

Preparation and Characterization of Biodegradable Thermoplastic Films Based on Collagen Hydrolyzate

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Received 17 November 2008; accepted 28 April 2009

DOI 10.1002/app.30670

Published online 3 November 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The increasing use of plastics and their nonbiodegradability have raised environmental awareness and hence there is a need for the development of environmentally friendly degradable materials. One of the ways to reach this goal is via the modification of the synthetic polymer, modified polyethylene (MPE), with protein, collagen hydrolyzate (CH). CH is a biopolymer isolated from hide/skin fleshing of untanned solid waste from the leather industry after enzymatic hydrolysis. An investigation on the blending of MPE with CH using polymer melt technique is reported. The resulting thermoplastic films were evaluated using Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA/DTA), and scanning electron microscope (SEM), in addition to simulated soil burial respirometric testing. It is interesting to note that CH easily blends with MPE, but like other biopolymers, it also has effects on the original mechanical

properties of the MPE. The CH addition in the blend significantly increases the biodegradation rate. The effect of CH on MPE biodegradability has been investigated. About 53% biodegradation is observed, after 24 days, when the polymer is blended with 5% CH and about 63% biodegradation is found in the case of polymer blended with 20% CH. Although MPE/CH thermoplastic film with 40% CH have shown better performance in biodegradation, the mechanical strength properties were rather poor in this case. The optimum thermoplastic film composition for blending of CH with MPE is about 10–20 wt % CH, which retains an acceptable range of compatibility, mechanical strength, and biodegradability. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 115: 3230–3237, 2010

Key words: biodegradation; collagen hydrolyzate; thermoplastic film; polyethylene

INTRODUCTION

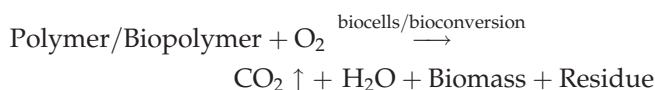
In recent years, there has been an ever increasing interest in the development of biodegradable polymers owing to the growing problem of waste disposal of plastics.^{1,2} Most synthetic plastics do not decompose naturally, which has caused the serious environmental problem of pollution from the waste polymers. Biodegradable polymers are polymers which completely degrade by the action of biological organisms such as proteins (for example, collagen hydrolyzate) and carbohydrates. This type of biopolymer is completely biodegradable, consequently, it is effective for the volume reduction of synthetic polymers waste by partial degradation. However, another type of biodegradable polymers are not completely degrade, which is called the biodisintegratable polymers.³ Industrial and technological applications reported for plastics are as follows⁴: packaging (41%), construction of buildings (20%), electric insulation (9%), automobile parts (7%), agriculture (2%), and miscellaneous (21%). Thus, the increasing use of plastics as packaging materials and their nonbiode-

gradability has raised an environmental awareness for the development of low cost environmentally friendly biodegradable materials through polymer modification. One of the ways to reach this goal is by using renewable, natural, low cost, and easily available biopolymers such as starch,^{5,6} cellulose,⁷ protein (collagen).^{8–11} The majority of thermoplastics currently used as packaging materials are based on polyolefins, such as high density polyethylene (HDPE), low density polyethylene (LDPE), and polypropylene (PP). Moreover, for low value items such as shopping bags, agriculture mulch films, and food packaging (foils and thermoformed items) recycling would be neither practical nor economical. Blends of synthetic and natural polymers have been used in recent decades to develop new materials, which are called bio-artificial polymeric materials. Their capability of combining good physical and mechanical properties with biocompatibility characteristics was used for the purpose of making new materials for biomedical applications.¹² Collagen hydrolyzates (CH) was previously prepared by alkaline and enzymatic hydrolysis of chrome leather waste which condensate with long-chain carboxylic acids and their chlorides at atmospheric pressure.¹³ A number of authors have reported on the chemical and enzymatic treatment of leather solid waste and the composition of leather industry waste are polymeric

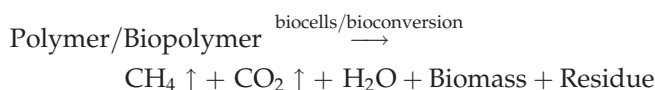
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materials which are biodegradable.^{14,15} The fundamental concept for biodegradation of these waste materials under aerobic and anaerobic conditions is illustrated as follows¹⁶:

Under aerobic condition:



Under anaerobic condition:



Leather solid waste can be processed into various useful products such as glue, gelatin, and collagen for medical applications. Edible sausage casings, cosmetics, fodder, and foods are also being produced.¹⁷ Fleshings and shavings represent the most important by-products of the leather industry. They are obtained in the fleshing/shaving phase of the hide/skin and are mainly comprised of raw collagen. Collagen hydrolyzate was positively investigated for blending with polymeric matrices with positive outcomes.¹⁸ Blending of proteins (CH) with synthetic polymer (MPE) represents the aim of this article which deals with the study of the tendency to form biodegradable thermoplastic film. The effect of CH addition on biodegradation rate and film formation were ascertained by means of respirometric test simulating soil burial experiments. Film samples were also characterized for thermal and mechanical properties, in addition to the SEM-morphology.

EXPERIMENTAL

Materials

The thermoplastic polyolefin elastomer produced by copolymerization of polyethylene and 1-octene obtained by Dow Chemical UK. It has melting

TABLE I
Biological and Physical Properties of CH
(Mean \pm SD, $n = 3$)

Properties	Determination
pH	5.8–6.2
Total nitrogen	11.33 g/100 g
Chromium	3 mg/kg
Sodium chloride	17.62 g/100 g
Sodium sulphate	3.09 g/100 g
Sodium carbonate	3.55 g/100 g
Density	0.4 kg/dm ³
Average molecular weight	1530 Dalton

TABLE II
The Content of the Most Important Amino Acids in CH
(Mean \pm SD, $n = 3$)

Type of amino acids	Concentration (g/100 g)
Hydroxyproline	5.0
Proline	11.86
Arginine	0.21
Phenylalanine	0.13
Lysine	5.23
Glycine	26.57
Leucine	3.84
Methionine	7.50
Tyrosine	0.42
Valine	0.55
Aspartic acid	2.79
Glutamic acid	6.38

temperature around 110°C, melt flow rate 5.0 g/10 min, density 0.87 g/cm², and molecular weight about 50,000 Dalton. Collagen hydrolyzate (CH) was supplied by Santa Croce Sull' Arno, Pisa, Italy, in a powdered form. It was obtained by lyophilization after fleshing solid waste of untanned bovine derma. Before use, CH was characterized in terms of biological and physical properties, including the amino acid content, as shown in Tables I and II. The average value for each test was taken for three samples to confirm the results, (Mean \pm SD, $n = 3$).

Preparation of biodegradable thermoplastic films^{19,20}

Thermoplastic films were prepared by mixing the ingredients in a Brabender plastograph, as shown in Table III. The content of CH in the binary blends MPE/CH was varied between 5 and 40 wt % CH, based on the total blend weight. The symbols of the blends indicate the compositions in CH. The resulting materials were placed in 0.42 mm thick steel frame between Teflon sheets under hydraulic press

TABLE III
Processing Behavior for Biodegradable Thermoplastic Composite Binary blends

Film ^a code	Ingredient concentration (wt %)	
	MPE	CH
MPE	100	0
MPE/5CH	95	5
MPE/10CH	90	10
MPE/20CH	80	20
MPE/40CH	60	40

^a Each blending Process was Carried Out in a Brabender Polymer Melt Instrument Under the Following Operation conditions: Temperature 120°C, Rotor Speed 50 rpm and Mixing Time of 5 min.

for compression molded heated at 130°C and 100 bar pressure applied, then kept for 5 min allowing for complete melting. Each composition was prepared in replicate for three films.

Characterization

FTIR spectroscopy

The thermoplastic films were examined using Perkin–Elmer Fourier transform infrared spectroscopy under certain conditions such as: scan resolution: 4 cm⁻¹, scan rate: 2 mm s⁻¹, range: 4000–600 cm⁻¹ and mode: transmission.

Thermal analysis

Thermogravimetric analysis (TGA/DTA) of the thermoplastic films were carried out using Perkin–Elmer 7 series thermal analyzer at heating rate of 10.0°C/min over the temperature range of 30–1000°C.

SEM morphology

The structural features of the prepared composite films were investigated, at high magnification ($\times 1000$) and resolution by means of energetic electron beam, using JEOL-5600 LV scanning electron microscope (SEM). Before observation, the fractured surfaces were coated with Au with SEM coating device (Edward spotter coater). Three micrographs were taken from different zones of each surface film under investigation.

Mechanical properties

Dog-bone shaped samples, stamp cut from the prepared films, were tested to assess the effect of film composition on the tensile properties and *E*-modulus by means of ASTM D638 tensile test (Tinius Olsen) at 10 mm/min. The average value for each test was taken for three samples to confirm the results (Mean \pm S.D., $n = 3$).

Simulated soil burial respirometric test²¹

The biodegradation tests were carried out in Erlenmeyer flasks containing a multistate substrate having the following composition: the bottom position of each flask was filled with 20 g perlite (hygroscopic aluminosilicate). The core was filled with a mixture of 20 g milled perlite and 10 g soil and the top was covered with other 20 g perlite. Film samples and control were placed in the core. Carbon dioxide produced within the incubation time from the test flasks flushed with CO₂-free air was determined as percent of theoretical quantity of CO₂ from the test material, deduced by its carbon content, corrected for the

amounts of CO₂ produced in the blanks (soil mixtures without any further carbon source) during the incubation time. The carbon dioxide produced was determined by adsorption in a solution of barium hydroxide (0.025N) and retitration with 0.05N HCL. Each test was carried out for three samples and average value was taken to ensure the results (Mean \pm S.D., $n = 3$).

RESULTS AND DISCUSSION

Morphology of pure MPE- and MPE/CH-blended thermoplastic films

Films of pure MPE are transparent but after blending with powdered CH, the modified polymer films display colors from pale white to light yellow. The change in color of the MPE is due to the presence of CH. The CH was obtained from fleshing solid waste of bovine derma which is the by-product of the preliminary steps of hide/skin treatment (soaking, bathing, unhearing, liming/deliming, pickling) before the chromium tanning process. The resulting CH was characterized as shown in Tables I and II. CH has average molecular weight, density, and total nitrogen about 1530 Dalton, 0.4 kg/dm³, and 11.33 g/100 g, respectively. In addition to, CH contains some amino acids with high concentrations such as proline and glycine (26.57 and 11.86 g/100 g, respectively). On the other hand, it contains some essential amino acids with low concentrations such as arginine, lysine, leucine, and methionine (0.21, 5.23, 3.84, and 7.50 g/100 g, respectively). This indicates that CH is low molecular weight hydrolyzed protein. Also, CH has chromium percentage about 3 mg/kg, this may be due to the metal contamination during the processes. However, this is a minimum acceptable level from environmental view and we can say that this novel biodegradable films could be used only in the packaging of the agricultural products and in the purification of waste water as membrane filter. Powdered CH is light yellow in color and so the colors and textures of the polymer film are changed according to the content of CH, as shown in Table III. The morphologies of the pure MPE and their film blends are shown in Figure 1. The blending nature of CH with MPE was studied by SEM. Very smooth MPE surface can be observed which indicates the purity of MPE. However, in its modified form, the protein-based biodegradable polymer shows some rough granular structure on the smooth surface of MPE, which increases with increasing the concentration of CH inside the polymer matrix. It can be concluded that at low CH concentration, the compatibility between MPE and CH is quite good.

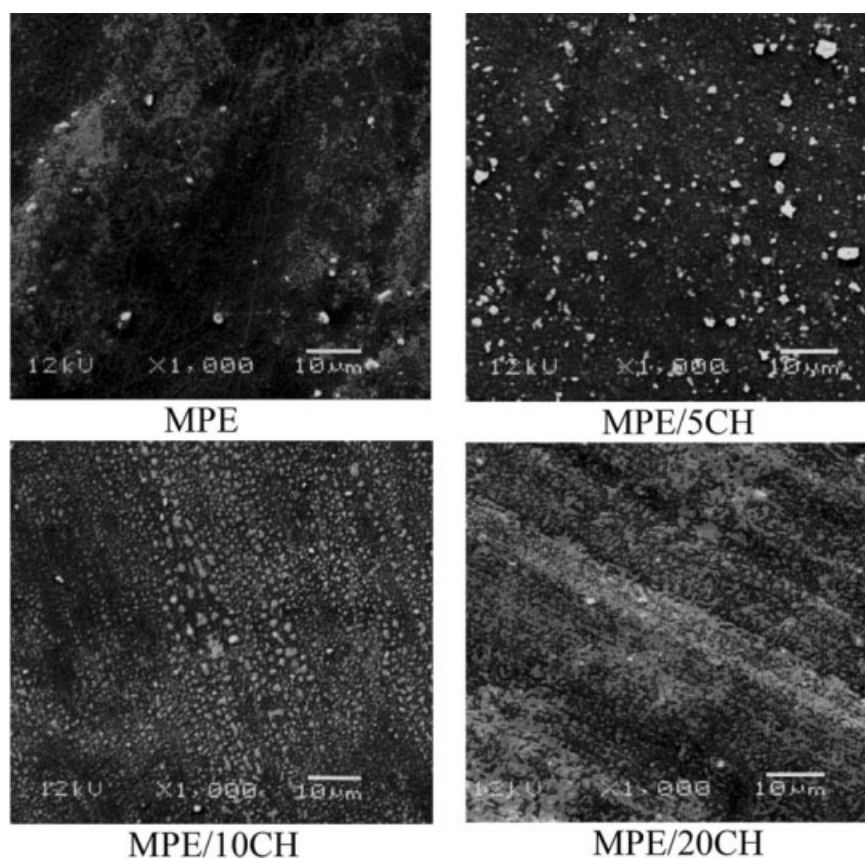


Figure 1 SEM- micrographs ($\times 1000$) of biodegradable thermoplastic films.

Physical structures of pure CH- and MPE/CH-blended thermoplastic film

The FTIR spectra were obtained to compare the physical structures of pure CH and its blended forms. It can be seen from Figure 2 (a,b) that due to blending the individual physical structure of MPE and CH do not significantly change, indicating that no new functionality have been developed and only the heights of the peaks have changed. The observed changes in peak heights are due to variations in the (mixing) concentration of MPE and CH in the blended polymer films. Polypeptides and proteins exhibit several characteristic absorption bands in the IR region of the spectrum. These occur in the spectra of all secondary amides and have been commonly labeled amides²² A, B, I, II, III, IV, V, VI, and VII. The presence of one peak at 1640 cm^{-1} corresponds to C=O stretching band of the amide I resulted in dramatically changes in the molecular structure of collagen hydrolyzate. On the other hand, the characteristic bands at 1520 and 1200 cm^{-1} correspond to amide II and III, respectively.²³ The amide II absorption is made up of amide N—H bending vibrations and C—N stretching vibrations (60% and 40% contribution to the peak, respectively), the amide III peak is complex, consisting of components

from C—N stretching and N—H in plane bending from amide linkages. The broad band centered about 3320 cm^{-1} is usually regarded as a complex band as a result of overlapping of the deformation stretching of the —OH group of water and the —NH group of collagen hydrolyzate. Figure 2(b) shows the FTIR spectra of MPE/CH film. It was illustrated that the characteristic bands at 2850 and 2910 cm^{-1} were appeared corresponding to the aliphatic hydrocarbon chains of MPE (—CH, —CH₂).

Thermal behavior of pure MPE- and MPE/CH-blended thermoplastic films

TGA/DTA data were obtained and recorded to compare the thermal behavior of pure MPE and their blends with different CH concentrations (10 and 40 wt %), as shown in Table IV and Figure 3. To give an interpretation to the observed thermal properties, it is necessary to describe the known aspects about the primary structure of collagen. It is accepted that to a rough approximation collagen is a block copolymer composed of sequences in which mainly α -amino acids are present including glycine at every third position (soft blocks), and sequences mainly made up of the imino acids proline (Pro) and

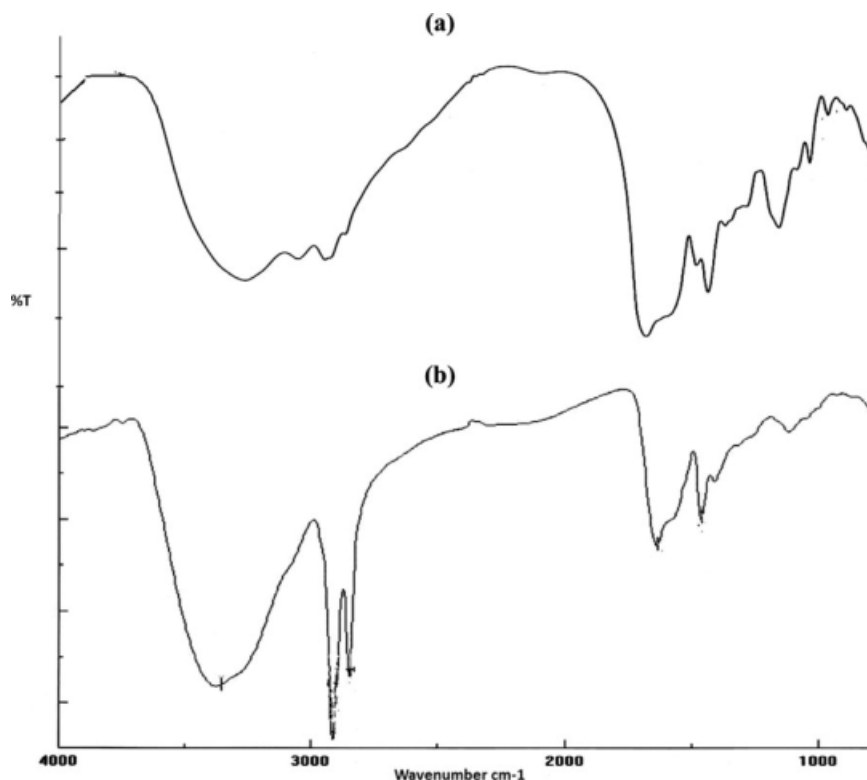


Figure 2 FTIR spectra of (a) CH and (b) MPE/CH.

hydroxyproline (Hypro), including glycine (Gly) at every third position (rigid blocks). Thus, the rough model²⁴ may be represented as follows:



where A and B denote any of the α -amino acids present in collagen.

TGA curves of the blends are specific with respect to the mixing ratio of the components that determines the phase distribution and the size of the dispersed phase in the continuous matrix. TGA data illustrated that two thermogravimetric steps had occurred. The first was assigned to CH decomposition, while the second one to MPE decomposition. It can be readily seen that the weight losses of the blends at 50–223°C, and 224–231°C are lower than the recorded values of 3.1 and 5.5 wt % for MPE/10CH, and respectively MPE/40CH blends. At

low CH concentration (10–20 wt %), the bound water molecules were decomposed firstly around 50–120°C, followed by the low molecular weight polypeptide fragments. The changes in the thermal properties are significant in the second thermogravimetric step. There are no changes in the characteristic temperatures (232–454°C), but the weight losses of all the blends are smaller (95 and 93 wt %, for MPE/10CH and MPE/40CH, respectively) relative to CH-free (99.1 wt % for MPE). It can be concluded that the thermal behavior results from the super imposition of the evaporation of volatiles present in the composite with the transition related to that of collagen hydrolyzate. In other words, the behavior of the binary blends depends on reciprocal solubility, the distribution of the CH in the MPE matrix or the distribution of the formed separated phases, in addition to the heating at various temperatures.

TABLE IV
TGA Data of the Prepared Biodegradable Thermoplastic Films (Mean \pm SD, $n = 3$)

Film code	Weight loss (wt %)		
	50–223°C	224–231°C	232–454°C
MPE	–	–	99.1
MPE/10CH	3.1	–	95
MPE/40CH	–	5.5	93

Mechanical properties of pure MPE- and MPE/CH-blended thermoplastic films

Mechanical properties evaluated on the basis of the stress and elongation at yield and at break, in addition to E -modulus parameters, as shown in Table V. It was observed that elongation linearly increases with CH content until 20 wt %, relative to pure MPE. High CH concentration (40 wt %) leads to an increasing in melting flow index²⁵ (MFI), which is

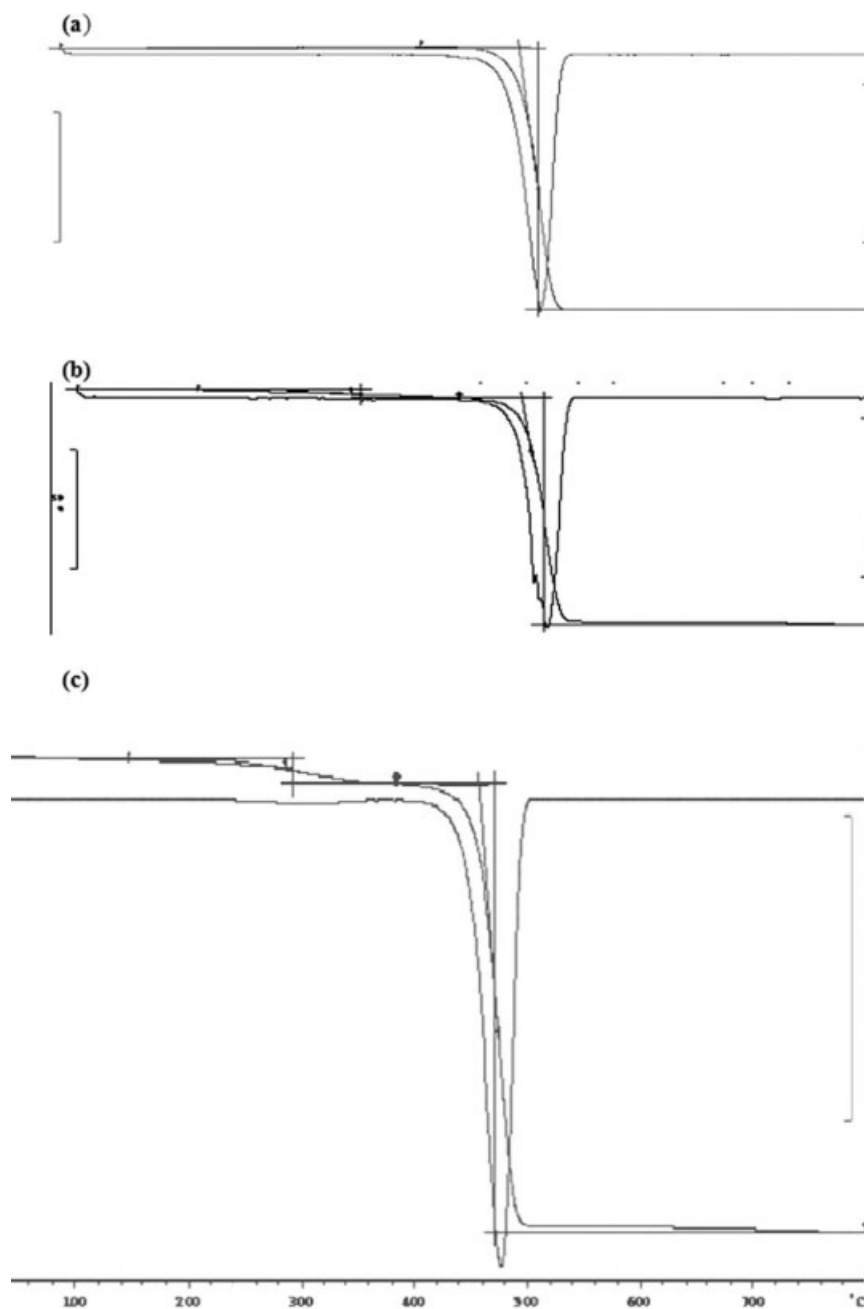


Figure 3 TGA/DTA diagram of (a) MPE, (b) MPE/10CH and (c) MPE/40CH.

TABLE V
Mechanical Properties of the Prepared Biodegradable Thermoplastic Films (Mean \pm SD, $n = 3$)

Film code	Mechanical properties				
	Stress at yield (MPa)	Stress at break (MPa)	Elong. at yield (%)	Elong. at break (%)	<i>E</i> -modulus
MPE	1.8	8.0	30	686	12.0
MPE/5CH	1.8	7.8	38	740	10.5
MPE/10CH	1.6	7.5	52	743	10.2
MPE/20CH	1.6	7.4	67	746	9.5
MPE/40CH	1.4	7.4	67	740	9.0

correlated with the mutual interactions within component of the blend (MPE and CH). In other words, stress at (yield and break) and elongation at break of all MPE/CH blends are slightly lower and higher than that of CH-free (MPE) equivalent, respectively. Thus, CH in the investigated ratios has plasticizing effect on MPE in accordance with the previous studies on cast films based on PVA and gelatin waste from pharmaceutical industries.¹¹ Chiellini et al. showed that PVA and animal gelatin blends have two glass transition temperatures corresponding to each plasticized component, thus suggesting the formation of immiscible blends within the composition range studied (10–70%). It can be concluded that no problems were observed during processing of model blends and extruded tapes. Films with CH showed faster mineralization in comparison with parental CH-free films. As evidenced by the results, the film containing CH has undergone significantly faster biodegradation in comparison with CH-free film.

Biodegradation of the prepared thermoplastic films

One of the main purposes of this study was to investigate the influence of CH addition on the biodegradation rate and extent of MPE/CH binary blends. Biodegradation respirometric experiments were carried out under a simulated soil burial test²¹ in accordance with the main goal of this study that is the realization of eco-compatible mulching blends. Films containing variable amounts (5–20 wt %) of CH showed different biodegradation extents not exceeding 63% of mineralization after 24 days of incubation, whereas pure CH film sample undergoes about 69% biodegradation in the same time frame, as shown in Figure 4. The results herewith reported clearly evidenced a positive influence of CH on the biodegradation rate and extent of MPE/CH blended film, however, the results were obtained by increasing amounts of CH. Thus, by considering the possibility of modulating the environmental duration of the blended films without crosslinking agent was assessed in order to find the more suitable blend compositions for obtaining a reliable self fertilizing mulching film. The reduction in biodegradation rate and extent of CH-based thermoplastic films caused by the addition of MPE (synthetic polymer) could be tentatively attributed to a physical entanglement exerted by the MPE on the protein. On the other hand, CH seems did not increase the biological degradation of MPE in comparison with MPE-free film, in the presence of soil microflora, as revealed by the results recorded in the presence of different films having decreasing amount of CH, such as MPE/5CH film. The fairly limited and slow mineralization of MPE under these incubation conditions aimed at simulating soil burial was also ascertained most

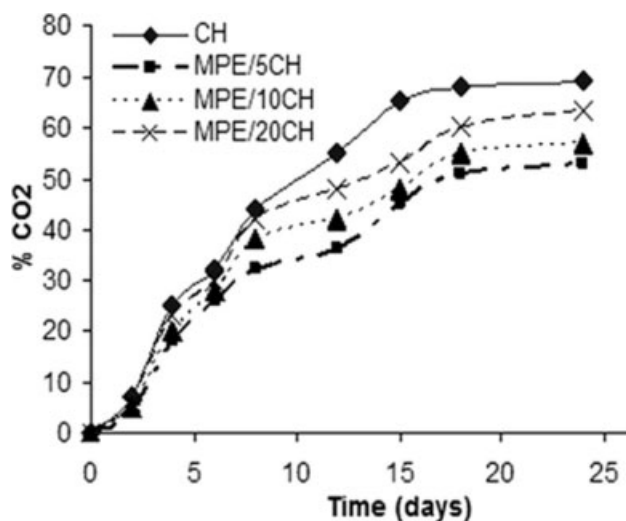


Figure 4 Biodegradation curves of MPE/CH biodegradable thermoplastic films at different time intervals.

likely due to the strong and almost irreversible adsorption of the synthetic polymer to the soil matrices, especially clay.

CONCLUSIONS

Process integration of leather manufacturing industry waste has high potential for larger economic returns through enzymatic retrieval of biodegradable polymer (BDP) and other value-added products using process biotechnology. The retrieved collagen hydrolyzate (CH) and BDP have already gained lots of agro-industrial applications in different countries. Modified polyethylene (MPE) matrix showed a capability to incorporate CH. The relatively low molecular weight of CH allowed a good blending with MPE as shown by SEM. Thermal stability of CH was compatible with MPE through melting processing and compression molding. Elongation of MPE/CH thermoplastic films increased with increasing the concentration of CH, until 20 wt %, while stress does not have a significant affect for valuable specific applications in the packaging and agriculture sectors. The study of SEM-morphology, thermal and mechanical properties of the prepared thermoplastic films based on MPE and CH demonstrates that the addition of CH up to 20% in the formulations yields blends with good thermal processability, transparency, cohesiveness, and flexibility in addition to products with valuable practical mechanical properties. It is clear that the reutilization of collagen hydrolyzate derived from the leather industry for the production of biodegradable thermoplastic films appears feasible and promising. The obtaining of these positive results, in absence of crosslinking or compatibilizing agents allows for low cost and simple processing procedure.

The author wish to thank prof. D.R.K. Harding at IFS, Faculty of Sciences, Palmerston North, New Zealand, for his skilful assistance in the final revision of this work.

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