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Effects of additives on the mechanical properties, hydrophobicity and water uptake of thermo-moulded films produced from sunflower protein isolate

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Abstract

The mechanical properties and water resistance of thermo-moulded films made from a sunflower protein isolate plasticized with glycerol (ratio 2:1, w/w) were studied. Various cross-linked or hydrophobic components (aldehydes, plant tannins, alcohols and fatty acids) were added and the properties of the films obtained with those of control films (containing no additives other than the plasticizer) were compared. The use of octanoic acid resulted in high tensile strength (7 MPa), whereas the use of octanol resulted in great increase in tensile elongation (54%). Plant tannins gave films with properties similar to those obtained with aldehydes, but without the toxicity. The network of proteins formed by thermo-moulding is water-resistant, regardless of which additives were used or their nature. © 2002 Published by Elsevier Science Ltd.

Keywords: Sunflower proteins; Film; Cross-linking

1. Introduction

Due to the growing demand for plastics and the problems associated with their disposal, there is an increasingly urgent need for new biodegradable materials [1,2]. Potential candidates for use in the fabrication of biodegradable films include soy proteins [3–8], wheat gluten [9], corn proteins [10,11], myofibrillar proteins from fish [12] and pea proteins [13,14]. Two processes are currently used to synthesize these films: the wet method, which involves the solubilization of proteins and a plasticizer in a solvent followed by the formation of a protein network on evaporation of the solvent; and the dry method, which is based on the thermoplastic characteristics of proteins and combines the use of pressure and heat to plasticize protein chains. The cross-linking between these chains results in a homogeneous and resistant material. Although the dry method requires more equipment, it has two major advantages: it is closer to industrial implementation [15] and it considerably decreases the solubility of the resulting films by creating a highly cross-linked protein network.

The large number of reactive groups present on the residues comprising the protein chains has led many researchers to carry out cross-linking experiments to increase the mechanical properties and to decrease the solubility of films formed by the wet method [5]. Aldehydes have been used for this type of reaction, particularly formaldehyde, glutaraldehyde and glyoxal [5,8,16]. However, although highly reactive, these components have a major disadvantage: their toxicity. This must be taken into account when synthesizing biodegradable materials. All of the aldehyde used must be cross-linked in a permanent protein network and the fate of the aldehyde in the environment at the end of the material's life must be considered.

This paper concerns the thermo-moulding of storage proteins isolated from sunflower seeds (*Helianthus annuus*). The main objective was to study the effect of various additives on the mechanical properties and water solubility of thermo-moulded films. Biodegradable plant tannins were used as complexing agents and their efficiency compared with that of aldehydes. Fatty alcohols and fatty acids were also incorporated into the proteins to improve their water resistance.

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2. Materials and methods

2.1. Materials

The sunflower protein isolate (ISFP) was extracted in alkali from industrial sunflower oil cake according to a protocol developed in the laboratory [17]. The protein was extracted by adding 2 kg of oil cake to 20 l of sodium hydroxide solution, pH 12. The mixture was stirred mechanically and heated at 50 °C for 20 min. Liquids and solids were then separated by centrifugation (4500 g, 15 min). The liquid phase (filtrate) was acidified to pH 4.8 with concentrated (50%, w/v) sulfuric acid. During this process, the proteins precipitate at their isoelectric points. The precipitated proteins are collected by centrifugation (4500 g, 10 min) and dried in a ventilated oven at 40 °C for 48 h. The precise composition of the oil cake used as the raw material and the final isolate are summarized in Table 1. The glycerol (99.5%) used as the plasticizer, and the formaldehyde (99%), glutaraldehyde (50%, w/v in water), glyoxal (99%) and fatty alcohols and acids (99–99.5%) were 'reagent' grade and were purchased from Aldrich (St Quentin Fallavier, France). Natural tannins and gallic acid were purchased from SCRD (Grauhlet, France). All of the reagents were used as supplied, with no additional treatment.

2.2. Film formation

Ten grams of dry ISFP were used for each film. The ISFP/glycerol ratio was set to 2:1, which corresponds to an initial quantity of 5 g of anhydrous glycerol per film. This ratio results in supple, smooth, homogeneous and perfectly coherent films. A preliminary study showed that a ratio of less than 3:1 led to the formation of fragile, heterogeneous films. A ratio greater than 2:2 led to a major loss of plasticizer during the thermo-moulding process and resulted in sticky films. A ratio of 2:1 was used throughout the experiment, so that the mechanical properties and water uptake of the resulting films could be directly compared. The amount of additive used varied from 1 to 6% of the total mass of the glycerol plus the protein isolate. Five grams of glycerol were weighed out, and the required amount of additive was then added to the glycerol, with mechanical stirring. This mixture was then combined with the ISFP, and subjected to thermo-moulding at 155 ± 3 °C for 2 min 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. After cooling for about 5 min, the film was removed from the mould, weighed and stored in a controlled-environment chamber at 25 °C and 60% relative humidity (RH), as described in the European Standard NF EN ISO 291 [18]. The first mechanical tests were carried out after storage in this chamber for 48 h.

2.3. Mechanical properties

A TA-XT2 texture analyser (RHEO Stable Micro Systems, London, UK) was used to assess the mechanical properties of the films. The test samples, obtained with a punch, 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 recorded the mean value. A speed of 1 mm/s was used, with an initial grip separation of 55 mm, as described in standards ISO 527-2 [19] and ISO 527-1 [20].

2.4. Glycerol and water content

The glycerol content of the films was measured by high performance liquid chromatography (HPLC) with an ICS chromatograph (Toulouse, France) in isocratic mode. The operating conditions are listed in Table 2. The solutions were prepared by immersing three film samples, each 2 cm in diameter, into 100 ml UHQ water and incubating at 50 °C for 30 min. The assay was standardized with 1, 2, 3, 4 and 5 g/l glycerol solutions, obtained by diluting a 50 g/l stock solution. The heating time was set at 30 min based on the results obtained for the kinetics of glycerol extraction from films, which indicated that all of the glycerol was extracted during this time.

The glycerol and water content was measured by gravimetry. Identical film samples (2 cm diameter) were

Table 1
Characteristics of the sunflower oil cake and ISFP

	Sunflower oil cake	ISFP	Determination method
Moisture (%)	10 ± 2	6 ± 1	105 °C/24 h oven-drying
Ash (%)	7.6 ± 0.4	2.4 ± 0.4	Incineration (525 °C/5 h)
Proteins ($F = 6.25$)	34.4 ± 1.0	90 ± 1	Kjedhal
Lipids (%)	1.0 ± 0.5	0.6 ± 0.4	Soxhlet extr. (hexane)
Cellulose (%)	22.3 ± 2.0	0	ADF–NDF [25,26]
Lignin (%)	5.2 ± 1.0	1.7 ± 1.0	ADF–NDF [25,26]
Hemicellulose (%)	9.8 ± 1.0	0	ADF–NDF [25,26]
Phenolic compounds (%)	5.7 ± 0.80	4.8 ± 0.8	UV spectrophotometry (Folin–Ciocalteu reagent)

Mean values and standard deviations calculated from three measurements.

Table 2
Chromatographic conditions used to determine the glycerol content of the protein films

Type of column	H^+ CAR-H ion exclusion resin (SARASEP)
Temperature of the column	35 °C
Eluant	0.004N H_2SO_4 solution
Flow rate	0.8 ml/min
Maximum pressure at the head of the column	25 bar
Volume of the injection loop	20 µl
Detector	Refractometer

weighed on their removal from the controlled-environment chamber, and were then placed in a ventilated oven at 105 °C for 72 h. Films were dried to constant weight. Tests on control films showed that after this treatment, only 1% of the glycerol used to make the film remained in the sample.

The water content was calculated as the difference between the percentage weight lost during evaporation and the percentage of glycerol as determined by HPLC. This calculation was validated by determining the amount of dry matter in parallel, at room temperature, with control films (protein isolate/glycerol ratio 2:1). Identical samples were placed inside a vacuum desiccator containing phosphorus pentaoxide and weighed every 24 h until a constant weight was reached (5–8 days). The mean water content of the thermo-moulded films was 10.1 ± 2.2%, regardless of the type of additive used. The mean water contents of the films for each additive are listed in Table 3.

2.5. Measurement of surface hydrophobicity

Surface hydrophobicity was assessed by measuring contact angle (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 analyser was used to measure the angle formed between the base, constituted of the surface of the film in contact with the drop of water, and the tangent to the drop of water. The mean hydrophobicity value for the surface of each film was calculated from five measurements on the film.

Table 3
Water content of the films as a function of the additive used

Type of additive	Mean water (%)
None	11.2 ± 1.6
Gallic acid	9.9 ± 2.1
Chestnut tannin	9.9 ± 2.2
Gambier tannin	10.1 ± 0.6
Tara tannin	10.4 ± 1.8
Octanoic acid	5.7 ± 2.3
Octanol	11.1 ± 2.0
Decanol	10.0 ± 1.1
Dodecanol	10.0 ± 2.2
Tetradecanol	12.0 ± 1.5
Hexadecanol	9.0 ± 2.0
Octanol	11.1 ± 2.0
Formaldehyde	9.3 ± 2.6
Glutaraldehyde	10.1 ± 2.2
Glyoxal	9.4 ± 1.7
Mean	10.1 ± 2.2

Mean values and standard deviations of five measurements.

2.6. Measurement of solubility in water and water uptake

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

3. Results and discussion

3.1. Properties of the control films

The control films were produced without additives. They contained 10 g of dry ISFP and 5 g of anhydrous glycerol. After 48 h of storage at 25 °C and 60% RH, the mean tensile strength and tensile elongation were 2.8 MPa and 37.6%, respectively, for a water content of 11.2 ± 1.6%. These films were brown, supple and smooth. Determination of the amount of glycerol in the film after thermo-moulding gave a final amount of 4.60 ± 0.08 g of plasticizer per film. This value did not seem to vary over time (Fig. 1). The protein content of the films was determined by the Kjedhal nitrogen assay (amount of protein = amount of nitrogen × 6.25) and was 10.07 ± 0.03 g. These films were used as a reference for subsequent experiments.

3.2. Changes in glycerol content over time

All of the mixtures used for thermo-moulding contained 5.0 g of anhydrous glycerol. The amount of glycerol in the films after moulding was between 4.3 and 4.6 g per film, regardless of the type of additive used (Fig. 1). Certain films (control films and films containing certain amounts of aldehydes) appeared to be ‘sticky’ after seven days of

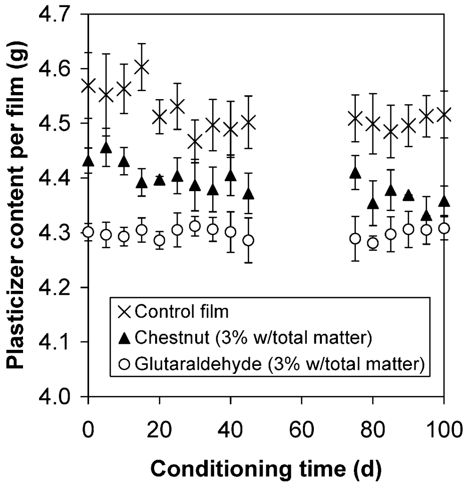

Fig. 1. Variation of plasticizer content during conditioning at 25 °C and 60% relative humidity. Average and standard deviation values of three experiments are plotted.

storage in controlled conditions. Glycerol loss during storage was therefore investigated. Glycerol content of the films was determined every five days for 45 days on samples that had been wiped to remove the stickiness and any glycerol that had been eliminated from the network. No significant change was observed in the glycerol content of the films over the 45 days. Tests carried out over a longer period (100 days) gave similar results. The 'stickiness' of the films is therefore more likely to be caused by surface hydration due to the hydrophilic nature of the glycerol rather than to the loss of glycerol.

3.3. Mechanical properties of the films

Aldehydes are the agents most frequently used to cross-link proteins during the production of films by the wet method and, to a lesser extent, by the dry method. Aldehydes were tested in this study as a reference for cross-linking agents. Three tannins were then compared with the aldehydes.

Curves of changes in tensile strength and tensile elongation over time were constructed (Figs. 2 and 3, respectively). The addition of 1.5% glutaraldehyde increased the tensile strength from 2.8 to 5.2 MPa (equivalent to an 85% increase) with no loss of elongation. Such increases in tensile strength were observed for additions of up to 3% glutaraldehyde. Such an increase in values relating to the mechanical properties of cross-linked protein films due to the addition of glutaraldehyde was previously demonstrated by Park et al. [21]. Although comparison with this study is difficult, the experiments carried out by Park et al. on films cast from soy protein isolates showed increases in tensile strength of 47% (from 8.3 to 12.2 MPa) for an aldehyde content equivalent to 2% of the total mass. Conversely, the addition of larger amounts of this aldehyde resulted in a decrease in the stress at break of the films made from thermo-moulded ISFP, with no corresponding decrease in elongation capacity. The form in which the compound was used may account for this. An aqueous solution of glutaraldehyde containing very few monomers was used. These molecules are mostly polymerized in the form of linear chains of varying lengths. Thus the bonds formed between the protein chains by aldehyde cross-linking may be relatively long. Although they can make the network rigid, they tend to increase the distance between the protein chains, thereby preventing the formation of both the bonds that stabilize the network in the control films (for example, disulfide bridges). The material is therefore rendered fragile by the presence of this aldehyde in too large an amount.

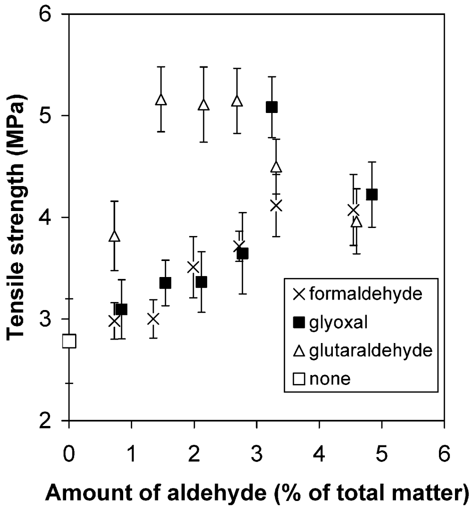

Fig. 2. Variation of tensile strength as a function of aldehyde amount in ISFP films. Average and standard deviation values of five experiments are plotted.

The other two aldehydes used also increased the tensile strength of the materials formed if used at concentrations of

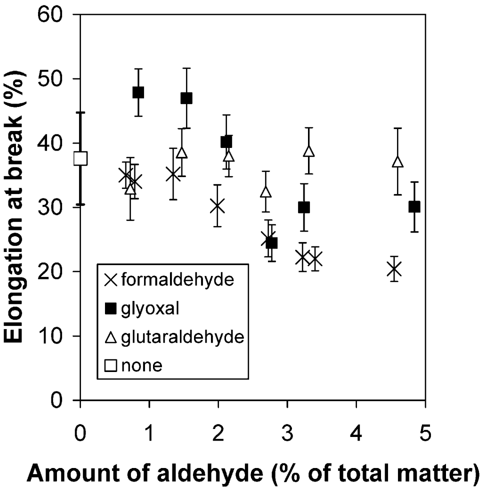

Fig. 3. Variation of elongation at break as a function of aldehyde amount in ISFP films. Average and standard deviation values of five experiments are plotted.

up to about 3%. A maximum was observed for these two compounds at 3% (5.2 MPa for glyoxal and 4.3 MPa for formaldehyde). Beyond this limit, the resistance of the films decreased for glyoxal and reached a plateau for formaldehyde. It is probable that 3% is the maximum quantity of formaldehyde that can enter the network. Beyond this limit, the free formaldehyde evaporates during thermo-moulding due to its extremely high volatility (boiling point = −20 °‴ C). Excess glyoxal seemed to be trapped within the protein structure and to behave like a plasticizer.

The addition of glutaraldehyde seemed to have little effect on the tensile elongation of protein films; the addition of up to 2% glyoxal slightly increased elongation capacity, but the addition of larger amounts had a negative effect. However, the addition of 3.5% formaldehyde made the network sufficiently rigid to decrease elongation by 40% with respect to control films. Elongation remained stable for the addition of larger amounts of formaldehyde, which seems to be consistent with the tensile strength results.

Two complex natural tannins and gallic acid were also used (Figs. 4 and 5). The major advantages of these substances over the aldehydes used are that they are non-toxic and entirely biodegradable. The chestnut tannin gave the largest gain in tensile strength, although the increase was not as great as that observed with glutaraldehyde: tensile strength reached 4.2 MPa for 3.5% tannin. However, tensile elongation steadily decreased, to as low as 28% for a concentration of 2%. Films containing 6% tara tannin had similar tensile strength (4.4 MPa) if experimental error was taken into account. Beyond a certain percentage, depending on the nature and the molecular weight of the tannin used, a considerable decrease was observed in tensile strength, probably due to the amount of tannin present weakening the interactions stabilizing the protein network. Concentrations of up to 1.5% gallic acid, which was used as a control for the effect of tannins on the protein isolate, also improved mechanical properties (with a maximum tensile strength of 3.8 MPa). Beyond this concentration, gallic acid led to a strong decrease. Furthermore, like the other tannins, gallic acid decreased tensile elongation.

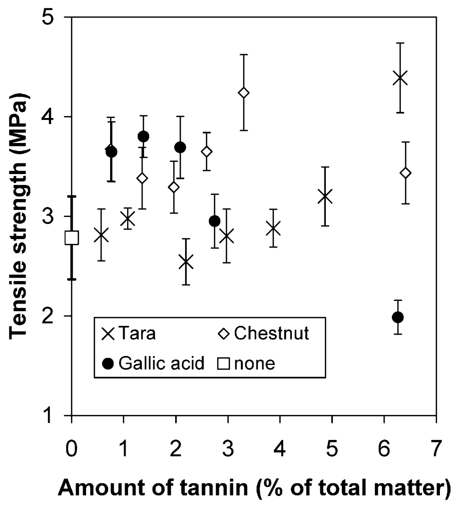

Fig. 4. Variation of tensile strength as a function of tannin amount in ISFP films. Average and standard deviation values of five experiments are plotted.

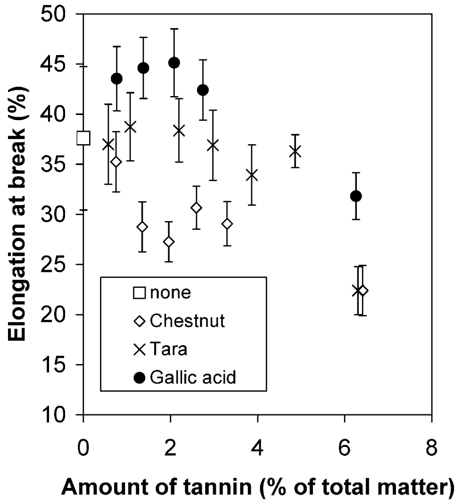

Fig. 5. Variation of elongation at break as a function of tannin amount in ISFP films. Average and standard deviation values of five experiments are plotted.

Thus, tannins gave films with lower mechanical properties than those obtained with aldehydes, probably because they act through weak interactions rather than covalent bonds in the case of aldehydes. Furthermore, their extremely low volatility at the processing temperature prevents elimination of the non-bound tannin fraction. Increasing the percentage of tannins beyond the maximum amount that can be bound to the network would therefore load the film with additional tannin, damaging its coherence. Therefore, at concentrations above a certain limit, tannins make the films fragile.

Fatty alcohols (octanol, decanol, dodecanol, tetradecanol and hexadecanol) were used to increase surface hydrophobicity and to decrease the solubility of the films. The mechanical properties of all of the films made with these compounds were also checked (Figs. 6 and 7). Surprisingly, the addition of these compounds seemed to favour the creation of new interactions between the protein chains. Indeed, although the presence of these compounds corresponds to an excess of plasticizer, films containing 3% or more octanol, decanol or dodecanol clearly had better mechanical properties than control films. The presence of up to 4% octanol in a film increases its tensile elongation, by a maximum of 2%. The addition of low percentages of decanol and dodecanol do not have a significant effect on the tensile elongation of the film, but causes an increase of tensile strength. On the other hand, just 0.5% of tetradecanol or hexadecanol causes a major decrease in tensile elongation even if tensile strength is increased slightly. This apparently surprising increase in the mechanical resistance of films containing low percentages of octanol, decanol or dodecanol may be due to the surface-active role

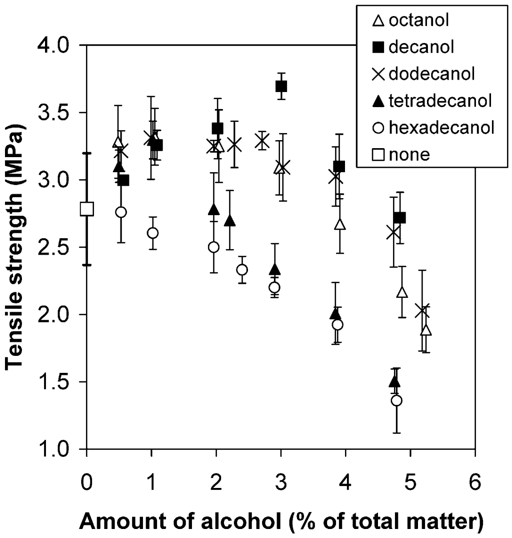

Fig. 6. Variation of tensile strength as a function of fatty alcohol amount in ISFP films. Average and standard deviation values of five experiments are plotted.

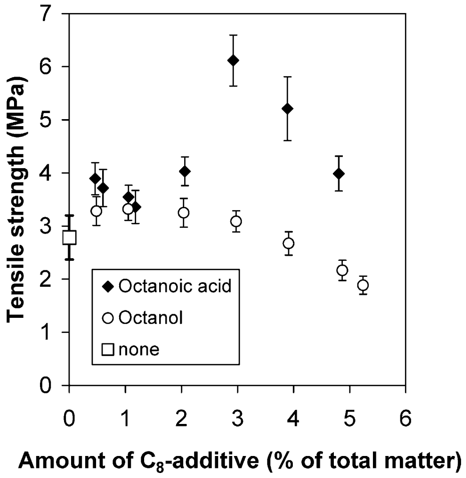

Fig. 8. Variation of tensile strength as a function of C_8-additive amount in ISFP films. Average and standard deviation values of five experiments are plotted.

of these alcohols. Their presence in the film affects the organization of the protein network: the increase in stability may be due to the creation of new hydrophobic interactions between the protein chains 'surrounded' by the fatty alcohol molecules. Conversely, higher concentrations of fatty alcohols act as plasticizers and decrease the mechanical resistance of the films. The alcohols with the longest chains (tetradecanol and hexadecanol) probably migrate poorly within the structure due to their high molecular weights.

Similarly, the addition of octanoic acid (Figs. 8 and 9) leads to a dramatic increase in tensile strength—a concentration of 3% results in films with a tensile strength of 7 MPa. This increase in tensile strength is countered by a marked decrease in tensile elongation, which nonetheless remains above 18%. This suggests that the presence of octanoic acid in the film promotes the creation of interactions stronger than those that normally stabilize the molecule. Previous studies on corn zein have shown that the protein chains can adopt a particular spatial organization in materials plasticized with fatty acids, which may improve the properties of the product [22,23]. Similarly, Di Gioia et al. [11] observed an oriented structure and an improvement in the properties of materials produced corn gluten plasticized with octanoic acid. Therefore, this compound seems to promote the extension of protein chains and their arrangement in parallel, thereby increasing the number of possible interactions between chains. The presence of another highly hydrophobic component may change the conformation of the proteins during thermo-moulding, leading to the formation of new, low-energy bonds (e.g. hydrophobic bonds). Films containing octanoic acid are also the least prone to water absorption during storage

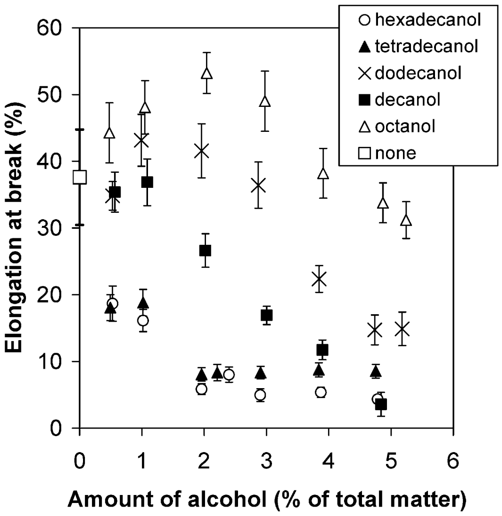

Fig. 7. Variation of elongation at break as a function of fatty alcohol amount in ISFP films. Average and standard deviation values of five experiments are plotted.

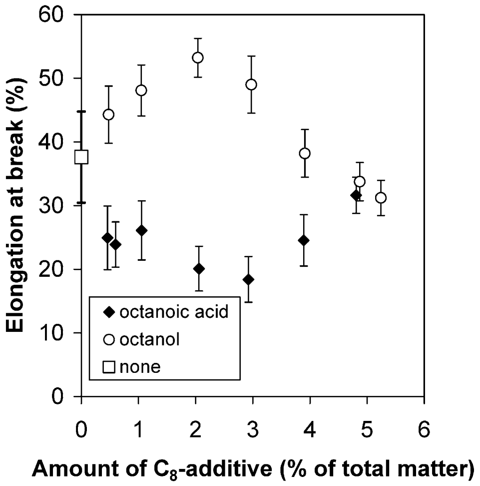

Fig. 9. Variation of elongation at break as a function of C_8-additive amount in ISFP films. Average and standard deviation values of five experiments are plotted.

(mean = 4.9% water). This may also account for these films having the best mechanical properties.

3.4. Solubility of the films formed

In this case, solubility measurements provided no essential information about possible differences in the properties of films as a function of the type and quantity of additive used. Two findings seem to be particularly important as far as control films are concerned. First, almost all the glycerol (>98%, as measured by HPLC) present in the films is extracted by immersing the films in water for 24 h. Second, over 99.5% of the dry matter of the film (corresponding to the protein network) remains insoluble. This results in the perfect maintenance of the structure of the samples studied. This results from the plastication of protein chains during the thermo-moulding process, which differentiates this process from the casting step of the wet method. This process results in a homogeneous network containing protein chains linked by disulfide bridges and weak hydrophobic interactions. The coextracted polyphenols present in the ISFP (when oxidized these compounds form highly reactive quinones) constitute a non-negligible quantity of potential cross-linking agents that bind with various strengths to the protein chains during thermo-moulding, accounting for the excellent maintenance of the three-dimensional network formed in water.

The same phenomenon was observed with the films formed from aldehydes: the protein network was insoluble whereas almost all of the glycerol was released. The films containing fatty alcohols also behaved similarly, except that the fatty alcohols remained trapped in the film during the solubilization phase and evaporated during the final drying step (105 °C, 24 h). The tannin-containing films behaved like the control films: the glycerol dissolved in the water and the tannin/ISFP network was virtually insoluble.

3.5. Water uptake and surface hydrophobicity of the films

All of the swelling values cited in this section reflect both the water that entered the protein network and was absorbed by the film and the glycerol that escaped from the network via osmosis into the water in which the film is immersed. However, these values can all be compared because the amount of glycerol in the films was identical regardless of the additive used.

Of the three aldehydes studied (Fig. 10), glutaraldehyde gave the best results. The level of swelling was considerably lower than that for control films, with 15% water uptake at a concentration of 0.7% glutaraldehyde versus 32.0% water uptake for control films, and a minimum of 9.5% with 4.7% cross-linking reagent. Formaldehyde resulted in similar levels of water uptake, with a minimum of 14.1% for 2% addition. Beyond this concentration, water uptake reached a plateau, consistent with the formation of a network strongly cross-linked with short bonds. It was observed that there was a slight decrease in water uptake as the concentration of glyoxal was increased. A minimum of 22% water uptake was reached with 3.2% glyoxal. Surface hydrophobicity was not modified by the addition of glutaraldehyde or glyoxal. Conversely, the addition of formaldehyde increased the contact angle to 39 ± 3.3° for a concentration of 1.5% versus 27 ± 2.4° for control films (Table 4). The water resistance of the films was thus quite considerably increased by adding formaldehyde, in terms of both surface hydrophobicity and lower levels of water uptake. This is probably accounted for by the formation of a denser protein network (especially at the surface), slowing the absorption of water by the glycerol trapped in the film.

Water uptake was also increased by adding all the tannins tested (Fig. 11). The most efficient tannin was gallic acid (only 5% swelling for a concentration of 6%), probably due to its small size, which facilitates its migration within the

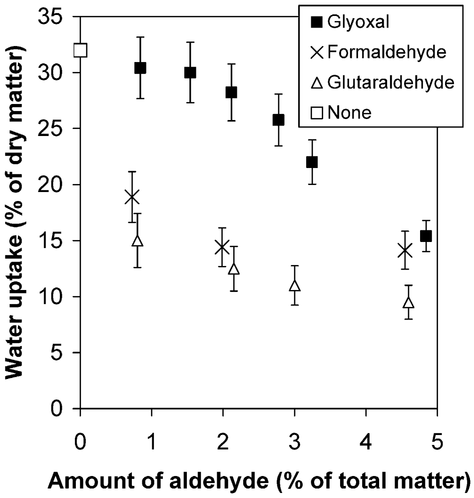

Fig. 10. Variation of water uptake as a function of aldehyde amount in ISFP films. Average and standard deviation values of five experiments are plotted.

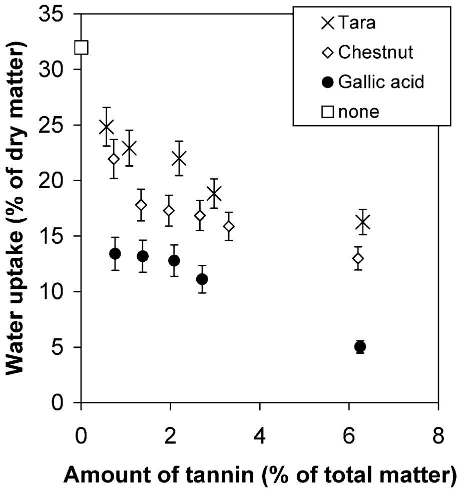

Fig. 11. Variation of water uptake as a function of tannin amount in ISFP films. Average and standard deviation values of five experiments are plotted.

Table 4
Contact angle values according to the type of additive

Additive	Amount of additive (% mass)	Contact angle (deg)	Tensile strength (MPa)
None	0	17.9 ± 2.4	2.8 ± 0.4
Formaldehyde	3.3	42.0 ± 4.0	4.1 ± 0.3
Decanol	0.6	95.1 ± 4.9	3.0 ± 0.1
Tara tannin	6.3	51.2 ± 2.9	4.4 ± 0.4
Octanoic acid	6.0	43.5 ± 1.9	4.0 ± 0.3

Mean values and standard deviations of five measurements.

protein network and increases its reactivity. Furthermore, for an equal mass, the reaction mixture contains more gallic acid molecules and so the number of reaction sites is higher. Greater efficiency was therefore expected. The chestnut tannin was the second most efficient, with a minimum of 13.0% solubility for films containing 6% of this tannin. The tara tannin was the least efficient of the tannins, with a minimum of 16.3% for a concentration of 6%. Tannins were particularly effective at decreasing the solubility of films, particularly with respect to aldehydes, even though they use non-covalent cross-linking.

Fatty alcohols did not significantly decrease water uptake (Fig. 12). Although these molecules halved the swelling of the films, this is not sufficient to justify their use in preference to other additives (tannins, aldehydes). However, the addition of decanol conferred remarkable surface hydrophobicity properties on the films produced. The addition of 1% of this fatty alcohol gave contact angles of over 90° versus only 27° for control films (Table 4). Thus, the surface of the films formed may be considered highly hydrophobic according to the classification system proposed by Mozes et al. [24].

The addition of octanoic acid leads to a remarkable decrease in water uptake with respect to that of control films (Fig. 13). This would seem to increase the density of the network and is consistent with there being an increase in the number of these interactions. Surface hydrophobicity was greater than that of the control films (50° with a concentration of 2% versus 27° in the controls), but not so high that the film could be classified as highly hydrophobic. Thus, the results obtained with this compound were satisfactory because it offered the best compromise between the various properties desired. This compound also has the considerable advantage of being non-toxic for humans and plants, a vital quality if it is to be used in biodegradable films for use as agricultural mulches, for example. Another of the properties of this compound is also of potential value: it has anti-fungal activity. Thus, if introduced into films in known, moderate quantities, this compound may slow degradation of the film in the soil, thereby providing a means of controlling biodegradation.

4. Conclusions

This study identified genuine alternatives to the aldehydes currently widely used to cross-link and to improve the qualities of protein films. These natural compounds, collectively known as tannins, confer on film's mechanical properties similar to those conferred by aldehydes, whilst markedly decreasing their solubility. They are also

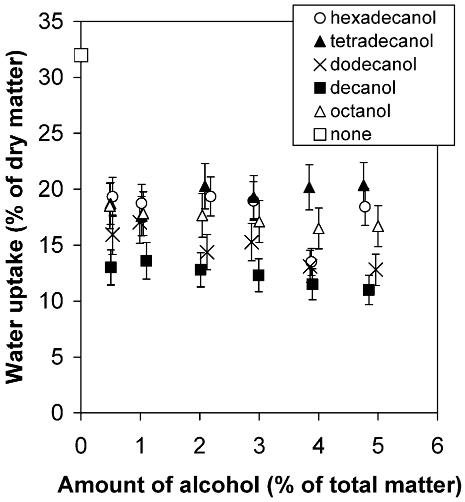

Fig. 12. Variation of water uptake as a function of fatty alcohol amount in ISFP films. Average and standard deviation values of five experiments are plotted.

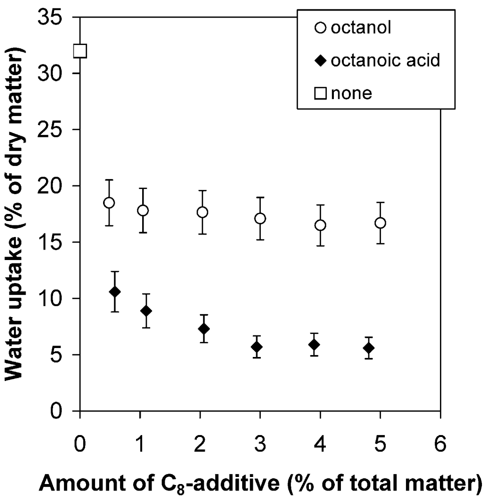

Fig. 13. Variation of water uptake as a function of C_8-additive amount in ISFP films. Average and standard deviation values of five experiments are plotted.

biodegradable, a major advantage if they are to be used in the production of films destined for biological degradation at the end of their lives.

The use of 5 g of fatty alcohols increased the hydrophobicity of our films to various extents. The contact angles obtained with decanol (> 90°) were particularly impressive. In addition, these alcohols had no negative effects on the mechanical properties of the films, as one might have feared given that these compounds are potential plasticizers.

Finally, very good results were obtained with octanoic acid, in terms of both improvements in mechanical properties and decreases in the water uptake of films. This is particularly interesting because this component is non-toxic for humans and possesses anti-fungal activity, which opens up new possibilities for its use, for example in the production of films for food use (e.g. packaging) or films for which controlled degradation is a necessary feature.

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