Fennel Waste-Based Films Suitable for Protecting Cultivations

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Biodegradable, flexible, and moisture-resistant films were obtained by recycling fennel waste and adding to fennel homogenates the bean protein phaseolin that was modified or not modified by the enzyme transglutaminase. All films were analyzed for their morphology, mechanical properties, water vapor permeability, and susceptibility to biodegradation under soil-like conditions. Our experiments showed that transglutaminase treatment of the phaseolin-containing fennel waste homogenates allowed us to obtain films comparable in their mechanical properties and water vapor permeability to the commercial films Ecoflex and Mater-Bi. Furthermore, biodegradability tests demonstrated that the presence of the enzyme in the film-casting sample significantly influences the integrity of such a product that lasts longer than films obtained either with fennel waste alone or with a mixture of fennel waste and phaseolin. These findings indicate the fennel−phaseolin film prepared in the presence of transglutaminase to be a promising candidate for a new environmentally friendly mulching bioplastic.

Introduction

The use of plastic mulching films in agriculture has increased in the last few decades because the films promote the reduction of weed killers, allow a lower irrigation frequency, and shorten harvesting times as a result of induced higher soil temperature. In contrast, a large portion of them is left on the field or is burnt by farmers, with obvious negative consequences for the environment. Thus, the use of biodegradable materials is a challenging alternative for enhancing sustainable and environmentally friendly agricultural activities in mulching film-mediated cultivations.[1]

Biodegradable polymers can be classified, according to their origin, into natural and synthetic polymers. The main biopolymers from mineral origins are polyesters and polyvinylalcohols, whereas biopolymers from natural origins include polysaccharides, proteins, and polyesters produced by microorganisms.[2] Mulching films made of one or both types of polymers have already been produced. BASF in Germany has recently developed a film trademarked as Ecoflex[3] made of a biodegradable plastic that can be broken down by a multitude of common microorganisms in the soil and compost. In addition, innovative biodegradable starch-based mulching films have been commercialized by Novamont (Italy).[4]

For natural polymer-based film preparation, it could be advisable to recycle byproducts of the agricultural industries that usually contain carbohydrates and proteins.[5] In this way, new value could be added to several agricultural byproducts with the concomitant goal of managing lower volumes of wastes and reducing environmental pollution. Thus, our research addressed the recycling of raw fennel homogenates in order to use them as a glucidic source for the preparation of polysaccharide−protein composite films. Fennel (*Foeniculum vulgare*, Mill) is an aromatic plant belonging to the Apiaceae family and is common to the Mediterranean. It can be an annual, biennial, or perennial herb and is divided into two subspecies *piperitum* and *capillaceum* that include three varieties *vulgare*, *dulce*, and *azoricum*. The first two varieties are used to flavor foods and liqueurs, and the third one, which was used for the present work, is extensively cultivated as a vegetable.[6] This variety is characterized by a round-shaped edible part that can reach an average diameter of 12 cm and a length of 15 cm. This part, called grumolo, is made of rigid, white leaf sheaths, of which the more external ones are discarded, representing at least 30% of the total production, which reaches about 70 000 tons/year in Southern Italy.[7] In summer, fennel waste can increase up to 50%, making its disposal even more expensive.

As the protein component for our composite films, we used phaseolin extracted from common beans because such a protein was shown to be able to act as a substrate for transglutaminase (TG, E.C. 2.3.2.13).[8] This enzyme catalyzes the formation of isopeptide bonds between endoprotein glutamine and lysine residues.[9] We recently demonstrated that it is possible to obtain edible films made of carbohydrates and TG structurally modified proteins that are endowed with different mechanical and permeability characteristics.[10−13] In this article, we report the preparation of fennel waste films cast in the absence and in the presence of phaseolin either unmodified or modified by TG and their characterization with regard to possible application in agriculture. Thus, besides studying water vapor permeability and mechanical properties, the biodegradability behavior of all three types of films was investigated.

Experimental Sections

Methods. *Film-Forming Procedure.* The outer parts of fennel were washed, cut into pieces of about 1 cm^3 in size, suspended in water at a concentration of 1 g mL^{-1}, and boiled at 95 °C for 30 min. The

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resulting material was treated using a kitchen juice extractor with a centrifugal filter having a cutoff of 200 μm (Centricon, Ariete). The strained material was used for film preparation. Phaseolin was isolated from *Phaseolus vulgaris* beans by using the ascorbate−NaCl procedure described by Sun and Hall.[14] The purified protein was dissolved in distilled water at a concentration of 80 mg mL^{-1}.

Microbial TG was prepared by dissolving the commercial preparation (Ajinomoto Co., Japan) in distilled water. The specific activity of the enzyme was 92 U/g. An estimation of enzymatic activity was carried out by a colorimetric hydroxamate according to Pasternack et al.[15] Prior to film casting, the solutions were degassed under vacuum to remove bubbles.

Three different kinds of films were prepared: fennel waste-based films (FW films), fennel waste-based films made in the presence of phaseolin (FW/Ph films), and fennel waste-based films made in the presence of TG-modified phaseolin (FW/Ph/TG films). Films were cast by pouring the solution into 5-cm-diameter polystyrene Petri dishes. For FW film preparation, 15 mL of fennel solution was spread onto the plates, whereas for FW/Ph films, fennel solutions (15 mL) were mixed with 2.3 mL of phaseolin solution and gently mixed. Finally, FW/Ph/TG films were obtained by adding 8.8 U of the enzyme to the final solution of fennel and phaseolin. All of the samples were allowed to dry at 37 °C overnight under dry air circulation. Dried films were peeled off of the casting surfaces intact and were conditioned at 50% RH and 25 °C for 48 h before being tested.

Protein Determination. Protein determination was carried out by Biorad protein assay (Biorad), using bovine serum albumin as the standard.[16]

Film Characterization. *Thickness.* The film thickness was measured using an electronic digital micrometer with a sensitivity of ±2 μm (Metrocontrol, Srl, model HO62). Film strips were placed between the jaws of the micrometer, and the gap was reduced until the minimum friction was measured. The mean thickness (mm) was determined from the average of measurements at five locations.

Scanning Electron Microscopy (SEM). SEM, used to characterize film surfaces and their cross sections, was used as described by Mariniello et al.[9] In particular, film samples were cut into of 9 mm^2 pieces using a sharp razor blade. Then, the film pieces were immersed in 6% glutaraldeyde−0.1 M phosphate buffer at pH 6.8 for 48 h at room temperature. After being washed in phosphate buffer for 1 h, the film strips were dehydrated in a graded series of ethanol solutions (15−100%), blocked with an epoxy resin, and made conductive with a 60:40 gold−palladium mixture deposited by a Hummer spotter coater to a thickness of 400 Å. For films subjected to bacterial degradation, the samples were washed with distilled water, dried under vacuum, and then observed by SEM.

The samples were examined with a scanning transmission electron microscope (Philips Electronics, Mahwah, NJ, model X20). Three different samples of each type of film were subjected to SEM, and five different micrographs of each sample were taken. Micrographs were obtained at 400× magnification.

Film Mechanical Properties. The tensile strength, elongation to break, and modulus were measured with an Instron universal testing instrument (Instron Engineering Corp., Canton, MA, model 4301) according to ASTM[17] and following a procedure very similar to that described by Mariniello et al.[9] Film samples were cut into 10 to 11 mm wide and 100 mm long strips using a sharp razor blade. The strips were equilibrated overnight at 50 ± 5% RH and 23 ± 2 °C in an environmental chamber. Five samples of each film type were tested. Each film strip was placed between the pneumatic jaws of the Instron that were pre-set to give an original gauge reading of 90 mm, and the strips were then stretched at a rate of 30 mm min^{-1} until sample failure occurred. Measurements of the load (N) and deformation (mm) were used to calculate the tensile strength (maximum load placed on the sample divided by the cross-sectional area) and the elongation to break (deformation of the sample at maximum load divided by the original gauge length).

Finally, the tensile modulus was determined automatically from force versus deformation measurements via simple extension.

Film Water Vapor Permeability. The film water vapor permeability (WVP) was evaluated by a gravimetric test according to ASTM E96[18] by means of a Fisher/Payne permeability cup (Carlo Erba, Italy) as described by Di Pierro et al.[10] Briefly, 3 g of silica gel was introduced into each cup, and a film sample disk with a diameter of about 6 cm was placed on top of the cup and sealed by means of a ring kept in place by three tight clamps. The film area exposed to vapor transmission was 10 cm^2. The cups containing silica gel were weighed and then placed in a desiccator containing a saturated KCl solution that provided a constant water activity of 0.8434 at 25 °C. The desiccator was stored in a Heareus thermostated incubator at 25.0 ± 0.1 °C. Every 24 h, the cups were weighed until a constant increase in weight was achieved. The water vapor transmission rate through the film was estimated by the linear portion of the diagram obtained by plotting the weight increment of the cup as a function of time. It was assumed that steady state was reached once the regression analyses made by using the last four data points resulted in $r^2 \geq 0.998$.

The WVP was calculated from the following equation

$$WVP = \frac{X}{A\Delta p}\frac{dm}{dt}$$

where dm/dt is the slope of the cup weight versus time once steady state was reached, X is the film thickness, A is the film exposed area, and Δp is the water vapor pressure across the film. By assuming that the vapor pressure inside the cup, due to the presence of silica gel, can be taken as equal to zero, Δp becomes equal to the vapor pressure inside the desiccator given by the product of the water activity and vapor pressure (P_0) at 25 °C (P_0 = 3.167 kPa).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Twenty milligrams of each film sample was dissolved in 250 μL of sample buffer (15 mM Tris−HCl, pH 6.8, containing 0.5% w/v SDS, 2.5% v/v glycerol, 200 mM β-mercaptoethanol, and 0.003% w/v bromophenol blue), boiled for 5 min, and then centrifuged for 10 min at 13 000*g*. Twenty microliters of each supernatant was analyzed by SDS-PAGE. SDS-PAGE experiments were carried out under reducing conditions on slab gels (5% stacking and 12% separating gels) as described by Laemmli.[19] Electrophoresis was performed at constant current (40 mA for 1 h and 70 mA for another 2 h), and the proteins were stained with Coomassie Brilliant Blue R250. Biorad Precision Protein Standards were used as molecular weight markers.

Biodegradability Tests. *Film Degradation in a Soil Environment (in Vivo Experiments).* The three different types of films were sterilized by UV exposure (30 min at 15 cm from UV lamp) and completely covered by a layer (~5 to 6 cm) of clay−loam soil from the University farm Torre Lama (Salerno, Italy). The chemical and physical characteristics were 42% sand, 27% loam, 31% clay, lime traces, 1.57% organic matter, 0.09% total N, pH 7.1; the soil−water contents at field capacity (in situ) and at −1.5 MPa were equal to 27.6 and 13.6% by weight, respectively; and the bulk density was equal to 1.25 t m^{-3}.

The soil was activated by water spraying up to complete adsorption (approximately 10% of the soil's total weight). After 7 and 21 days of incubation at 30 °C, total soil microflora was evaluated by microbial counts on PCA.

Isolation and Identification of Microrganisms. Strain isolation was carried out in 2006 from soil coming from the La Colombaia organic farm, Capua (CE), Italy. The soil was neutral (pH 7.0) clay−sand and had low contents of organic matter (1.5%) and nitrogen (1.0 ‰). Thirty-five isolates from countable plates of a plate count agar (PCA) (Oxoid) were screened for cellulosolytic and pectinolytic activity according to procedures proposed by Kluepfel[20] and Jayasankar and Graham,[21] respectively. Cellulosolytic and pectinolytic isolates were checked for cell and colony morphology, catalase, and gram reaction as well as for its spore-forming capability. Tryptone soya broth (Oxoid) was routinely used as the cultivation medium. Eight strains were then selected on the basis of cellulosolytic and pectinolytic capabilities for in vitro

experiments. (See below.) Among them, four strains showing degradation activity on fennel films were harvested in sterile Ringer solution by centrifuging at 10 000*g* for 1 min. DNA was extracted by InstaGeneTM Matrix (Bio-Rad Laboratories, Hercules, CA) according to the supplier's recommendations. Surnatant containing DNA was stored at −20 °C or promptly used for 16S rRNA amplification experiments.

Synthetic oligonucleotides described by Weisburg et al.[22] (fD1, 5′AGAGTTTGATCCTGGCTCAG3′ and rD1, 5′AAGGAGGTGATCCAGCC3′) were used as primers in the presence of *Taq* polymerase, following author instructions. PCR conditions consisted of 30 cycles (1 min at 94 °C, 45 s at 54 °C, and 2 min at 72 °C) plus 1 additional cycle at 72 °C for 7 min as a final chain elongation. The presence of PCR products was controlled by 1.5% w/v agarose−Tris borate EDTA gel electrophoresis at 7 V cm^{-1} for about 1 h. Obtained 16S rRNA PCR fragments were purified from 1.5% w/v agarose gel by a Perfectprep gel cleanup extraction kit (Eppendorf srl; Milan, Italy) according to the supplier's instructions. The DNA sequence was determined by the dideoxy chain-termination method.[23] Research for DNA similarity was performed with the National Centre of Biotechnology Information GenBank.[24]

Microbial Growth on Films (in Vitro Experiments). The three different types of films were placed on plates containing tryptone soya agar (TSA, Oxoid) or agar (0.75%, Oxoid) alone in order to evaluate the microbial utilization of films with or without additional nutrients. Films were sterilized by UV exposure as reported above.

Eight strains were selected on the basis of cellulosolytic and pectinolytic capabilities. Cells from overnight cultures were harvested, washed in sterile Ringer quarter-strength solution, and resuspended in the same diluent up to the second level of the McFarland scale. Cell suspensions were spotted (10 μL) onto the films placed in plates containing the above-mentioned media.

Spots of sterile Ringer solution and cell suspension of *Lactococcus lactis* subsp. *lactis* LC1 were used as a negative control.

Film Degradation Monitored through the Microbial Respiration Rate. The microbial respiration rate, measured as the development of CO_2,[25] was monitored for each type of hydrocolloid film. Films were placed in hermetic recipients containing a trap with 1 N NaOH and covered with 50 g of soil used for bacterial isolation. The soil was then activated by spraying water onto the surface up to complete adsorption. Each kind of film was inoculated in triplicate. Moist soil alone was used as a negative control.

After 1, 2, 3, and 7 days of incubation at 30 °C, 5 mL of NaOH solution was titrated with 0.1 M HCl, using phenolphthalein as a pH indicator and 1 mL of 1.5 M $BaCl_2$ so as to catch developed CO_2 by forming $BaCO_3$.

CO_2 could be expressed as μg/g d.m. by the following equation

$$C\mathrm{CO}_2 = (A - B)MECPs$$

where

A = μL of HCl employed to titrate NaOH in the trap used as a negative control

B = μL of HCl employed to titrate NaOH in the trap

M = HCl molarity

E = 6 = equivalent weight to express data as carbon

C = mL of NaOH in the trap/mL of NaOH used for titration

Ps = hydrocolloid film plus soil dry weight

Results were expressed as the percent of mineralization of total carbon.[25] Film weights and carbon percentages are as follows: FW film, 0.28 g ± 0.01 and 74.3% ± 1.5; FW/PH film, 0.38 g ± 0.02 and 82.3% ± 1.01; FW/Ph/TG film, 0.5 g ± 0.07 and 95.8% ± 0.6.

Statistical Analysis. Microsoft Excel 2002 was used for all statistical analyses. The data were subjected to analysis of variance, and the means were compared using the Student's *t* test. Differences were considered to be significant at $P < 0.05$.

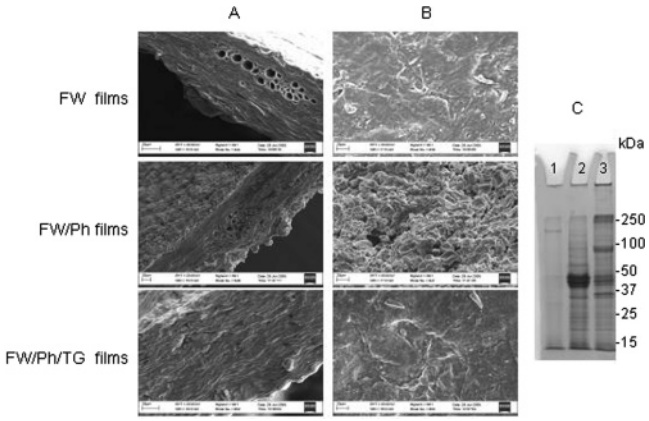

Figure 1. SEM micrographs (at 1000× magnification) displaying the cross-sections (A) and surfaces (B) of FW, FW/Ph, and FW/Ph/TG films. Twenty milligrams of each type of film was solubilized in 250 μL of sample buffer, boiled for 5 min, and centrifuged for 10 min at 13 000*g*. Twenty microliters of each sample supernatant was analyzed by 12% SDS-PAGE, followed by Coomassie staining (C). Lane 1, FW film; lane 2, FW/Ph film; lane 3, FW/Ph/TG film.

Results

In the present investigation, fennel raw homogenates were used as a carbohydrate source to prepare hydrocolloid films containing or not containing phaseolin, a globular protein occurring in the cotyledons of the bean *Phaseolus vulgaris*. In addition, some fennel−phaseolin films were cast in the presence of microbial TG as a cross-linking agent because we have recently demonstrated that phaseolin is able to act as an effective acyl donor and acceptor substrate for the enzyme.[8]

Hence, three kinds of films were prepared: a fennel waste homogenate-based film (FW), a fennel waste homogenate−phaseolin-based film (FW/Ph), and a fennel waste homogenate−phaseolin-based film made in the presence of TG (FW/Ph/TG). All three types of films were flexible and easily removed from the plates. To establish whether the films differed in their textures, SEM analyses were carried out. The micrographs reported in Figure 1 represent a cross section (panel A) and surface (panel B) of the films. Significant differences in the film network structure were observed upon analyzing the three film types. It is evident that FW and FW/Ph/TG films have surfaces that are similar to but smoother than that of FW/Ph films. In fact, it is possible to observe the presence of phaseolin crystals of quasi-cubic symmetry on the surface of the film containing unmodified phaseolin. These crystal morphologies are common to a large number of other varieties of seed-storage proteins[26] when extracted under acidic conditions.[27] Interestingly, SEM revealed that, in the film obtained in the presence of TG, phaseolin crystals are not evident, suggesting that the action of the enzyme on the globular protein affects its 3D structure. Moreover, the film cross section allows us to observe in all of the samples the presence of cellulose microfibrils, typical of the plant cell wall, arranged in parallel orientation and forming sheet-like overlapped layers. Furthermore, in both FW and FW/Ph films it is possible to note, in association with the cellulose fibers, the presence of deep holes representing the tracheary elements (tracheids and vessel members) that plants use for support and for the transport of water and minerals. These elements are totally absent in the cross section of the FW/Ph/TG film that, on the contrary, shows a very homogeneous structure. To characterize the three samples further, films were solubilized and analyzed by SDS-PAGE (Figure 1, panel C). The results indicate that the TG reaction carried out in the

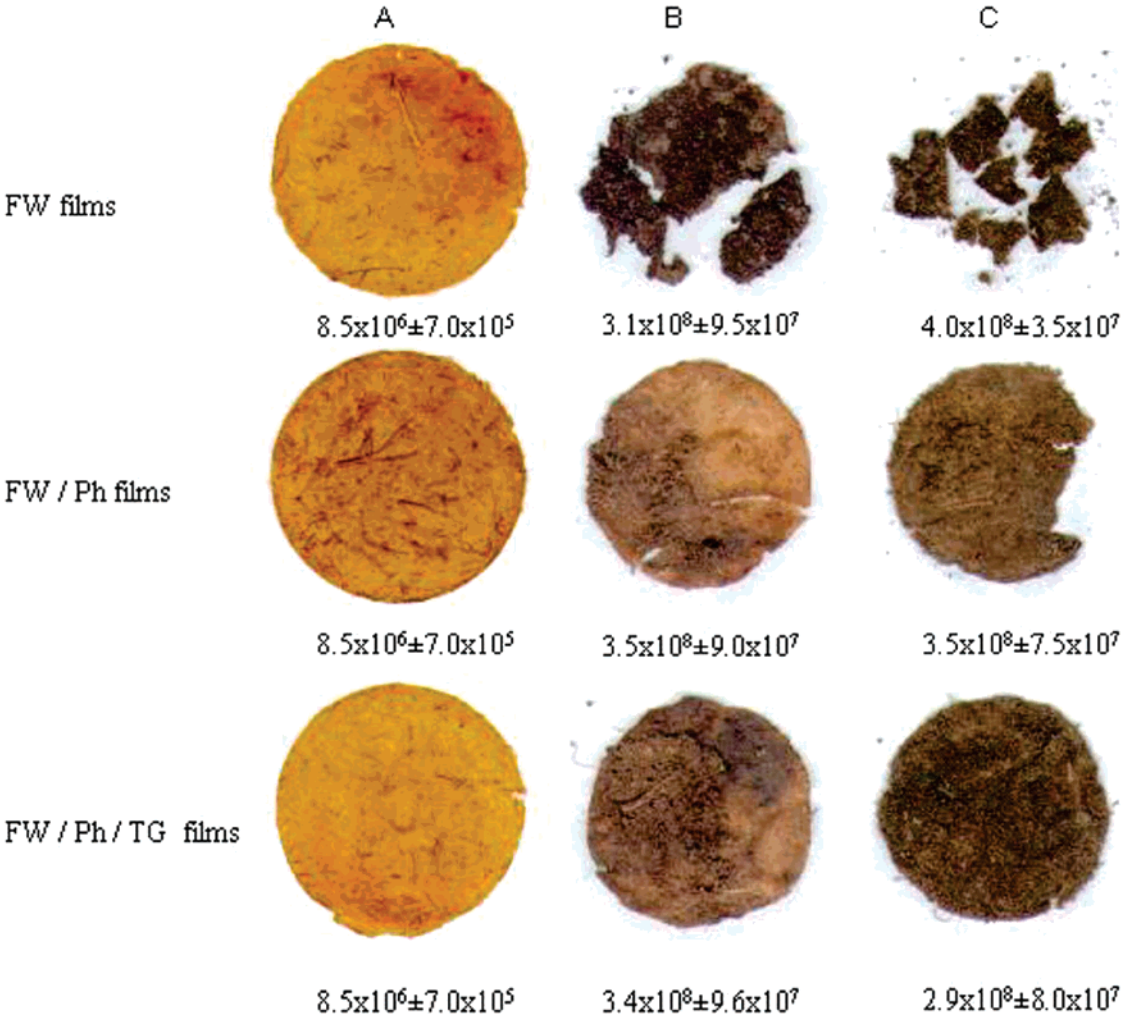

Figure 2. FW, FW/Ph, and FW/Ph/TG films 0 (A), 7 (B), and 21 (C) days after Torre Lama soil exposure.

casting system gives rise to phaseolin polymerization as shown by the formation in the FW/Ph/TG film of polymers of about 100 and 250 kDa (panel C, lane 3).

To assess the behavior of produced films as biodegradable products, in vivo and in vitro tests have been carried out. For in vivo experiments, the three kinds of films have been buried in soil from the Torre Lama farm having a composition typical of soil used in traditional agriculture. Figure 2 reports qualitative analysis results obtained by visual inspection of film surfaces, together with quantitative results corresponding to bacterial counts at the beginning of and 7 and 21 days after film exposure to soil. As an overall observation, it is evident that FW films were severely damaged after just 7 days of exposure (panel B) and after 21 days the level of damage is even more evident, showing larger breaks (panel C). In the presence of phaseolin, the disruption of the film is clearly slower, and samples tend to maintain their integrity for a longer time. Furthermore, it is worth noting that, when phaseolin was TG-modified, the films appear almost completely intact and do not dissolve even after 21 days of exposure to soil. The microorganisms counts in the soil, reported in Figure 2 under the film images, indicate that the presence of films increased the level of microorganisms, suggesting that Torre Lama soil contains bacteria that is able to degrade the buried films. In particular, the number of soil bacteria at the beginning ($8.5 \times 10^6 \pm 7 \times 10^5$) increased approximately 100 times after 7 days, independent of the type of buried film. Similar microbial counts were detected after 21 days of film exposure to soil.

Table 1. Cellulosolytic and Pectinolytic Activities for 35 Strains Randomly Isolated from the Soil of Organic Farm La Colombaia[a]

isolate	cellulosolysis	pectinolysis
7[b]	++	++
3, **5**, **8**, **34**, **38**, 39	+	++
1, **6**, 23	+	+
22	+	−/+
9	−/+	−/+
2, 33, 36	−	+
4, 13	−	−/+
29	+/−	−
14	−/+	−
10, 11, 12, 16, 17, 19, 21, 25, 26, 27, 28, 30, 31, 37	−	−
32	−	n.g.[c]
35	−	n.g.

[a] Results were roughly quantified by measuring the diameters of clear zones surrounding colonies. [b] Strains in bold were selected for further experiments. [c] n.g. indicates no growth.

For in vitro biodegradability studies, soil from the organic farm La Colombaia was chosen. Thirty-five microbial isolates were preliminarily screened for cellulosolytic and pectinolytic activities. Eight strains were selected because they revealed significant cellulosolytic and pectinolytic activities by measurement of the clear zone around the colonies (Table 1). By cell and colony morphologies, gram reaction, catalase activity, and spore production, four strains could be presumptively referenced

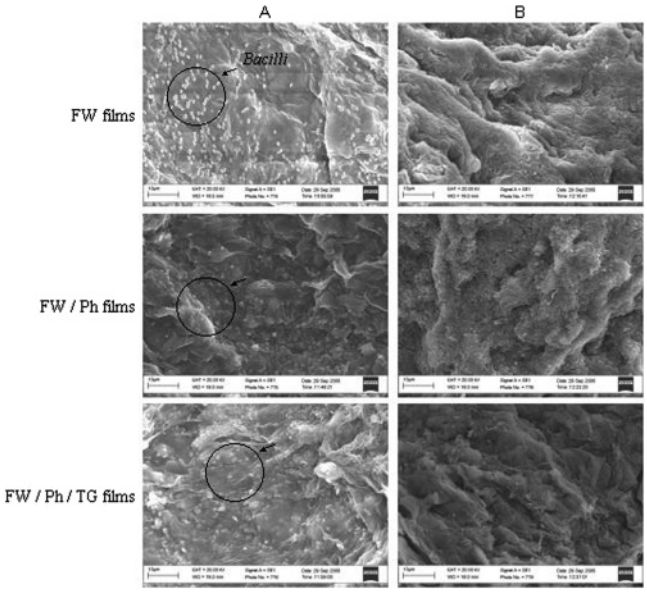

Figure 3. SEM micrographs (at 400× magnification) displaying the surface of FW, FW/Ph, and FW/Ph/TG films after 5 (A) and 60 (A) days of exposure to La Colombaia soil microflora.

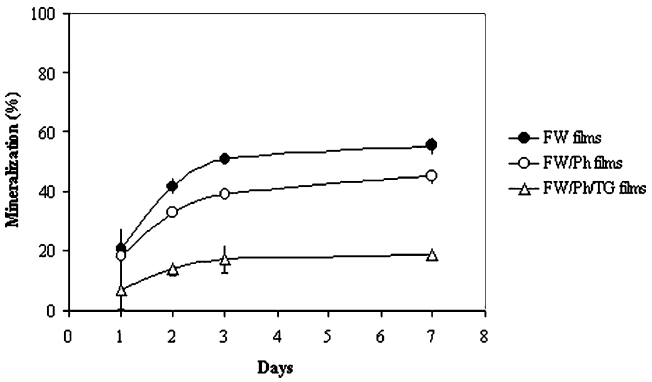

Figure 4. Carbon rate of mineralization by the three different types of films exposed to the soil from the La Colombaia organic farm after 1, 2, 3, and 7 days of incubation.

to the genus *Bacillus* spp. (1, 6, 7, 8) and four to the *Actinomyces* group (3, 5, 34, 38).

With the aim of understanding whether strains selected on the basis of enzymatic performance could develop on films without any additional nutritive enrichment, spots with microbial cell suspension were performed both on film simply placed on agar as an inert support and on film placed on plates of TSA, which is a medium that is able to provide nutritional support. After 48 h of incubation at 37 °C, films were completely covered by colonies both on TSA-containing agar and agar alone, and the incubation was extended up to 5 and 60 days. *Bacillus* spp. appeared to be the unique microrganism developed on agar. In fact, as shown in Figure 3, SEM analyses revealed only bacteria possessing a *Bacillus* spp. typical shape, whereas no *Actinomyces* were observed even after 60 days of incubation.

From SEM micrographs, it is also evident that *Bacillus* strains grow more easily on FW films than on FW/Ph films. Moreover, films containing TG-modified phaseolin appeared to be even less metabolized by *Bacillus* spp. coming from La Colombaia farm soil. These results were in line with those obtained from microbial respiration rate experiments (Figure 4). In fact, the percent of carbon mineralization, monitored after 1, 2, 3, and 7 days of incubation, indicates a slightly higher microbial utilization of FW films when compared to that for films containing phaseolin. Moreover, FW/Ph/TG films were still less utilized by La Colombaia *Bacillus* strains.

At this point, it was useful to identify such *Bacillus* wild strains by 16S rRNA sequencing. MacDNAsis 3.0 alignment multiple function was used to evaluate the correlation among 16S rRNA sequences and to compare the obtained sequences with those available on GenBank.

Strain 6 presented a 99% degree of similarity level with *Bacillus* (*B.*) *mojavensis* (AB021191), strain 8 exhibited a 99% degree of similarity with *B. subtilis*, reference strain DSM6405^T, and strain 7 showed a 99% degree of similarity with *B. licheniformis* (DQ234853). Strain 1 could be referred to only the genus *Bacillus* spp. because homology in 16S rRNA sequences with species in the GenBank did not reach 98%, a value regarded as evidence of separate species.[28,29]

Further film characterization was addressed to investigate their permeability and mechanical resistance, which are important features for possible applications in agriculture. Table 2 shows that casting in the presence of phaseolin led to a greater thickness compared to that of the film obtained with the fennel raw homogenates alone. In addition, a lower WVP was observed in the phaseolin-containing films, probably as a consequence of electrostatic interactions between fennel pectin carboxyl residues and the phaseolin positive charges. This effect could explain not only the reduction of the segmental mobility of the protein chains but also their solubility in water and the following WVP decrease through the fennel carbohydrate matrix.[30] The film WVP was further decreased when phaseolin was structurally modified by TG, suggesting that the enzyme-mediated phaseolin cross linking reduced the free volume inside the film, thus improving protein network stability and influencing the barrier properties of the water vapor.

Film mechanical properties and integrity under stress conditions were evaluated by measuring the tensile strength, the elongation to break, and the modulus, which is a measure of film deformation resistance. The results reported in Table 2 indicate that films containing phaseolin showed an increased tensile strength and modulus in comparison to films made in the absence of protein and that the presence of the enzyme in the casting solutions led to the production of films that are more resistant to deformation. Table 2 also contains properties of Ecoflex, Mater-Bi-Z, and low-density polyethylene (LDPE) with the aim of comparing fennel waste-based films to these well-known materials. All data refer to samples analyzed without exposing them to humidity. It can be noted that the FW/Ph/TG film modulus is higher than that exhibited by Ecoflex[31] and comparable to those reported for both Mater-Bi and LDPE.[32] Conversely, FW/Ph/TG films offer a poorer barrier to water vapor than do commercial materials.[3,31,32]

Discussion

The use of biodegradable films in agriculture is strongly advisable for reducing pollution due to plastic mulching films. Therefore, many efforts are being made to develop films that are both biodegradable and environmentally friendly. Commercially available products have been proposed by BASF with Ecoflex[3] and by Novamont with starch-based Mater-Bi.[4] Both are bioplastics containing different additives to improve mechanical and permeability performance.[4,33] Our investigation was undertaken to discover new biodegradable products obtained by recycling agricultural waste, the disposal of which pollutes

Table 2. Functional Properties of Fennel Waste-Based Films and of Some Commercially Available Materials

			mechanical properties		
film	thickness (mm)	WVP ($cm^3\ mm\ m^{-2}\ day^{-1}\ kPa^{-1}$)	modulus (MPa)	tensile strength (MPa)	elongation to break (%)
FW	0.2508 ± 0.007	68.45 ± 1.1	122.87 ± 0.8	1.9 ± 0.1	7.3 ± 0.58
FW/Ph	0.3034 ± 0.0.22	55.24 ± 2.3	166.77 ± 26.6	3.3 ± 0.3	8.0 ± 1.43
FW/Ph/TG	0.3188 ± 0.019	48.20 ± 2.1	203 ± 3.9	4.0 ± 0.5	9.0 ± 1.64
Ecoflex		0.14−0.03[c]	100[a]	30.3[a]	820[d]
Mater-Bi-Z		33.33[b]	100−600[b]	20−50[b]	200−600[b]
LDPE		0.5[b]	150−300[b]	8−10[d]	15−600[d]

[a] See ref 31. [b] See ref 32. [c] See ref 3. [d] See ref 4.

the environment. To this aim, we used fennel waste homogenates and the globular protein phaseolin extracted from common beans. Phaseolin possesses a trimeric structure[34] and is able to act as a TG substrate because it contains both glutamine and lysine reactive residues.[8] In fact, our experiments demonstrated that phaseolin and TG addition to the film-forming mixtures is effective at improving several properties of this novel Bioplastic. Phaseolin cross linked by TG was probably responsible for the smoother structure of the films, as assessed by SEM analyses, and better resistance after 21 day burial tests compared to the same properties of FW/Ph and FW films. Microbial characterization studies demonstrated that *Bacillus* strains were able to digest all of the examined films whereas, according to SEM evidence, *Actinomyces* strains were unable. In vitro and in vivo tests revealed that the biodegradability of TG-modified phaseolin-containing films was always reduced. These findings suggest that the fennel waste-based films containing TG-modified phaseolin are effective at persisting in the soil and preserving its biodegradability; as a consequence, cultures such as short-cycle crops are protected. The determined film permeability features confirm their possible application to crops such as zucchini, asparagus, and lettuce that need to be protected by a mulching sheet with a high water vapor transmission rate. It has been reported that the WVP of composite films varies greatly with the orientation of the molecules[35] and their polarity and density as well as with the high level of chain-to-chain packing.[36] In this respect, it is noteworthy that the FW/Ph/TG film seems to resemble the Mater-Bi-based one and Ecoflex has lower permeability values (Table 2). Similarly, by comparing modulus values, it is notable that the FW/Ph/TG film is resistant to deformation, as is Mater-Bi, whereas it was 2-fold more resistant than Ecoflex. Mater-Bi is known to be made of corn-extracted starch to which different synthetic polymers (i.e., poly-(ϵ-caprolactone) and/or polyvinyl alcohol) are added with the aim of increasing the flexibility and resistance to moisture. However, Ecoflex is an aliphatic−aromatic copolyester made of modular units including 1,4-butanediol, adipic acid, and terephthalic acid.[3] Novamont and BASF produce different types of these materials by both adjusting components and/or adding special additives.[4,33] Conversely fennel-based films are prepared by recycling fennel waste and adding to their homogenates phaseolin, a protein available in large quantities, and the cross-linking enzyme TG. Addition to the film-forming mixtures of either plasticizers or other additives, such as synthetic polyesters, could provide fennel-based biodegradable polymers with further potential for wider and environmentally friendly use in agriculture.

Of course, additional studies are required to establish how these materials could be generated on a large scale and thus to determine production costs.

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