Preliminary Study of Formation of Films from Cellulose-Enriched Agricultural By-Products

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ABSTRACT: Two agricultural by-products, sugar-beet pulp and wheat bran, have been examined for their suitability to be transformed into bioplastics by esterification by lauroyl chloride. Influence of cellulose content was studied on eleven samples enriched in cellulose after chemical or enzymatic removal of pectins from sugar-beet pulp and heteroxylans from wheat bran. After a pretreatment by immersion in 0.5 mol/L sulfuric acid, esterification was carried out with lauroyl chloride. Neither the amount of cellulose nor the extraction treatment had a marked influence on the formation of plastic. A film could be obtained from all the wheat-bran samples, including samples with low cellulose content, but only from one sugar-beet pulp sample. The crystallinity of the cellulose in sugar-beet pulp and wheat bran were different. The nature of cellulose could be responsible for the failure of sugar-beet pulp residues to form plastic. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 331–337, 1998

Key words: sugar-beet pulp; wheat bran; cellulose; esterification; lauroyl chloride; degree of substitution; lignin; films

INTRODUCTION

Synthetic plastic waste is becoming a serious problem because most is dumped or burnt after use. Plastics obtained from plant sources can be good candidates for replacement of synthetic plastic if they are "biodegradable" and possess similar physical properties. In particular, cellulose derivatives are widely used by various industries[1] (food, pharmaceuticals, tobacco, detergent, textiles, adhesives, etc.) and cellulose-based films have been extensively studied because of their efficient oxygen and hydrocarbon barrier properties.[2–4]

The esterification of cellulose by fatty acids (acetic, propionic, butyric, etc.) has been widely studied and used in industry[1] (thermoplastics, textiles, films, resins, etc.). Long-chain fatty-acid esters of cellulose have been less studied, although they have interesting properties[5–8]; we have shown that cellulose can be esterified by lauroyl chloride, and that the esterified products obtained after evaporation of solvent at room temperature have filmogenic properties.[9] As the chain length of the fatty acid increases, its reactivity towards cellulose diminishes,[1,10] and an acidic pretreatment[5] or an alkaline activation[7,8] is needed before esterification.

Sugar-beet pulp and wheat bran, two important and very cheap agricultural by-products, contain, respectively, about 750 and 650 mg/g (w/w) of cell wall polysaccharides.[11,12] Polysaccharides consist largely of pectins and cellulose in sugar-beet pulp, and heteroxylans and cellulose in

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wheat bran. These by-products also contain some lignin[11,12] (80 mg/g in wheat bran and 20 mg/g in sugar beet pulp). It is possible to extract pectins or heteroxylans by enzymes or by other means, and the residues are especially rich in cellulose, more than 500 mg/g (w/w).[13,14]

Beet pulp and wheat bran may be interesting as sources of cellulose and could form films after esterification by fatty acid. In this article, we have therefore studied the influence of cellulose content of the sugar-beet pulp and wheat bran on the esterification reaction, using various extraction methods.

MATERIALS AND METHODS

Materials

Destarched wheat bran, sugar-beet pulp, and two "industrial" enriched-cellulose residues, recovered after acidic treatment with sulfuric acid of wheat bran (W-ind) and sugar-beet pulp (B-ind), were provided by ARD (Pomacle, France).

Extraction of Pectins from Sugar-Beet Pulp

Acidic Extraction

Sugar-beet pulp (50 g) was stirred with 0.05 mol/L hydrochloric acid (350 mL) at 85°C for 30 min. The residue was recovered by filtration (pore diameter < 15 μm) and washed with water until washings reached pH 6. The residue (B-ac) was washed with 95% ethanol (3 times, 300 mL), then acetone (3 times, 300 mL), and dried in an oven at 40°C overnight.

Alkaline Extraction

Sugar-beet pulp (50 g) was stirred with 0.05 mol/L sodium hydroxide (400 mL) at 4°C for 30 min. The residue was recovered by filtration (pore diameter < 15 μm) and washed with water until washings reached pH 7. The residue (B-alk) was washed with 95% ethanol (3 times, 300 mL), then acetone (3 times, 300 mL), and dried in an oven at 40°C overnight.

Enzymatic Extraction

Enzyme preparation SP 584 (Novo Nordisk, A/S, Bagsvaerd) rich in various pectinolytic activities and mostly devoid of cellulolytic activities[15] was added to sugar-beet pulp (150 mg of proteins/15 g of pulp) suspended in 450 mL de-ionized water. The suspension was stirred for 120 h at 40°C and 150 mg of additional enzymatic proteins were added in the medium at 24 and 48 h. The reaction was stopped by immersion in boiling water for 15 min and the residue was recovered by filtration (pore diameter < 15 μm). The residue (B-enz) was washed with distilled water, with 95% ethanol (3 times, 300 mL), then with acetone (3 times, 300 mL), and dried in an oven at 40°C overnight.

Chemical Extraction of Heteroxylans from Wheat Bran

Four samples (W-alk1–W-alk4) were obtained by alkaline treatments. Destarched wheat bran (50 g) was stirred with 1 to 2 mol/L potassium hydroxide solutions (500 mL) for different extraction times (0.5 to 5 h), at various temperatures (65 to 100°C). The residues were recovered by filtration (pore diameter < 15 μm) and washed with distilled water until washings reached pH 7. The residues were washed with 95% ethanol (3 times, 300 mL) and acetone (3 times, 300 mL), then dried in an oven at 40°C overnight.

"Delignification" of Wheat Bran and Sugar-Beet Pulp

Samples (50 g) were stirred with 1 mol/L sodium hydroxide (500 mL) and 14 mL (130 volumes) hydrogen peroxide[16] at 70°C for 1 h. The medium was adjusted to pH 7 with 13 mol/L hydrochloric acid and the residue was recovered by filtration (pore diameter < 15 μm) and washed with distilled water until washings reached pH 6.5. The residues (W-ox, B-ox) were washed with 95% ethanol (3 times, 300 mL) and acetone (3 times, 300 mL), then dried in an oven at 40°C overnight.

Esterification of Cellulose

The sample (3 g) was immersed at room temperature in 0.5 mol/L sulfuric acid (50 mL) for 1 min (Fig. 1). The residue was recovered by filtration and dried at 40°C until the initial weight was recovered.[5]

Then the residue (3 g) was ground and stirred with toluene (50 mL), lauroyl chloride (42 mL), and pyridine (15 mL) at 80°C for 5 h. The ester was precipitated with 98% ethanol (100 mL) and recovered by filtration. The esterified material was dissolved in toluene and precipitated again with 98% ethanol (100 mL) to remove residual

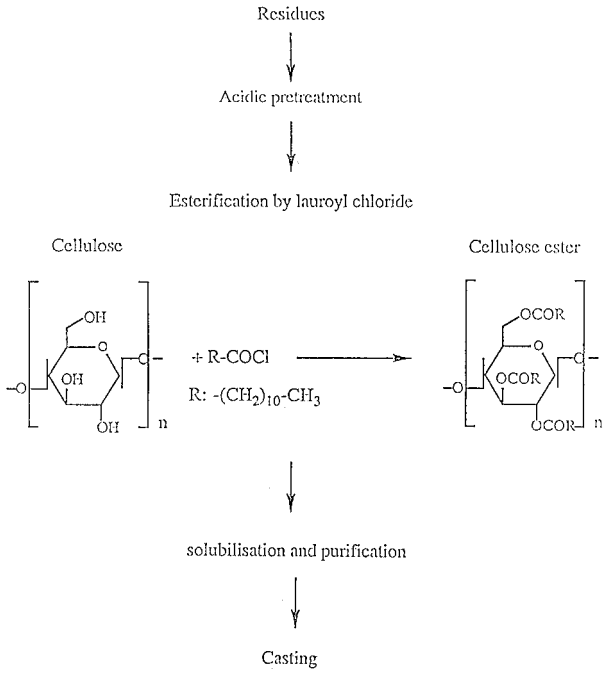

Figure 1 Chemical modification of enriched-cellulose residues.

pyridine and excess lauroyl chloride. Cellulosic ester was then dissolved in dichloromethane, chloroform, or toluene, and insoluble particles were removed by filtration. Filmogenic properties were evaluated by visual inspection of the esterified product after evaporation at room temperature. The product (film, individual particles, heterogeneous product) was stored under vacuum for 2 days to remove any remaining traces of solvent.

General Methods

All results are expressed relative to the dry matter content determined by drying at 120°C for 3 h.

Individual neutral sugars were analyzed by gas–liquid chromatography (GLC)[17] after total hydrolysis and derivation of the monomers into alditol acetate. Conditions of pre-hydrolysis and hydrolysis were optimized for each product. Beet-pulp products were treated first for 3 h in 13 mol/L sulfuric acid at room temperature, then 1 h in 1 mol/L sulfuric acid at 100°C, whereas wheat-bran products were hydrolyzed first for 2 h in 13 mol/L sulfuric acid at room temperature, then 2 h in 1 mol/L sulfuric acid at 100°C.

Cellulosic glucose was estimated by the difference between glucose content as measured by GLC with (total glucose) and without (noncellulosic glucose) prehydrolysis.

Uronic acids were quantified by colorimetry using *m*-hydroxybiphenyl as chromogen[18] after hydrolysis (30 min in 13 mol/L sulfuric acid at room temperature, then 3 h in 1 mol/L sulfuric acid at 100°C). Standards were galacturonic acid and glucuronic acid for sugar-beet pulp and wheat bran, respectively.

Proteins ($N \times 6.25$) were determined by a semi-automatic Kjeldhal procedure.

Lignin was quantified by the method of Klason.[19]

Ash was measured after incineration overnight at 500°C then for 2 h at 900°C.

X-ray diffraction powder diagrams were recorded using a transmission technique with an XRG 3000 X-ray generator (INEL, Orleans, France) equipped with a quartz monochromator (λ = 0.15405 nm) and a curve position sensitive detector (CPS 120, INEL, Orleans, France). The working conditions were 40 kV, 30 mA, recording time: 2 h.

Lauric acid was released after saponification of esterified product (0.3 g) in a 0.25 mol/L NaOH (10 mL) in solution in 95% ethanol, during 16 h at 30°C. NaOH excess was titrated with 0.1 mol/L hydrochloric acid and the amount of lauric acid was estimated by the difference between total NaOH and excess NaOH. A blank was prepared in the same conditions.

The degree of substitution in the text refers to the molar ratio between lauric acid and cellulose as measured in the initial sample.

RESULTS AND DISCUSSION

Composition of the Residues

Native Wheat Bran and Native Sugar-beet Pulp

Cellulose-enriched materials were obtained by extraction of pectins from sugar-beet pulp and of arabinoxylans and lignin from wheat bran. The main constituents of beet pulp (Table I) were sugars (~ 750 mg/g), mainly arabinose (~ 200 mg/g) and galacturonic acid (~ 200 mg/g) from pectin, and glucose (~ 240 mg/g), mostly from cellulose. Destarched wheat bran (Table II) was also rich in sugars (~ 600 mg/g), mainly arabinose (~ 130 mg/g) and xylose (~ 210 mg/g) from arabinoxylan, and glucose (~ 210 mg/g), mostly from cellulose. Lignin represented about 80 mg/g in wheat bran, a value higher than in sugar-beet pulp (~ 20 mg/g). These compositions are in agreement with previously published data.[12,13,20]

Table I Sugar-beet Pulp Residues Composition (mg/g)

	Beet Pulp	B-ind	B-ac	B-alk	B-ox	B-enz
Treatment	No	Acid (Industrial)	Acid	Alkaline	Alkaline Peroxide	Enzymatic
Yield of residue (mg/g)	1,000	nd	781	827	448	244
Rhamnose	30	8	29	22	23	5
Arabinose	201	38	190	200	222	15
Xylose	13	22	17	15	26	36
Mannose	13	22	13	11	17	30
Galactose	54	16	57	51	62	11
Glucose	239	480	248	223	459	545
Galacturonic acid	198	101	157	154	70	24
Cellulose	207	456	239	196	441	519
Pectins[a]	483	163	433	427	377	55
Lignin	18	20	25	40	nd	nd

nd, not determined.
[a] Pectins = rhamnose + arabinose + galactose + galacturonic acid.

Cellulose-enriched Sugar-beet Pulp

Various extractions were tested to remove pectins, with the aim of increasing cellulose content (Table I). Three chemical extractions and one enzymatic hydrolysis were used; a cellulose content varying from ~ 200 to ~ 520 mg/g was obtained, and the amount of residues left varied from ~ 240 to ~ 830 mg/g. The most efficient treatment was enzymatic hydrolysis which removed ~ 96% of pectins and gave a cellulose content of about 520 mg/g in the residue, as previously reported.[15] The chemical extractions had a lower efficiency: ~ 30% of the pectins were removed after acidic (B-ac) or alkaline (B-alk) extraction, and 60% with alkaline peroxide treatment (B-ox). Rouau and colleagues[21] obtained a similar result after extraction with the hydrochloric acid, although the treatment was repeated three times. Glucose content in B-ind was much higher than in B-ac, although both residues were obtained after acidic treatment.

The alkaline peroxide treatment increased the amount of glucose to ~ 440 mg/g. Thibault and Rouau,[20] using a similar treatment, obtained a residue with a glucose content of ~ 500 mg/g. The amount of galacturonic acid was still high after chemical extraction, as well as the amount of arabinose. About 20% of glucose was lost, probably due to oxidative degradation of the cellulose molecule in the alkaline medium, known as oxydative peeling.[22]

Cellulose-Enriched Wheat Bran

To remove arabinoxylans from destarched wheat bran, various alkaline extractions were tested (Table II). Cellulose content (~ 220–420 mg/g) and yield of residues after extraction (~ 330–490 mg/g) depended on the concentration of alkali and the temperature, whereas the extraction time did not influence the cellulose content (236 mg/g of cellulose in W-alk1 after 30 min of treatment and 244 mg/g of cellulose in W-alk3 after 5 h of treatment). With higher temperature (100°C) and concentration (2 mol/L), cellulose content increased (W-alk4). Chanliaud and associates[23] observed similar results with maize bran, although the yields of extraction were much higher.

Lignin was almost completely removed after extraction with alkaline peroxide. This treatment increased the cellulose content of the residue (~ 420 mg/g) but the arabinoxylan content of the residue was still important, about 335 mg/g. The cellulose was probably degraded by oxidative "peeling" during the alkaline peroxide treatment. The "industrial" residue W-ind was enriched in cellulose (~ 380 mg/g) and its lignin content was also high (about 170 mg/g), compared with the other wheat bran residues.

Activation and Esterification of Cellulose-Enriched Residue

Before esterification, the cellulose-enriched residues had to be submitted to an acidic pretreat-

Table II Destarched Wheat Bran Residues Composition (mg/g)

	Wheat Bran	W-ind	W-alk1	W-alk2	W-alk3	W-alk4	W-ox
Treatment	No	Acid Industrial	KOH (1 mol/L)[a]	KOH (1 mol/L)[b]	KOH (1 mol/L)[c]	KOH (2 mol/L)[d]	Alkaline Peroxide
Yield of residue (mg/g)	1,000	nd	489	446	453	329	330
Arabinose	126	14	145	199	118	124	120
Xylose	209	73	207	252	200	155	215
Mannose	5	14	5	5	5	4	7
Galactose	12	3	12	12	11	11	13
Glucose	206	404	269	275	280	350	460
Glucuronic acid	46	15	32	31	29	23	10
Cellulose	156	379	236	219	244	317	416
Arabinoxylan[e]	335	87	352	451	318	279	335
Lignin	76	169	104	108	106	56	42

nd, not determined.
[a] 65°C, 0.5 h.
[b] 65°C, 2 h.
[c] 65°C, 5 h.
[d] 100°C, 1 h.
[e] Arabinoxylan = arabinose + xylose.

ment by immersion in sulfuric acid. Without this pretreatment, the residues failed to be esterified by fatty-acid chloride as previously stated,[5] suggesting that sulfuric acid catalyzed the esterification. This treatment probably increased the accessibility of the functional groups of glucose. However, more studies are necessary to understand the influence of this pretreatment, notably its effect on the degree of polymerization of the cellulose.

All samples from sugar-beet pulp, whatever their cellulose content, formed only insolubles particles and no films, however B-alk gave a heterogeneous product (Table III).

Films were obtained with wheat bran residues

Table III Result of Esterification of Sugar-beet Pulp Enriched in Cellulose

Sample	Aspect	Degree of Substitution
Beet pulp	particles	2.2
B-ind	particles	1.5
B-ac	particles	2.0
B-ox	particles	2.1
B-enz	particles	1.1
B-alk	heterogeneous product, nonpurified	1.0

W-alk1, W-ox, and W-ind (Table IV). Clear, flexible, and only slightly colored (yellow) films were obtained after a filtration step necessary to eliminate residual particles. Cellulose content in W-alk1, W-ox, and W-ind was above 300 mg/g, and the amounts of esterified product varied from $\sim$ 180 to $\sim$ 700 mg of cellulosic film per g of initial sample. The degree of substitution (DS) varied from 1.1 to 2.85. The low yield of esterified product may be due to the elimination of the non-esterified materials during the filtration step. In samples with a cellulose content lower than 300 mg/g (including destarched wheat bran), only heterogeneous products can be formed. The insoluble particles were probably constituted by unsubstituted cellulose or by other components such as arabinoxylans and lignin. Lignin and arabinoxylans may also be esterified because their constituting monomers bear hydroxyl groups. Antal and Micko[24] reported that hemicellulose, lignin, and cellulose can be substituted to various extents. However, the esterification of lignin probably did not lead to the formation of films.

Film-forming ability, yield of esterified product, and DS were not simply related to the cellulose content. The results showed that, for example, samples with the highest amount of cellulose did not have the highest yield of esterified product: W-ind had a lower content of cellulose than W-ox, but its yield of esterification was higher. It is

Table IV Results of Esterification of Wheat Bran Enriched in Cellulose

Sample	Result	Yield in Esterified Product (mg/g of initial sample)	Degree of Substitution
Wheat bran	heterogeneous product, nonpurified	nd	1.5
W-alk1	heterogeneous product, nonpurified	nd	1.6
W-alk2	heterogeneous product, nonpurified	nd	0.8
W-alk3	heterogeneous product, nonpurified	nd	1.6
W-alk4	filmogenic product, purified	179	2.2
W-ox	filmogenic product, purified	433	2.9
W-ind	filmogenic product, purified	707	1.1

nd, not determined.

therefore likely that other parameters (presence of noncellulosic material, nature of the cellulose, accessibility, etc.) may be critical in the formation of cellulosic films.

Influence of Noncellulosic Compounds

The arabinoxylan content influenced neither the yield of esterified product nor the DS; W-alk4 and W-ox presented comparable amounts of arabinoxylans ($\sim$ 280 mg/g against $\sim$ 335 mg/g), whereas the amount of arabinoxylans in W-ind was $\sim$ 90 mg/g. In contrast, it is likely that the lignin content affects the esterification (Fig. 2): the DS of the cellulose decreased when lignin content of the samples increased. This relation was valid also for samples that did not form films.

During esterification, lignin could be a competitor for the esterification reagents and/or could have a major influence on cellulose accessibility, as it can form an amorphous network with embedded cellulose microfibrils. Wang and Tao[7] esterified pure cellulose with long-chain fatty acids (mixtures of palmitic, stearic, linoleic, and linolenic acids) and obtained a high yield ($\sim$ 2.45 g/g). Therefore, it is possible that the low yield obtained might be due to the noncellulosic components of the residue, which were probably esterified and eliminated but also prevented the esterification of the cellulose.

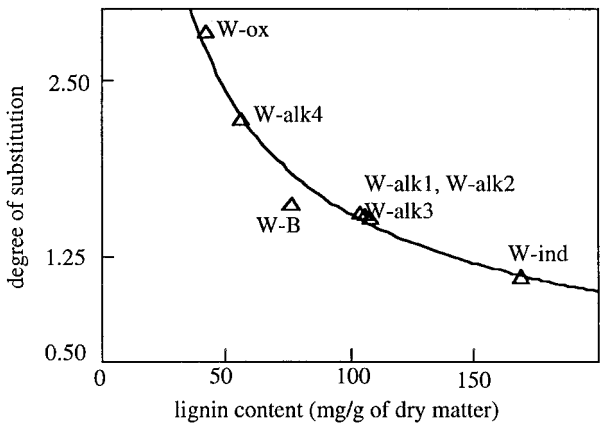

Figure 2 DS of cellulosic esters in relation to the amount of lignin.

Influence of the Cellulose Origin

Two cellulose-rich residues, W-ox from destarched wheat bran and B-ox from sugar-beet pulp, were submitted to the same extraction by alkaline peroxide and their cellulose contents were similar ($\sim$ 420 mg/g W-ox, $\sim$ 440 mg/g B-ox); however, in contrast to W-ox, B-ox did not give film. This discrepancy may be ascribed to differences in the nature of cellulose. The crystallinity and the nature of the cellulose from these two samples were therefore studied by X-ray diffraction. The corresponding diagrams are shown in Figure 3. The crystallinity of both samples was rather low, but cellulose from sugar-beet pulp had a lower crystallinity than cellulose from wheat bran. Moreover, cellulose from sugar-beet pulp seemed to contain a greater amount of cellulose II (mercerized cellulose), as shown by the diffraction angles (2θ) at 20.4 and 22.1 degrees, whereas wheat-bran cellulose was native cellulose, as shown by the diffraction bands at 14.9 and 16.3 degrees.

The failure of sugar-beet pulp residues to form cellulosic films, whatever the cellulose content, might therefore be explained by differences in the nature (degree of polymerization, crystallinity) of

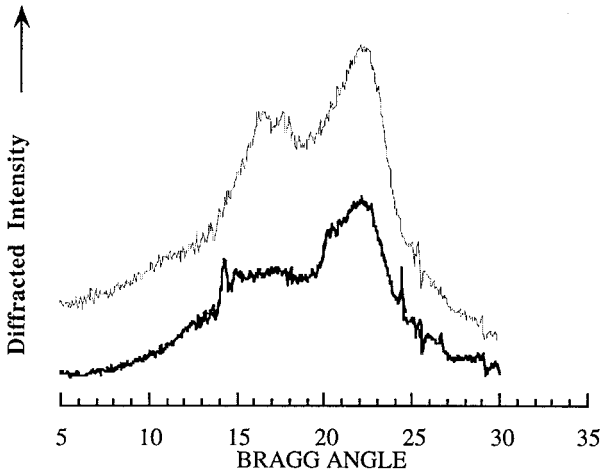

Figure 3 X-ray diffractograms of cellulosic residues: (——) W-ox from wheat bran; (▬▬) B-ox from sugar-beet pulp.

the cellulose between sugar-beet pulp and wheat bran.

CONCLUSION

Our preliminary results showed that it is possible to obtain films from agricultural by-products such as wheat bran. The formation of cellulosic films depended primarily on the origin of the cellulose. Cellulose from wheat bran formed films for cellulose content $> \sim 300$ mg/g, whereas cellulose from sugar beets did not form films, at least under our conditions, whatever the cellulose content. Cellulose content is not the only factor affecting the film formation, however it has an influence on the yield of esterified product. Lignin, which decreased the accessibility of the cellulose to the fatty acid, probably influenced the DS of modified cellulose.

The acidic pretreatment is a prerequisite and an important step. It will be investigated in detail in further studies including, for instance, the role of the nature and concentration of acid, and the drying step. In this way we hope to increase the yield of esterified material.

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