins have mainly focused on commercially available proteins like soy protein[1-6] and whey protein[7]. On the other hand,

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sunflower oil cake is an inexpensive source of proteins and it has been recently demonstrated that such proteins can be alkali extracted to yield an isolate composed mainly of globulin and albumin[8]. Because their most common setting process, casting, is not easily industrializable, the present study focused on thermo-mechanical techniques. In addition, the combined effects of pressure and temperature allow for the formation of stronger interactions than with casting.

It was therefore the objective of the present study to use sunflower proteins as raw materials for the preparation of thermo-moulded biodegradable films.

Because plastics made from protein alone are stiff and brittle, various plasticizers were tested and their influence on some physical properties of sunflower protein-based films were examined.

Materials and Methods

Reagents

The sunflower protein isolate was extracted from sunflower oil cake by an alkaline method on semi-industrial scale[8]. We obtained an isolate containing 85% of proteins.

Reagent grade glycerol, ethylene glycol (EG), diethylene glycol (DEG), triethylene glycol (TEG), tetraethylene glycol (TEEG), polyethylene glycols (PEG 400, 1000, 3000), propylene glycol (PG) and polypropylene glycols (PPG 400, 1000, 3000) were purchased from Aldrich (St Quentin Fallavier, France). They were used without further treatment.

Film preparation

The protein isolate and the selected polyhydric alcohol were mixed mechanically in order to obtain a homogeneous powder. 10 g of dry sunflower protein isolate (ISFP) were used for each film. Various plasticizer to protein ratios were used, with the aim of obtaining a supple, smooth, homogeneous and perfectly coherent film. This mixture was then subjected to thermomolding at 150°C±3°C for 4 minutes (3 min preheating ; 30 s at 100 MPa ; 30 s at 200 MPa) between two Teflon®-coated aluminum plates, using a heated hydraulic press (model OA274, Pinette Emidecau Industries, Chalon sur Saône, France). A 0.25 mm aluminum frame was placed between the two plates to mark the edges of the film and to control the thickness. We obtained smooth black films.

Film conditioning

Before testing, film specimens were removed from the mold, weighed and stored in a climate-controlled room at 25°C and 60% relative humidity (R.H.), as described in the European norm NF EN ISO 291[9]. The first mechanical tests were carried out 48 hours after the beginning of storage.

Mechanical properties

A TA-XT2 texture analyzer (RHEO Stable Micro Systems, London, UK) was used to assess the mechanical properties of the films. The test samples were 65 mm long and 5 mm wide. Their thickness was measured at five points with a digital micrometer (model IDC-112B, Mitutoya Corp., Tokyo, Japan) and the mean value was recorded. Stress-strain measurements were carried out to determine tensile strength (σ_{max}) and elongation (ε_{max}) at break value. A speed of 1 mm/s was used, with an initial grip separation of 55 mm, as described in norms ISO 527-2[10] and ISO 527-1[11].

Water uptake and solubility

Samples (2 cm diameter) were cut out of the film, weighed and immersed in demineralized water for 24 hours at 25°C. The surfaces of the samples were then dried mechanically and the samples were weighed in a Petri dish to minimize water exchange with the atmosphere. The samples were then dried for 24 hours in an oven at 105°C and weighed again. The difference between the sample mass after immersion and the initial mass of the dried sample was used to calculate water uptake. The difference between the initial mass of the dried sample (dry matter) and the mass after immersion and drying gave the quantity of soluble matter in the film.

Surface hydrophilicity

Surface hydrophilicity was assessed by contact angle measurements (DigiDrop, GBX, Romans-sur-Isère, France). A 4 µl drop of demineralized water was placed on the surface of the film with an automatic piston syringe and photographed. An image analyzer was used to measure the angle formed between the base, composed of the surface of the film in contact with the drop of water, and the tangent to the drop of water. The mean contact angle for the surface of each film was calculated from five measurements on the film.

Water vapor permeability

The water vapor permeability (WVP) of the films was determined as described in norm ASTM E96-95[12]. Film samples (7 cm diameter disks that had been conditioned for 48 h) were fixed above aluminum cups containing 15 g of calcium chloride. The whole device was weighed and then placed in a climatically controlled chamber (25°C, 60°C R.H.). The cups were then weighed at regular time intervals and a linear relationship between the quantity of water transferred per unit air and time was obtained. When corrected for thickness, the slope of the line gave the water vapor permeability. Five independent determinations were carried out for each quantity and each type of plasticizer. The WVP value of each film was the mean of these five values.

Scanning electron microscopy

The observations were carried out on a scanning electron microscope LEO 435VP (Cambridge, England). At the end of the molding process, the samples were cooled down (cooling rate: 30°C/min) and then dried at 60°C for 48 h in a vacuum dessicator containing phosphorus pentaoxide (P_2O_5), before being metallized and observed.

Plasticizer content

The plasticizer content of the films was measured by gas liquid chromatography (Glc) with a Hewlett Packard 5890 Series II chromatograph equipped with a WCOT fused silica Varian column (stationary phase = CP – FFAP CB for free fatty acid) (25m × 0,32mm × 0,30µm), an injector functioning in splitless mode (220°C), a flame ionization detector (250°C) and an helium supply (vector gas ; pressure=50 kPa).

The used temperature cycle consisted of one-minute isotherm at 120°C, followed by a temperature gradient with increases of 8°C/minute to 240°C (5mins). The solutions were prepared by immersing three film samples, each 2 cm in diameter, into 100 ml of UHQ water and incubating at 50°C for 30 minutes. The assay was standardized with 1, 2, 3, 4 and 5 g/L glycerol, 1,2-propanediol, EG, DEG and TEG solutions, obtained by adding the appropriate amount of each 50g/l plasticizer stock solution. The heating time was set at 30 minutes, based on the results obtained for the kinetics of plasticizer extraction from films, which indicated that all of the plasticizer had been extracted after this time.

Preliminary Study

Polyhydric alcohols were selected for this study because of their similar polarities to sunflower protein. Thus, different molecular weight polyhydric alcohols were tested as potential plasticizers for sunflower protein films.

Their efficiency and ability to produce homogeneous films was compared with that of glycerol, which is the most commonly used plasticizer for protein films. All the tested polyols in this preliminary study are quoted in the table 1.

Table 1. Ability of various polyhydric alcohols to act as plasticizers in the preparation of sunflower protein-based films.

Plasticizer	Film homogeneity	Plasticizer effiency	Results
Glycerol	+++	+++	OK
Ethylene glycol	+++	+++	OK
Diethylene glycol	+++	+++	OK
Triethylene glycol	+++	+++	OK
Tetraethylene glycol	+	+	Rejected
Polyethylene glycol 400	--	-	Rejected
Polyethylene glycol 1000	---	--	Rejected
Polyethylene glycol 3000	---	---	Rejected
Propylene glycol	+++	+	OK
Polypropylene glycol 400	-	0	Rejected
Polypropylene glycol 1000	---	---	Rejected
Polypropylene glycol 3000	---	---	Rejected

After this study, only five plasticizers were kept for more extensive analyses: Glycerol (G), Ethylene glycol (EG), Diethylene glycol (DEG), Triethylene glycol (TEG) and Propylene glycol (PG). This result may be explained by the small molecular weight and the higher polarity of these compounds. In order to obtain smooth and homogeneous films, amounts of plasticizer for detailed studies were chosen between 4 and 7 grams per 10 grams of sunflower protein isolate.

Mechanical Properties

Mechanical properties of sunflower protein films were tested after the forty eight hours conditioning period in a climate room at 60% relative humidity and 25°C.

The representative stress and strain at break curves of sunflower protein films are plotted in Figure 1.

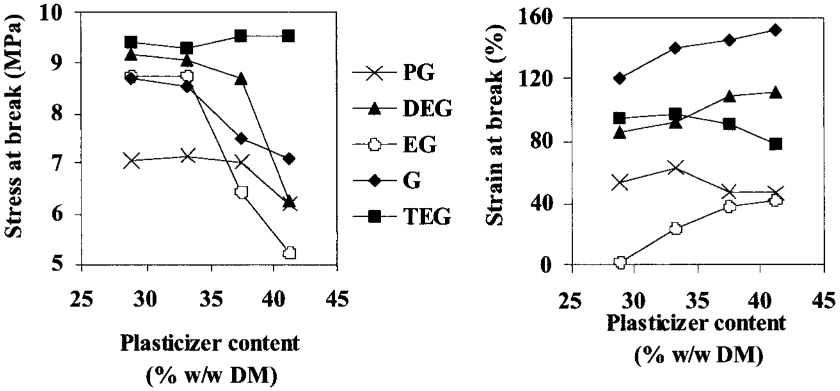

Figure 1. Evolution of stress and strain at break of sunflower protein-based films as a function of plasticizer nature and content.

The best stress at break was obtained with TEG (about 9.5 MPa) and the best strain at break with glycerol (about 100%).

For most of the films, it was noted that an increase in the content of plasticizer led to a decrease in mechanical resistance and an increase in elongation at break.

Water Uptake and Solubility

The solubility parameters of sunflower protein films were assessed by 24 hours immersion in water with stirring.

The water uptake, loss of dry matter and plasticizer content after immersion are summarized in table 2.

Table 2. Solubility parameters of sunflower protein films[a,b].

Plasticizer	Water Uptake[c] (%DM)	Loss of matter in water[c] (g/film)	Plasticizer content[c] (g/film)
Glycerol	85 (4)	5.1 (0.3)	0.1 (0.1)
Ethylene glycol	115 (8)	5.6 (0.6)	0.4 (0.3)
Diethylene glycol	121 (1)	4.8 (0.4)	0.4 (0.2)
Triethylene glycol	110 (2)	4.2 (0.2)	0.9 (0.2)
Propylene glycol	107 (7)	4.9 (0.1)	0.3 (0.2)

[a] Average and standard deviation values of five determinations are reported.
[b] Each film contained 5g of plasticizer at the beginning of the study.
[c] Values after immersion.

Water uptakes and losses of dry matter were determined gravimetrically and plasticizer contents were quantified by Glc analyses.

All the quoted values concern films containing 5 g of plasticizer at the beginning of the study. Water uptakes are about 100%. Concerning the losses of dry matter, all the values are about 5 g which correspond to the initial amount of plasticizer. In addition, plasticizer content values after immersion show that films containing TEG retain about 18% of this compound. For all the other plasticizers, the values do not exceed 8%.

According to these results, the dry matter solubilized in water is mostly made up of the plasticizer and probably some low molecular weight polypeptides. Then it was concluded that protein network is almost insoluble.

Surface Hydrophilicity

The third parameter evaluated for the sunflower protein films was the contact angle with water in order to evaluate the hydrophilicity of the film surfaces. All the results of contact angle measurements are grouped in table 3.

Table 3. Contact angle values for sunflower protein films[a,b].

Plasticizer	**Contact angle with water (deg)**
Glycerol	28 (3)
Ethylene glycol	35 (1)
Diethylene glycol	39 (2)
Triethylene glycol	38 (2)
Propylene glycol	44 (3)

[a] Average and standard deviation values of five determinations are reported.
[b] Each film contained 5g of plasticizer at the beginning of the study.

These results show clearly that the film surfaces were hydrophilic, regardless of the type of additive used. This result is logic and could have been expected, because of the hydrophilic nature of all the plasticizers used in this study.

However, it is noticeable that the use of DEG, TEG or PG produced less hydrophilic film surfaces, as indicated by the higher values of the contact angle.

Water Vapor Permeability

It was noted that the film water vapor permeability increases with the content of plasticizer (Figure 2). This first result could be explained by a modification in the proteic network density due to the increase in the free volume between the protein chains.

Further more, it is not surprising that the hydrophilic character of the film increases with the concentration of hydrophilic plasticizer. The values of water vapour permeability (between $2{\cdot}10^{-12}$ and $10{\cdot}10^{-12}$ $g{\cdot}m^{-1}{\cdot}s^{-1}{\cdot}Pa^{-1}$) were similar to those obtained for low density polyethylene and lower than those obtained for most of the other protein films, including sunflower protein films we prepared by casting.

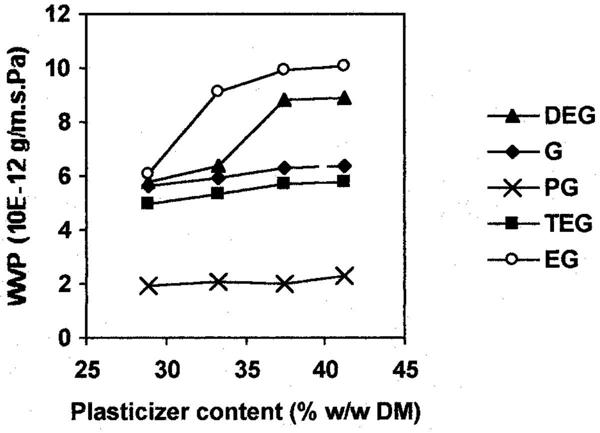

Figure 2. Evolution of the Water Vapor Permeability of sunflower protein films as a function of the plasticizer nature and content.

Scanning Electron Microscopy

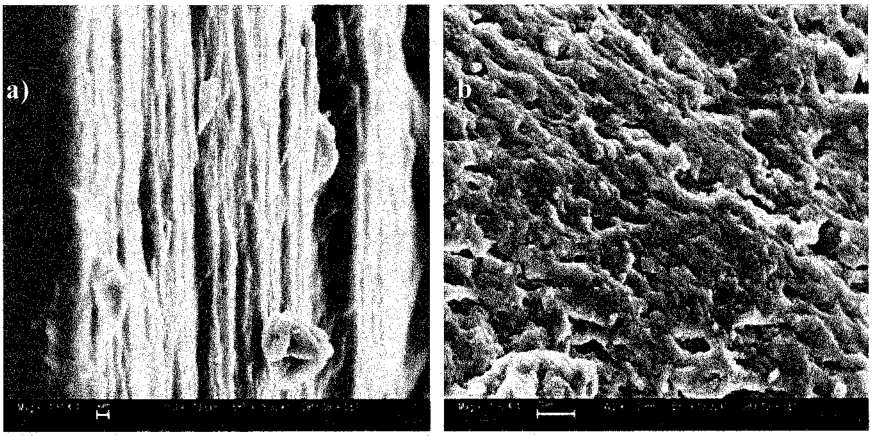

Figure 3. Scanning electron micrograph of the surface of sunflower protein-based side. a) thermo-molded films ; b) cast films.

Film morphologies were investigated by SEM. Micrographs of moulded films showed clearly a continuously thin slice morphology which indicated that protein chain adopted an oriented structure (figure 3a).

This orientation is characteristic of thermo-moulded sunflower protein films and results from the complete melting of the protein, contrary to that obtained in the case of cast films which show a non-oriented morphology, probably due to the uniform deposition of protein chains during the drying process (figure 3b).

Aging

Migration of plasticizers during time is a well known natural process which affects the film properties and depends on the plasticizer concentration and on the hydrophobic-hydrophilic balance of the plasticizer.

Solubility measurement have shown that all plasticizer was removed from the film and solubilized in water. These results showed clearly that the polyhydric alcohols used in this study were not strongly bound to the protein network.

Moreover, in the present study it was observed that the surface of some films became greasy during a long storage time. In order to know if this phenomenon was really due to the plasticizer migration from the bulk to the surface, a 3 month study of the evolution of the plasticizer concentration in films was carried out.

In addition, we investigated the film mechanical properties evolution during time as a function of the plasticizer content of the films.

Mechanical properties

Initially, mechanical properties were evaluated in comparison with mechanical properties without storage. Figure 4 shows the evolution of film stress at break over a period of three months.

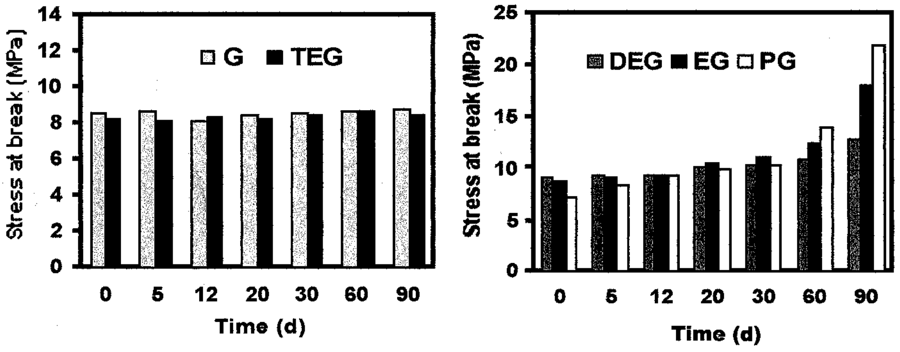

Figure 4. Evolution of sunflower protein film stress at break as a function of plasticizer nature and content, and storage time in a climate room (60% R.H., 25°C).

Concerning glycerol-plasticized films, no changes were noticed for this parameter during time. TEG-plasticized films showed a similar behavior. On the other hand, films containing DEG, EG and PG showed a dramatic increase of stress at break during time. Accordingly, the constant stress at break of films containing glycerol and TEG resulted in constant strain at break. Likewise, the increase of stress at break for EG, DEG and PG resulted in a decrease of the strain at break for the films plasticized with one of these compounds.

Plasticizer content

The curves showing the concentration evolution of each plasticizer in films containing 5 g of plasticizer at the beginning of the study are plotted in figure 5.

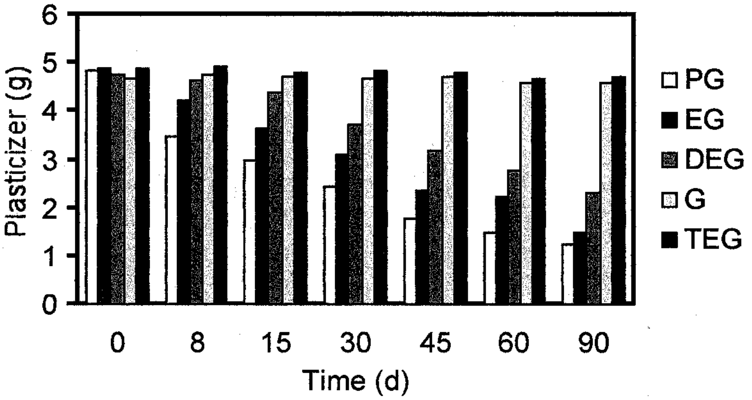

Figure 5. Evolution of the strain at break of sunflower protein films as a function of plasticizer nature and content, and storage time in a climate room (60% R.H., 25°C).

Not surprisingly, a dramatical decrease of plasticizer concentration was observed in films containing PG, EG or DEG. Very likely, the plasticizers were removed from the films by volatilization and, the consequent increase in stress at break may be due to stronger interactions between protein chains.

On the other hand, glycerol and TEG plasticized films keep about all the plasticizer amount during time. Accordingly, it was concluded that the decrease in mechanical resistance for some films is due to a loss of plasticizer during time.

Film thickness

The figure 6 show the evolution of film thicknesses during time. A decrease of this parameter was observed for films containing DEG, EG or PG, suggesting that the proteic network became more dense.

On the other hand, only a slight decrease of film thicknesses was observed for G- and TEG-plasticized films.

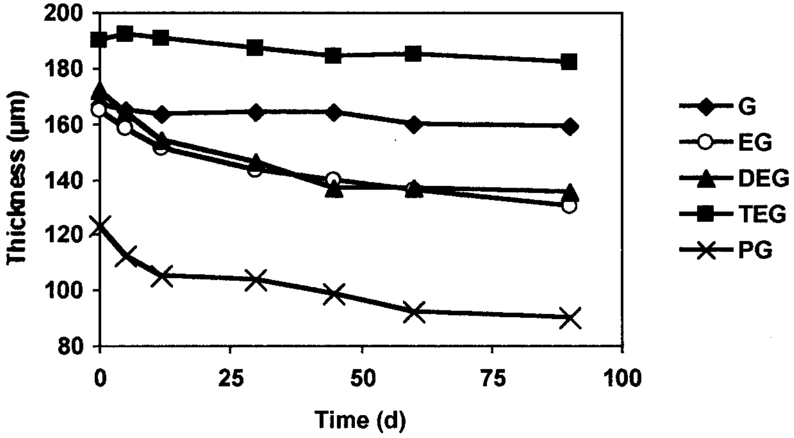

Figure 6. Evolution of sunflower protein film thickness as a function of plasticizer nature and content, and storage time in a climate room (60% R.H., 25°C).

All those results confirm the idea that the observed decrease in mechanical properties is really due to plasticizer volatilization.

Conclusion

In the present study, the thermoplastic potential of sunflower protein isolate was demonstrated. Two plasticizers were found very adapted to the plastification of sunflower proteins because of their ability to produce films with high stress and strain at break and their good resistance during time.

The use of glycerol resulted in the greatest strain at break and TEG resulted in the highest stress at break. In addition, it was found that the protein network is insoluble contrary to what obtained by casting.

These results open new outlets for the exploitation of sunflower residues.

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