Valorization of Maize Bran to Obtain Biodegradable Plastic Films

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ABSTRACT: Cellulose-enriched residues from maize bran can be transformed into plastic films after esterification of the cellulose by various fatty acid chlorides. Alkali pretreatment and acidic activation of the sample are necessary before the esterification reaction in order to form esters. The degradation by microorganisms of the plastic film was also studied. We showed that the isolated strain (*chromobacterium violaceum*) can hydrolyze modified cellulose plastic films. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 3040–3045, 1999

Key words: maize bran; cellulose; esterification; fatty acid; plastic films; biodegradation

INTRODUCTION

Cellulose is one of the most abundant of naturally occurring polymers. Modification of this polysaccharide is of great interest because this compound is found in abundance in agricultural wastes.

Plastics obtained from plant sources could be good candidates for replacement of synthetic plastics, if they are biodegradable and possess similar physical properties. In particular, cellulose derivatives are widely used by various industries[1] (food, pharmaceuticals, detergent, tobacco, textiles, etc.). It is well known that microcrystalline cellulose can provide, after chemical modifications, new biodegradable materials,[2,3] which could be used for packaging food products, for instance. Long-chain fatty acid esters of cellulose have been less studied, although they have interesting properties.[2,4] But to the best of our knowledge, there are only a few results on materials synthesized from polysaccharide wastes,[5] particularly from maize bran. This latter substrate is constituted mainly of heteroxylans (45%) and cellulose (13%).[6] It is possible to extract heteroxylans by alkali treatments, and the residue is especially rich in cellulose, more than 50%.

In the present work we report on the results of the esterification of cellulose included in crude maize bran with various fatty acid chlorides, e.g. lauric, octanoic and hexanoic. Plastic film degradation by microorganisms was also studied.

MATERIALS AND METHODS

Materials

All reagents and solvents were purchased from Aldrich Chemical Company and used without further purification. Maize bran was provided by ULICE (Riom, France).

Extraction of Heteroxylans and/or Lignin

- Treatment by KOH + NaBH$_4$

  Samples (80 g) of maize bran were stirred with 800 mL of 1.5M potassium hydroxide and 610 mg (1%) of sodium borohydride at 100°C for 2 h. The mixture was then centrifuged for 30 min at room temperature. The supernatant was disgarded, and the residue was washed with 800 mL of deionized water. This procedure was repeated three times. The resulting material was neutralized with acetic acid and then washed with ethanol (3 × 500 mL) and acetone (3 × 500 mL). After drying for 24 h at 40°C, the mass of cellulose-enriched residue obtained was 20 g (25% yield).

- Treatment by KOH + H$_2$O$_2$

  Samples (80 g) of maize bran were stirred with 800 mL of 1.5M potassium hydroxide and 22.4 mL (1%) of hydrogen peroxide at reflux. After 2 h, the workup was carried out as described above. The result was 12 g of cellulose-enriched substrate (15% yield).

- Treatment by KOH

  Samples (10 g) of maize bran, finely ground, were stirred with 200 mL of 2M potassium hydroxide at 70°C for 5 h. The cellulosic residue was recovered by filtration, and washed with 500 mL of distilled water until neutral pH. The residue was then dried at 30°C for three days. The result was also 1.5 g of cellulose-enriched residue (15% yield).

Cellulose Activation

We immersed 1 g of cellulosic-enriched residue at room temperature in 50 mL of 0.5M sulfuric or phosphoric acid for 1 min. The residue obtained by filtration was dried at 40°C until the initial weight was recovered.

Esterification

We ground 1 g of cellulosic residue (6 mmol, 1 eq) with a mechanical grinder and stirred it with toluene (30 mL), fatty acid chloride (30 mmol, 5 eq), and 5 mL of pyridine (60 mmol, 10 eq) at reflux for 5 h. The mixture was filtered, and pyridine was evaporated. The cellulosic esters were precipitated with 100 mL of ethanol (95%). The precipitate was recovered by filtration. The casting was realized by dissolution of the precipitate in the chloroform and by evaporation at room temperature.

Degree of Substitution

- Saponification

  After saponification the esters produced were analyzed for their fatty acid content using the back titration method, in which the reaction product (about 300 mg) was treated by 10 mL of 0.25M sodium hydroxide in ethanol at 30°C for 16 h.

- Elemental analysis

  Elemental analysis of carbon and hydrogen were carried out by combustion at the Service Régional de Microanalyse, Université Pierre et Marie Curie, Paris.

Sugar Composition

Individual neutral sugars were analyzed by gas–liquid chromatography (GLC)[7] after total hydrolysis and derivation of the monomers into alditol acetates. Cellulose-rich residues[8] were prehydrolyzed for 2 h in 13M sulfuric acid at room temperature, then hydrolyzed in 2M sulfuric acid at 100°C for 2 h. Cellulosic glucose was estimated by measuring the difference between glucose content using GLC with and without prehydrolysis.

Spectroscopy

Infrared spectra were recorded in cm^{-1} on a FTIR Perkin Elmer (Spectrum 1000) spectrometer.

NMR spectra were recorded using a Bruker DPX 400 apparatus (400 MHz for ^{1}H or 100 MHz for ^{13}C). Chemical shift was measured in ppm with TMS as the internal standard.

Microorganisms Selection

Different fragments of natural cellulose derived from leaf, stem, grass, or compost were added to different sterile flasks containing a sample (5 cm × 2 cm) of the plastic film in 10 mL of water. Growth was examined at room temperature. In each flask, an isolation was realized on nutritive agar-agar at room temperature for 48 h. Every
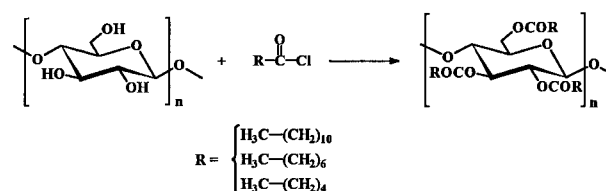
Figure 1 Esterification of maize bran by fatty acid chlorides.

Table I   Composition of Crude or Treated Maize Bran

Composition	Substrate			
	Crude Maize Bran	Maize Bran + KOH + 1% H_2O_2	Maize Bran + KOH + 1% $NaBH_4$	Maize Bran + KOH + H_2O
Arabinose	13.5	3.2	5.5	4.4
Xylose	24.9	7.7	11.6	8.4
Mannose	0.6	1.2	1.2	0.8
Galactose	3.7	1.2	2.1	1.9
Glucose	11.6	3.4	5.5	8.8
Cellulose	17.3	72.9	60.3	58.0
Glucuronic acid	3.8	1.7	2.0	3.0
Total doses	75.4	91.3	88.2	85.3
Proteins	4.5	0.3	0.2	0.5
Lignine	1.8	0.8	0.7	4.0
Ashes	0.8	1.3	0.9	3.1

Heteroxylans = arabinose + xylose + mannose + galactose + glucuronic acid.

species was transferred into another flask containing a second sample of plastic film (5 cm × 2 cm) in 10 mL of phosphate buffer. Flasks were maintained under agitation at room temperature for one month.

RESULTS AND DISCUSSION

Hydroxyl groups of the cellulose contained in the maize bran were esterified by fatty acid chlorides (Figure 1).

Some experiments made on crude maize bran have showed poor film forming properties of the plastic obtained after direct esterification of this material. Examination of the composition of crude maize bran (Table I) may support an explanation of these results as being based on the presence of large quantities of noncellulosic materials, mainly heteroxylans. We therefore did different treatments in order to eliminate the heteroxylans, lignin, and proteins, and to make available cellulose-enriched residues. These treatments consisted of an alkaline reaction followed or not by oxidative (H_2O_2)[9] or reductive $(NaBH_4)$[10] treatments. It can be seen from Table I that the ratio of heteroxylans was greatly diminished by alkaline treatment and that lignin was elimi-

Table II   Esterification by Lauroyl Chloride of Crude and Treated Maize Bran

Entry	Pretreatment	Activation	Lauroyl Chloride	Yield[a] (Aspect)	DS[b]	DS[c]
1		H_2SO_4 (0.5M)	5 eq	No plastic film	nd	nd
2	KOH (1.5M) + 1% H_2O_2	H_2SO_4 (0.5M)	5 eq	3% (Very thin plastic film)	nd	nd
3	KOH (1.5M) + 1% $NaBH_4$	H_2SO_4 (0.5M)	5 eq	24.5% (Sticky and thin plastic film)	1.7	1.6
4	KOH (2M) + H_2O	H_2SO_4 (0.5M)	5 eq	75% (Smooth and bright plastic film)	2.2	2.1
5	KOH (2M) + H_2O	H_2SO_4 (0.5M)	10 eq	54% (Smooth and bright plastic film)	2.3	2.2
6	KOH (2M) + H_2O	H_3PO_4 (0.5M)	5 eq	63% (Thick and bright plastic film)	1.8	2.1

[a] Yields are calculated from the ratio of cellulose in the sample and the degree of substitution when it is known.
[b] Degree of substitution determined by titration after saponification.
[c] Degree of substitution determined by elemental analysis.

Table III Esterification of Treated Maize Bran with Octanoyl and Hexanoyl Chlorides

Entry	Pretreatment	Activation	Acid Chloride	Yield[a] (Aspect)	DS[b]	DS[c]
1	KOH (2M) + H_2O	H_2SO_4 (0.5M)	Octanoyl 5 eq	80% (Brown and bright plastic film)	2.0	2.05
2	KOH (2M) + H_2O	H_2SO_4 (0.5M)	Hexanoyl 5 eq	74% (Yellow and bright plastic film)	1.9	1.85

[a] Yields are calculated from the ratio of cellulose in the sample and the degree of substitution when it is known.
[b] Degree of substitution determined by titration after saponification.
[c] Degree of substitution determined by elemental analysis.

nated by oxidative as well as by reductive treatments.

Prior to esterification, maize bran cellulose was activated by acidic treatment in order to make hydroxyl groups more accessible.[11] The cellulose was then esterified by fatty acid chloride in the presence of pyridine in toluene. After extraction and purification, the plastic residue was submitted to casting by evaporation at room temperature of chloroform. The degree of substitution (DS) of these materials was determined by back titration after saponification[12] and by elemental analysis of carbon and hydrogen. These results are summarized in Table II.

The esterification of samples treated to eliminate both heteroxylans and lignin (Table II, entries 2 and 3) gave comparable plastic films to those obtained by esterification of microcrystalline cellulose, but with a lower yield (25%). This may be explained by some degradation of cellulosic material.

The sample treated only with potassium hydroxide led to the best results (Table II, entry 4); the yield was 75%. The utilization of a large excess of fatty acid chloride (Table II, entry 5) resulted in a 54% yield, which was lower than the former, because elimination of fatty acid excess requires supplementary washings. Activation with other acids, such as phosphoric acid, (Table II, entry 6) did not give the best results.

All these results show that the treatment with KOH is sufficient and that only the presence of the heteroxylans (58%) in the maize bran prevents esterification and/or formation of plastic. The lignin in the treated sample does not seem to prevent the reaction. The use of excess fatty acid did not improve the yield (Table II, entry 5).

We have thereafter studied the variation of the length of the fatty acid chloride chain to observe the influence on its plastic film aspect. We obtained the esterification after alkali treatment and activation of the maize bran with octanoyl
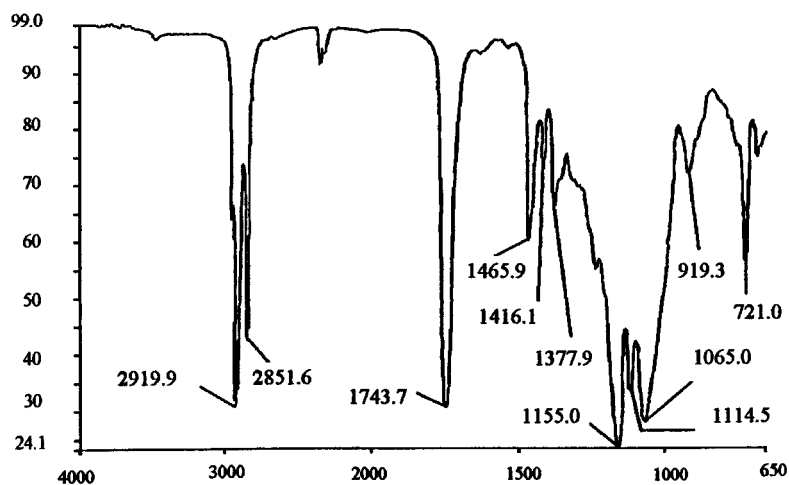
Figure 2 Infrared spectrum of plastic film.

Table IV   Band Assignments for Cellulosic Esters (Plastic Film)

Wave Numbers (cm^{-1})	Band Assignments
2851–2955	ν CH
1743.7	ν C=O (ester)
1465.9	δ CH$_3$
1416.1	δ_s CH$_2$
1377.9	δ CH
1155.0	ν_{as} C—O—C (bridge)
1114.5	ν_{as} (ring)
1065.0	δ C—O—C (pyranose ring)

Table VI   NMR-^{13}C of Plastic Film

C	δ (ppm)
C=O	172.0, 172.8, 173.4
Sugar	
C-1	101.0
C-2	84.0
C-3	71.0
C-4	84.0
C-5	69.0
C-6	60.0
Alkyl Chain	
$\underline{C}$H$_3$	14.1
$\underline{C}$H$_2$	29.7, 29.6, 29.4, 22.7

and hexanoyl chloride (Table III). In these two cases, we obtained plastic films with good yields (75 and 80%) and degrees of substitution on the order of 2. Regardless of the chain length fixed on the cellulosic residue, a plastic film was obtained.

The tensile strength and elongation of the plastic film obtained were determined, and glass transition was determined by the DSC method. The plastic showed a tensile strength of 2.3 Mpa and an elongation of 149%. The plastic aspect of these films could be explained by the samples having a T_g ($-10°$C) that was lower than room temperature.

The plastic films obtained have been analyzed by infrared and NMR spectroscopies. Figure II shows the infrared spectrum of the plastic film, obtained after esterification with the lauroyl chloride of the maize bran enriched in cellulose. Characteristic bands of the IR spectra are given in the Table IV.

When compared to the IR spectra of microcrystalline cellulose, we noticed the disappearance of the band at 3,340 cm^{-1}, characteristic of hydroxyl groups; the increase of the intensity of the bands

Table V   NMR-^{1}H of Plastic Film

| H	δ (ppm)
Sugar	
H-1	4.3
H-2	4.8
H-3	5.0
H-4	3.6
H-5	3.5
H-6	4.1
Alkyl Chain	
$\underline{C}$H$_3$	0.8
$\underline{C}$H$_2$	1.0–2.2

at 2,900 cm^{-1}, due to the presence of alkyl chains; and the presence of a new band at 1743.7 cm^{-1}, characteristic of a carbonyl group.

Signals observed in NMR spectroscopy of the proton and carbon-13 are given in Tables V and VI, respectively (Figure 3).

The ^{1}H-NMR spectra showed peaks between 0.8 and 2.2 ppm, corresponding to the alkyl groups and peaks of the anhydroglucose unit between 3.5 and 5.5 ppm. Moreover, no peaks were observed around 6–7 ppm and 10–13 ppm, showing the absence of aromatic and fatty acid residues (Table V). The ^{13}C-NMR spectra showed three peaks, at 172.0, 172.8, and 173.4 ppm, corresponding to the carbonyl groups linked to the sugar carbons C-2, C-3, and C-6, respectively (Table VI).

Alteration of Modified Maize Bran by Microorganisms

Four species of microorganisms have been observed (bacteria and fungi). After isolation and growth with plastic film as nutriment, the film colonization was observed for one species charac-
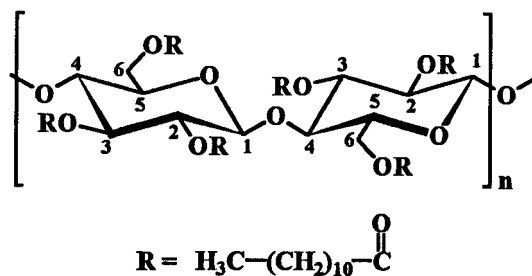
Figure 3   Numeration of carbons for NMR spectroscopy.

terized by a purple pigmentation. A pure clone of bacteria was obtained after a second isolation. These bacteria survived in a poor universal mineral liquid medium without protein and sugar and with the modified cellulose plastic film as one nutriment.

A significant modification in mechanical properties of the plastic film was observed. It was especially easy to reduce it to powder. It is noteworthy that a plastic film sample maintained in water as a reference during the same period was unchanged in its structural properties (same weight, same ^{1}H-NMR spectrum). On the other hand, the loss of weight was about 6% after one month. Probably, the excreted bacterial enzymes can degrade the plastic film, degradation products being one source of carbon for the microorganisms.

The isolated strain has been characterized as gram negative, catalase positive, aero-anaerobic and with a polar mobility. Oxydase was difficult to determine because this strain is pigmented. Meanwhile, our observations strongly support the idea that this strain belongs to a chromobacterium species. Thus, this strain can hydrolyze modified-cellulose plastic films. Optimal conditions of digestion are under investigation.

In conclusion, we have described a method for valorization of maize bran, which is an easily accessible, low-price substrate. Esterification of the bran after basic pretreatment and acidic activation formed esters that can be used as biodegradable plastic. In addition, prior elimination of lignin with reductive or oxidative agents was not necessary.

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