Enzymatic Hydrolysis of Zein−Wax-Coated Paper

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Enzyme hydrolysis of zein−wax-coated paper was investigated as part of an effort to develop a recyclable waxed paper product. Brown Kraft paper was coated with corn zein and paraffin wax, and the grease proofing and water vapor barrier properties of the paper were determined. Grease permeation was found to depend on the amount and the method of application of zein present in the zein−wax coating. Water vapor transmission values were strongly dependent on the amount of wax in the coating. We have shown that α-chymotrypsin was able to hydrolyze the zein and cleanly separate the coating from the paper fibers. Treatment with Tris buffer pH 8.5, containing 0.1% SDS and α-chymotrypsin (E:S ratio = 1:100) for 0.5 h effectively removed more than 97% of the zein−wax coating from the paper. Zein removal was followed by SDS−capillary electrophoresis of liquid samples taken during hydrolysis. SEM images of the extraccted paper indicated that the paper fibers were free of coating material.

Keywords: *Zein; paraffin wax; coating; paper; recycle; enzyme; hydrolysis; digestion; α-chymotrypsin*

INTRODUCTION

Agriculturally derived alternatives to polyolefin packaging material, currently used by the food industry, provide an opportunity to strengthen the agricultural economy and reduce importation of petroleum and petroleum products. Presently, waxed paper products are not recycled because wax particles cannot be cleanly separated from the paper fibers during mechanical pulping. Recyclable packaging paper materials using autodispersible waxes have been reported (Back, 1995). The author demonstrated, by incorporating small amounts of fatty acids and a nonionic surfactant into corrugated cartons, that wax dispersions could be removed during the recycling process under alkaline conditions. Corn zein coated paper is recyclable and has been suggested as an alternative to polyolefin materials (Trezza and Vergano, 1994). Grease resistance of corn zein coated paper was measured with respect to coating level, plasticizer addition and time of exposure. Proteases such as pepsin and α-chymotrypsin have been shown to be capable of digesting zein films at acid and nearly neutral pH values respectively (Yamada et al., 1995). Zein films, cross-linked with epichlorohydrin, were readily digested with α-chymotrypsin. In addition, urea-denatured α-zeins were almost completely hydrolyzed into small peptides by digestion with thermolysin at 37 °C (Yano et al., 1996), and papain was found to effectively hydrolyze zein in 70% aqueous ethanol at 25 °C (Saito et al., 1988).

In this study, we evaluated coating formulations composed of the corn protein zein and paraffin wax for grease proofing and water vapor barrier properties. This coating will be susceptible to clean separation from the paper by subjecting zein to enzymatic degradation. Conventional techniques will then be used to separate zein and paraffin wax from the paper fibers.

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MATERIALS AND METHODS

Materials. Corn zein F-4000 was obtained from Freeman Inc., Tuckahoe, NY; α-chymotrypsin (type II, from bovine pancreas; activity, 40−60 units per milligram of protein), from Sigma Chemical Co., St. Louis, MO; and paraffin wax, from Cullen Industries Inc., Huntingdon Valley, PA. A reciprocal shaking bath (Model 25) was obtained from Precision Scientific, Winchester, VA; and a BioFocus Capillary Electrophoresis System, from Bio-Rad, Hercules, CA.

Sample Preparation. Double-layer and single-layer techniques were used to coat the Kraft paper. For the double-layer technique, 1 g of corn zein was dissolved with heating into 30 mL of 95% (v/v) ethanol. The solution was then painted onto brown paper (16 in.^{2}) and dried under ambient conditions. Paraffin wax (3 g) was dissolved in 30 mL of hexane and painted on to the zein-coated paper. For the single layer technique, the zein solution was prepared at the same concentration in absolute ethanol and mixed with the wax solution to a final zein concentrations of 2 or 4% (w/w) with heating. For both techniques, the dry paper was weighed after each application to determine the amount of zein or paraffin wax deposited.

Testing. Water vapor permeability of the coated paper was determined using the method described in ASTM E96-80 (ASTM, 1980) and grease permeation by the TAPPI method T507 cm-85 (TAPPI, 1991).

Enzyme Hydrolysis. The zein coating was hydrolyzed by cutting the coated paper into 0.25 in. squares, placing them in a flask containing 25 mL of 10 mM Tris (pH 8.5), containing 0.1% SDS and α-chymotrypsin (E:S ratio = 1:100) and agitating them in a reciprocal shaking bath at 37 °C. Paper samples were typically coated with between 50 and 60 mg of zein per 16 in.^{2}. Small samples (0.1 mL volume) were taken periodically for hydrolysate analysis.

Removal of Zein−Wax Coating. Formation of zein hydrolysate was analyzed by SDS−capillary electrophoresis (CE) using the method described by Parris et al. (1997). All CE analyses were performed using a protein analysis kit (CE-SDS, Bio-Rad Laboratories, Hercules, CA). Samples (0.1 mL) were mixed with an equal volume of sample buffer and microfuged for 5 min at 12000*g*. Electrophoretic separations were performed using an uncoated fused silica capillary (24 cm × 75 μm i.d.) at 15 kV and 20 °C for 15 min. Samples

10.1021/jf980406j CCC: $15.00
Published on Web 09/24/1998

Table 1. Effect of Zein and/or Paraffin Wax on Water Vapor Transmission Rates (WVTR) of Coated Paper at 23 °C and a 50% Relative Humidity Gradient

sample	coating (g/16 in.^{2})	WVTR (g/m^2 day)
zein	0.09	1305
zein	0.10	1357
zein[a]	0.08	2030
wax	0.10	484
wax	0.13	440
zein−wax[b]	0.14	239
zein−wax[b]	0.20	201

[a] From Trezza et al. (1994). [b] Coating applied as a 2% solution of zein in paraffin wax.

Table 2. Grease Permeation Test on Effect of Coating Method[a] and Level and Staining Time

sample	coating (g/16 in.^{2})	time (h)	area stained (%)	area per hour (%)
1	0.132	1	56.9	56.9
2	0.115	2	59.7	29.9
3	0.131	2	87.5	43.8
4	0.152	2	63.2	31.6
5	0.123	2	56.9	28.5
6	0.171	2	56.2	28.1
7	0.129	3	95.8	31.9
8	0.128	4	95.8	24.0
				av 34.3 ± 10.77

[a] Coating applied as a 4% solution of zein in paraffin wax.

Table 3. Grease Permeation Test on Effect of Coating Method[a] and Level and Staining Time

sample	coating (g/16 in.^{2})	time (h)	area stained (%)	area per hour (%)
1	0.151	1	41.0	41.0
2	0.134	1	19.4	19.4
3	0.129	2	25.7	12.9
4	0.150	2	27.1	13.6
5	0.119	3	81.9	27.3
6	0.133	3	83.3	27.8
7	0.108	4	64.6	16.2
8	0.146	4	89.6	22.4
				av 22.6 ± 9.38

[a] Coating applied as a bilayer of zein then paraffin wax.

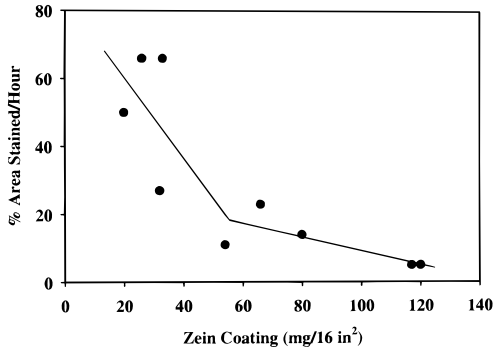

Figure 1. Effect of the amount of zein coating on area stained.

were injected for 5 s at 10 kV (electrophoretically) and proteins were detected at 220 nm. Zein protein and hydrolysate molecular weights were estimated using the BioSize software (BioRad Laboratories).

The mass of paraffin wax removed from the coated paper was determined gravimetrically. Residual wax on enzyme-treated paper squares was removed by extraction for 0.5 h with 30 mL of hexane in a reciprocal shaking bath at 37 °C. The squares were rinsed two more times with 15 mL of hexane, combined with the original extract, dried under nitrogen, and weighed.

SEM. Square pieces of paper, ~1 cm, were excised from experimental samples with scissors and mounted on aluminum specimen stubs using adhesive tabs and colloidal silver adhesive paint (Electron Microscopy Sciences, Ft. Washington, PA). Mounted samples were coated with a thin layer of gold by low voltage DC sputtering (Plasma Sciences, Lorton, VA) and viewed in a JSM 840A scanning electron microscope (JEOL USA, Peabody, MA) operated in the secondary electron imaging mode and integrated with a digital imaging workstation (Princeton Gamma-Tech, Princeton, NJ).

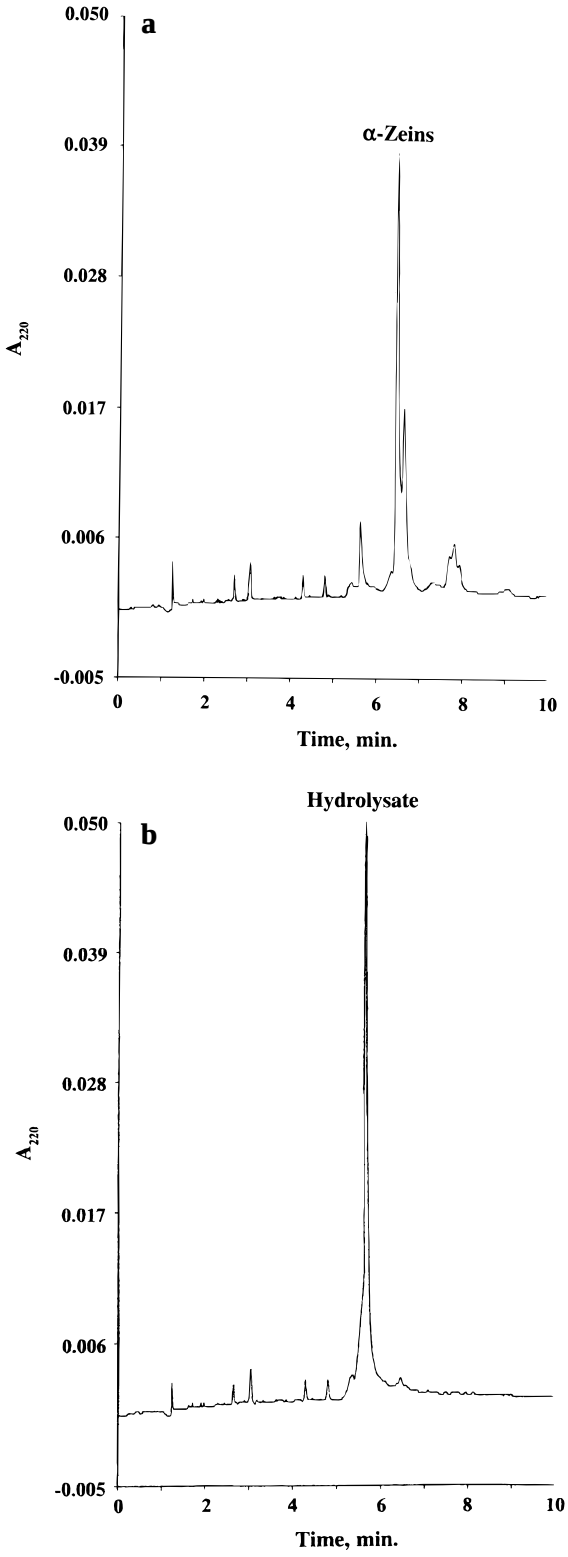

Figure 2. Electropherogram of extract from zein−wax-coated paper extracted with (a) 0.1% SDS and (b) 0.1% SDS + α-chymotrypsin after 1.5 h at 37 °C.

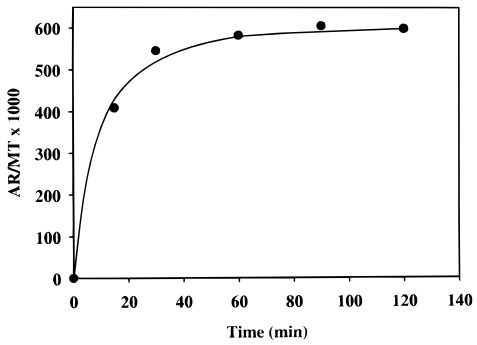

Figure 3. Amount of enzyme hydrolysate in extract from zein−wax-coated paper. AR/MT = area/migration time.

RESULTS AND DISCUSSION

Water barrier and grease permeation properties were measured on Kraft paper coated with zein, wax, and a combination of the two. Water vapor transmission rates (WVTR) for zein-coated paper were found to be comparable to those reported by Trezza et al. (1994) for approximately the same coating weight (Table 1). The WVTR for paper coated with paraffin wax were found to be significantly lower than those measured using the zein-coated paper. Coating the paper with a 2% solution of zein in paraffin wax reduced the WVTR by approximately half the values obtained for wax coated paper. Grease permeation was measured for zein−wax-coated paper prepared using either the single layer (Table 2) or the bilayer (Table 3) techniques. Both types of coatings, at various levels, were exposed from 1 to 4 h and the stained areas measured. Permeation values for the grease-stained coating were found to be lower using the bilayer coating technique and had a variance of $p = 0.036$ (compare Table 2 and Table 3). The bilayer coating technique was used for sample preparation for the remainder of the study. The dependence of the area stained on the amount of zein coating on the paper

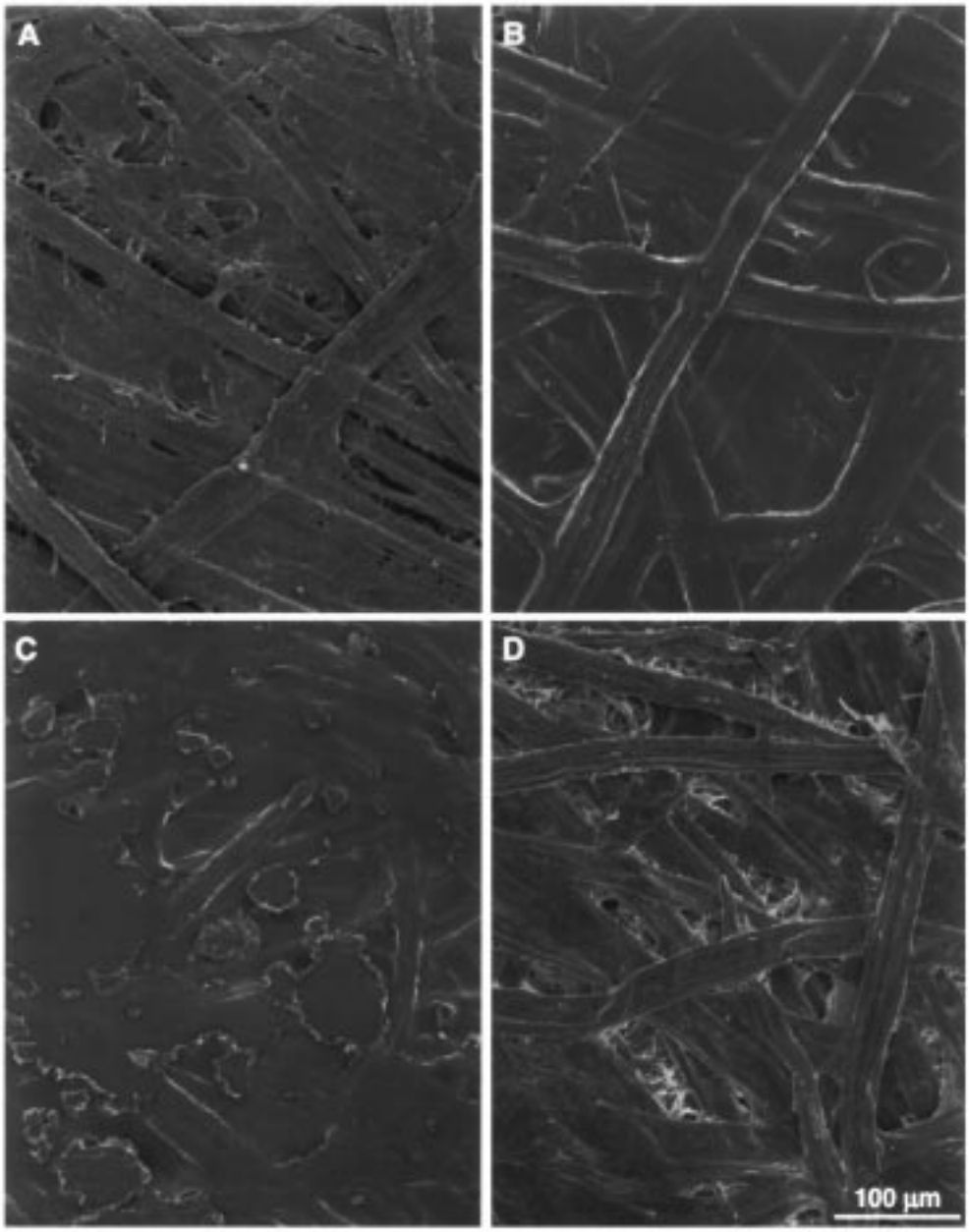

Figure 4. SEM images of (a) kraft paper, (b) zein-coated paper, (c) zein−wax-coated paper, and (d) paper after extraction with 0.1% SDS + α-chymotrypsin.

appeared to stop below 50 mg/16 in.^{2} (Figure 1). At higher concentrations of coating good grease resistance was produced. The data demonstrated that the zein layer of the bilayer coating contributes grease proofing and the wax layer water resistance (Table 1).

To enzymatically remove the coating, the zein must be soluble. Zein, a prolamine, is soluble in a variety of organic solvents and other aqueous solubilizing agents such as urea, guanidine hydrochloride, and SDS. Although proteases such as papain, pepsin, thermolysin, and α-chymotrypsin have all been shown to be capable of digesting zein under specified conditions, α-chymotrypsin was selected because hydrolysis can be carried out at relatively mild temperatures and at a nearly neutral pH. The effective enzyme was prepared in 0.1% SDS (at lower concentrations (0.01% SDS) hydrolysis of the zein in the coating did not occur). An SDS−CE electropherogram of zein extracted from the zein−wax-coated paper, in the absence of α-chymotrypsin, is shown in Figure 2a. The zein coating consisted primarily of two partially resolved α-zein proteins with migration times of 6.5 and 6.7 min and corresponding molecular weights of 19 and 22 kDa. The electropherogram of the extract containing α-chymotrypsin consisted of a single hydrolysate peak with migration time of 5.7 min and an estimated molecular weight of 14 kDa (Figure 2b). Effectiveness of the enzyme was determined by measuring area/migration time of the hydrolysate peak relative to incubation time (Figure 3). Hydrolysis of the α-zeins was relatively rapid and ~70% complete in 15 min. No further increase in the hydrolysate peak occurs after 1.5 h and the zein was completely extracted from the paper. The extract was turbid yellow consisting of suspended wax particles.

Quantitative removal of paraffin wax from the paper was determined gravimetrically, and the paper fibers were examined by SEM. Residual wax on the enzyme-treated paper was extracted with hexane as described earlier. In the absence of α-chymotrypsin, ~30% residual wax remained on the paper after treatment with the buffered SDS solution. Addition of α-chymotrypsin to the extracting solution significantly improved the removal of the wax coating and only 2.6 ± 0.9% ($n = 4$) residual wax remained on the paper after extraction. To ensure that greater than 97% of the wax coating was removed from the paper after enzyme treatment and that the paper was suitable for recycling, SEM digital images of the paper surfaces were prepared and recorded at 250× (Figure 4). At this magnification images of the Kraft paper clearly showed the weblike structure of paper fibers with hairy attachments on the fibers (Figure 4a). Images of the zein coated paper (Figure 4b) indicated that the coating was relatively uniform. The fibers were still visible but not the hairy attachments and in some areas few micrometer size and smaller holes were present. The paper fibers were barely visible in the wax−zein-coated paper and the wax coating lacked uniformity (Figure 4c). After extraction of the zein−wax-coated paper for 1.5 h with the buffered SDS + α-chymotrypsin solution, its SEM image more closely resembled Kraft paper (Figure 4a) than the image of the zein−wax-coated paper (Figure 4c). The paper fibers with hairy attachments are prominent, and the fibers do not appear to be coated with zein and wax particles.

In conclusion, gravimetric analysis and SEM images indicate that wax can be cleanly removed from paper fibers after enzyme treatment of zein−wax-coated paper. Research will be continued to determine if the fibers can be sheeted to re-form a paper substrate without intermediate drying.

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Received for review April 22, 1998. Revised manuscript received August 7, 1998. Accepted August 10, 1998.

JF980406J