Effect of Chemical Pretreatments on the Thermal Degradation of Corn Husk Lignocellulosics

Branka Barl,[1] Costas G. Biliaderis,* and E. Donald Murray

The thermal degradation of α-cellulose and xylan as well as native and chemically pretreated corn husk residues, under nitrogen atmosphere, was studied by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The DSC and TGA thermal profiles of corn husk residues reflected the pyrolysis of their main constituents. On thermal decomposition of these materials, single exothermic effects were observed at 238 °C (hemicellulose) and 317 °C (cellulose), suggesting that dehydration and charring reactions dominate the overall pyrolytic process. Evaluation of kinetic parameters (activation energy, reaction order) was carried out with nonisothermal TGA data and dynamic equations. Despite the chemical heterogeneity of the materials and complexity of the reactions involved, pyrolysis was found to obey first-order kinetics. Chemical pretreatments caused pronounced changes in both kinetics and DSC/TGA profiles. An attempt was made to relate the thermal behavior of these systems with the structural/compositional alterations brought about by the solvent during pretreatment. The X-ray crystallinity values were found to exhibit positive relationships with several TGA thermal parameters (maximum rate of weight loss, temperature at 10% weight loss, activation energy).

INTRODUCTION

Agricultural crop residues represent an abundant, inexpensive, and renewable resource of biomass for the production of liquid fuels and chemical products. Increasing research efforts have been directed to the enzymatic conversion of cellulose to glucose and subsequent fermentation to ethanol or assimilation into single-cell protein. The enzymatic hydrolysis of native lignocellulose (heterogeneous reaction) is, however, slow due to the structural and morphological features of the lignin–carbohydrate complex. Crystallinity, the presence of lignin and its association with the polysaccharides, and the limited specific surface area have been suggested as the major deterrents to the effective utilization of lignocellulosic residues (Cowling and Kirk, 1976; Chang et al., 1981; Puri, 1984). Several physical, biological, and chemical pretreatments are used to improve the accessibility and thus susceptibility of the solid substrate to cellulosic enzymes (Millet et al., 1975 and 1976; Avgerinos and Wang, 1983; MacDonald et al., 1983; Gharpuray et al., 1983; Yu et al., 1984; Vallander and Eriksson, 1985; Reid, 1985). Enhancement in the hydrolysis rates with such pretreatments is generally attributed to structural modification and/or selective removal of cell wall constituents. In addition to the enzymatic conversion processes, attention has been also given to the development of pyrolytic methods for converting cellulosic biomass to sugars and other low molecular weight compounds (Shafizadeh, 1971; Shafizadeh, 1983). However, application of pyrolytic processes to cellulose depolymerization is still hampered by the complexity of the thermal reactions and the lack of specificity in producing high yields of a particular product.

Studies attempting to characterize the pyrolytic behavior of cellulosic materials often involve thermoanalytical methods such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). These techniques are valuable tools to probe the extent and sequence of the physical/chemical transformations encountered under dynamic temperature–time heating protocols (Biliaderis, 1983). Although the thermal degradation of lignocellulosics is dependent on their structure and composition, to our knowledge very few studies (and only on purified cellulose) have attempted to relate the thermal behavior to the physicochemical properties of such systems (Shafizadeh and McGinnis, 1971; Basch and Lewin, 1973a,b; Rodrig et al., 1975; Cabradilla and Zeronian, 1976). The objectives of the work presented herein were, therefore, to examine the effect of various chemical pretreatments on the pyrolytic decomposition of corn husk residues using DSC and TGA and to explore any possible relationships between thermal properties and structural/compositional features of these materials. In an effort to elucidate the nature of the pyrolytic processes of complex cellulosics such as corn husk residues, the thermal behavior of two commercial samples of xylan and α-cellulose was also investigated.

MATERIALS AND METHODS

Corn husk residues were selected as the experimental material of the present study because they are moderately lignified as compared to other parts of the plant. The raw material had the following composition (d.b.): cellulose, 38.8%; hemicellulose, 44.5%; lignin (Klason), 7.1%; protein, 1.7%; ash, 2.8%. Cellulose and hemicellulose were determined according to Goering and Van Soest (1970) and lignin was determined by the TAPPI Standard T22 05-74 method (TAPPI, 1974), while protein and ash were determined by using AOAC (1975) methods. The air-dried husks were ground (1 mm) and subjected to various chemical pretreatments as specified in Table I. For temperatures above 100 °C an autoclave was used. Residual biomass solids after pretreatment were removed by filtration and washed several times with distilled water until acid or alkali free (pH 6.0–7.0). The residues were then air-dried, ground with a Wiley mill (<0.5 mm), and stored for subsequent analyses. Xylan (oat spelts) and α-cellulose were products of Sigma Chemical Co. (St. Louis, MO). The X-ray crystallinities were measured on ground samples placed on aluminum holders using a Philips PW 1051 diffractometer. An iron-filtered Co Kα radiation was used (voltage 36 kV, current 8 mA, range of 2θ 8–36°). The crystallinity index (CrI) was determined according to Segal et al. (1959). Crystallinity indices were reproducible with a measured standard deviation of 1.5%.

DSC was carried out in a nitrogen atmosphere (700 kPa) on a Du Pont 9900 thermal analyzer equipped with a 910 cell base and a pressure DSC cell. Samples of 3.2–3.8 mg were sealed into aluminum pans by a Du Pont pan crimper; the lids were reversed to minimize thermal lags and

Food Science Department, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

[1] Present address: Maize Research Institute, 11080 Beograd-Zemun, Slobodana Bajica 1, Yugoslavia.

0021-8561/86/1434-1019$01.50/0

Table I. Chemical Composition and Crystallinity Index of Corn Husk Residues

sample	pretreatment (solvent/temp, °C/time)	residue, % wt	hemicellulose, % in residue	cellulose, % in residue	CrI, %
i	native husk	100	45	39	51
ii	1% w/w H_2SO_4/55/2 h	90	44	41	57
iii	5% w/w H_2SO_4/85/2 h	47	8	69	61
iv	14.3% w/w H_3PO_4/50/72 h	66	28	49	59
v	0.5% w/v NaOH/121/15 min	57	29	58	61
vi	5.0% w/v NaOH/121/15 min	36	13	75	67

Table II. Thermal Analysis Data (DSC, TGA) of α-Cellulose, Xylan, and Corn Husk Residues

	DSC exoth peak, °C			kinetic parameters (TGA)				
sample[a]	I	II	III	temp range,[b] °C	E_a,[b] kJ/mol	n[b]	temp range,[c] °C	E_a,[c] kJ/mol
i	238	317	450	295–327	59 (–0.96)[d]	0.97	295–327	56 (–0.99)[d]
ii	266	334	437	292–325	95 (–0.97)	0.87	301–334	83 (–0.99)
iii		339	441	299–325	152 (–0.99)	0.99	302–334	135 (–0.99)
iv	275	332	434	290–326	86 (–0.98)	0.85	290–326	102 (–0.99)
v	232	315	438	303–326	101 (–0.94)	0.87	303–333	117 (–0.98)
vi	249	318	436	303–321	166 (–0.99)	0.94	303–331	147 (–0.99)
α-cellulose		317		326–346	178 (–0.98)	0.98	326–346	195 (–0.99)
xylan	240			263–296	148 (–0.99)	0.97	263–292	120 (–0.99)

[a] Samples i–vi as designated in Table I. [b] Freeman and Carroll equation. [c] Broido equation. [d] Numbers in parentheses are the correlation coefficients for the corresponding kinetic plots.

thus improve the reproducibility of the thermal profiles. Operation and calibration of the equipment were essentially as described by Biliaderis et al. (1985). The heating rate was 10 °C/min, and Ottawa sand was used as reference material. Each sample was run at least three times. Transition peak temperatures generally varied within ±1.0 °C for repeated analyses of the same sample. Data were recorded at 0.8-s time intervals and stored on floppy disks. A Du Pont 951 TGA unit connected to a Du Pont 1090 thermal analysis system was used for thermogravimetric analysis. The system was purged continuously with dry nitrogen at a flow rate of 120 cm^3/min. The heating rate was 1.5 °C/min. Each sample (14.5–16.8 mg) was run at least twice in aluminum pans. The temperature at which weight loss of 10% occurred (after removal of adsorbed water) as well as the maximum rate of weight loss (from the derivative curve) were directly obtained from the thermal curves. The data sampling rate was 1.0 s, and data were stored on floppy disks. Data analyses for both TGA and DSC were performed by Du Pont software analysis programs. Evaluation of the kinetic parameters from the TGA curves was performed by employing the dynamic kinetic methods of Broido (1969) and Freeman and Carroll (1958). The former employs the equation

$$\ln [\ln (1/y)] = -(E_\alpha/R)(1/T) + \text{const}$$

where y is the fraction of the number of initial molecules not yet decomposed, E_α is the activation energy, R is the gas constant, and T is temperature (K). The progress of the reaction(s) can be followed by continuous monitoring of the sample weight. Thus, y is determined as $y = (W_t - W_{inf})/(W_0 - W_{inf})$ where W_0 is the original weight, W_t is the weight at time t, and W_{inf} is the weight at the end of the reaction. A plot of ln [ln (1/y)] against $1/T$ provides the E_α from the slope of the curve. The final equation derived by the method of Freeman and Carroll is

$$\frac{\Delta \log (dx/dT)}{\Delta \log (a - x)} = n - \frac{E_\alpha}{2.303R} \frac{\Delta(1/T)}{\Delta \log (a - x)}$$

where a is the initial weight of the material, x is the amount that reacted at temperature T(K), dx/dT is proportional to the rate of the reaction (at a constant heating rate), and n is the reaction order. From this equation a plot of $\Delta \log (dx/dT)/\Delta \log (a - x)$ against $\Delta(1/T)/\Delta \log (a - x)$ provides an estimate of E_α and n from the slope and intercept, respectively. The kinetic parameters (E_α, n) were evaluated using the method of least squares for both equations. Estimates of E_α generally had a standard deviation of ±7.0 kJ/mol (Broido, 1969) and ±5.0 kJ/mol (Freeman and Carroll, 1958) for repeated analyses of the same sample and data taken over the same temperature range.

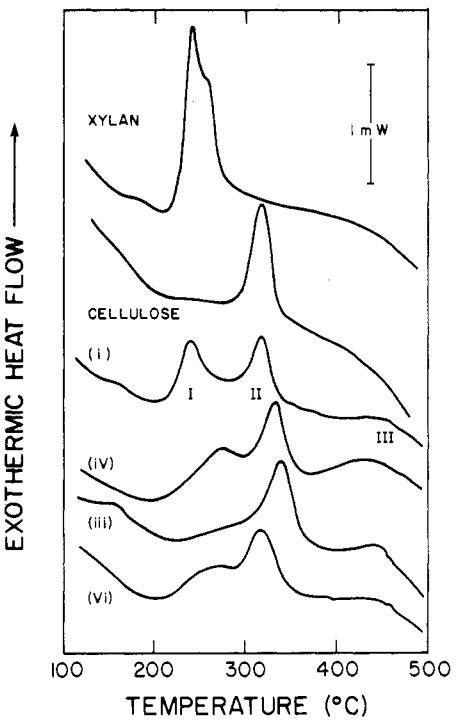

Figure 1. Typical DSC thermal curves for the pyrolytic degradation of xylan, α-cellulose, and corn husk residues. Sample weights from top to bottom (mg): 3.50, 3.58, 3.56, 3.20, 3.43, 3.67. Samples i, iv, iii, and vi as designated in Table I. Heating rate 10 °C/min.

RESULTS AND DISCUSSION

Figure 1 shows representative DSC decomposition curves for xylan, α-cellulose, and corn husk lignocellulosics. The general appearance of these profiles indicates that the recorded thermal events of the pyrolytic processes are exothermic. The peak temperatures [I, II, and III, as specified in curve (i)] for all samples are given in Table

II. The thermal curve of the native husk (i) is characterized by three exothermic peaks at 238, 317, and 450 °C, respectively. Interestingly, the peak temperatures of the first two transitions corresponded well to those of xylan (240 °C) and α-cellulose (317 °C). Since single exothermic effects were exhibited by both xylan and α-cellulose, the peaks I and II of sample (i) most likely reflect the decomposition processes of hemicellulose and cellulose components. Pretreatment of corn husk residues with alkali or acid resulted in solubilization or hydrolysis of the hemicellulose component as evidenced by their chemical composition (Table I). It is apparent from these results that such selective removal of the hemicellulose fraction is dependent on the conditions employed during pretreatment; i.e., it increases with temperature, time, and concentration of the solvent. From the DSC thermal curves (Figure 1), it was evident that there was a decrease in the magnitude of the transition I after removal of the hemicellulose fraction during solvent pretreatment. Although it has been difficult so far to measure accurately the areas of peaks I and II, because of the convolution of these transitions, DSC, using well-defined conditions, may prove a rapid and simple method to determine the relative proportions of the two main polymeric components present in native and treated cellulosics. Existing quantitative methods of analysis for cellulose, hemicellulose, and lignin are tedious and based on wet chemistry. However, additional development of the thermal methodologies is required to permit assessment of the analytical potential of DSC in quantitating these polymeric constituents. Following the second transition, a small exotherm (III) was observed at 430–460 °C for most of the corn husk residues. Secondary decomposition/charring reactions of cellulose and/or thermal degradation of lignin could account for this transition. Lignin has been shown to undergo major changes at temperatures above 350 °C (Shafizadeh and McGinnis, 1971; Bouchard et al., 1985). The fact that lignin decomposes mainly into aromatic compounds that are generally formed at higher temperatures than the nonaromatic oxygenated degradation products of cellulose (Shafizadeh, 1983) further supports the above argument.

At this point it is useful to emphasize that pyrolysis of polysaccharides is a complex process, consisting of many competitive and consecutive reactions. Furthermore, many physical and chemical factors such as temperature, pressure, size of sample, atmosphere, purity, and homogeneity of the carbohydrate material are important in affecting the degradation pathways. Most studies on purified cellulose and model small molecular weight compounds indicate that thermal degradation leads to a variety of products that can be derived by more than one pathway, as shown in the over-simplified scheme of Figure 2 (Shafizadeh and McGinnis, 1971; Shafizadeh et al., 1971; Shafizadeh and Fu, 1973; Shafizadeh and Lai, 1975; Furneaux and Shafizadeh, 1979; Liskowitz et al., 1980; Shafizadeh, 1983). The suggested mechanisms for molecular rearrangement/transformations that take place include cleavage of glycosidic linkages (via free-radical formation and/or transglycosylation), formation of anhydro sugars (mainly levoglucosan) and tar (via transglycosylation, condensation, and dehydration), and decomposition into carbonaceous char and volatiles (via dehydration, disproportionation, and fission reactions). Some of these reactions are endothermic (transglycosylation and volatilization of the depolymerization products) while others are exothermic in character (dehydration and charring). Furthermore, dehydration and charring are favored at low temperatures and in the disordered regions, while depolymerization by transglycosylation and levoglucosan (1,6-anhydro-β-D-glycopyranose) production take over at higher temperatures and usually occur in the crystalline parts of the molecule (Basch and Lewin, 1973b; Broido et al., 1973; Cabradilla and Zeronian, 1976; Shafizadeh, 1983). Overall, the extent to which dehydration and char formation competes with that of levoglucosan production will determine the net thermal response (DSC) of the system undergoing pyrolytic degradation. As such, the thermal behavior observed for the samples of the present study suggests that the decomposition pathway of dehydration and charring was predominant.

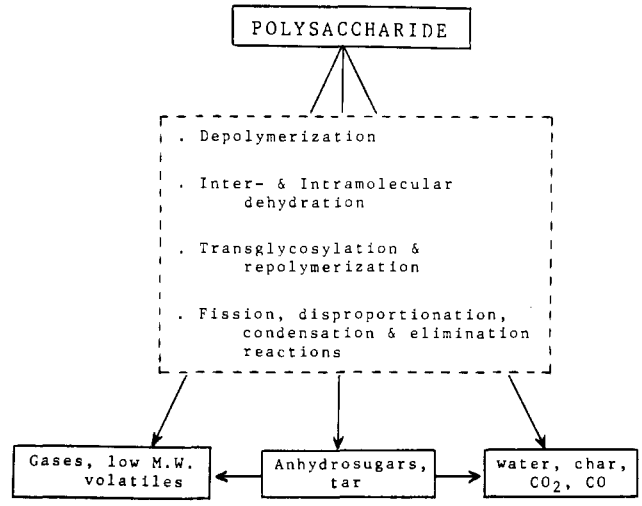

Figure 2. Generalized competing pathways for pyrolysis of carbohydrates.

Another interesting aspect of the DSC curves, within the series of samples examined herein, has been the response of the transition II peak temperature to the various chemical pretreatments. Hydrolysis of corn husk residues with acids imparted a marked increase (15–22 °C) in the temperature of this transition (samples ii–iv, Table II). On the other hand, alkali treatments had no significant effects on the pyrolysis temperature of the cellulose component (samples v and vi). These results could be explained by considering the semicrystalline nature of native cellulose and the action mechanism of the various solvents employed on the lignin–hemicellulose–cellulose complex during pretreatment. Although the exact microfibrillar structure of cellulose is still a matter of controversy (Chang et al., 1981; Buleon and Chanzy, 1982), all studies on crystallinity and accessibility of cellulosic materials indicate the existence of ordered (crystallites) and disordered (paracrystalline or amorphous) regions. It is noteworthy also to point out that the major source of stability in cellulose is hydrogen bonding. In the less ordered parts of cellulose, hydrogen bonding is not extensive and therefore thermal decomposition commences in these regions at lower temperatures and with high rates (Basch and Lewin, 1973a, 1973b; Cabradilla and Zeronian, 1976). In this respect, after heterogeneous acid prehydrolysis, a significant increase in the crystalline material would be expected since the less ordered regions are more amenable to hydrolysis. Furthermore, upon partial removal of amorphous chain segments (i.e., tie molecules), chain mobility on the exposed faces of the crystallites is enhanced and thus perfection (annealing) may occur. Overall, mild acid treatments such as those employed in the present studies would likely alter the proportion and/or perfection of the crystalline material sufficient enough to improve its resistance to pyrolysis. Unlike the acid–cellulose system, alkali treatments, as applied to lignocellulosic residues, cause selective removal

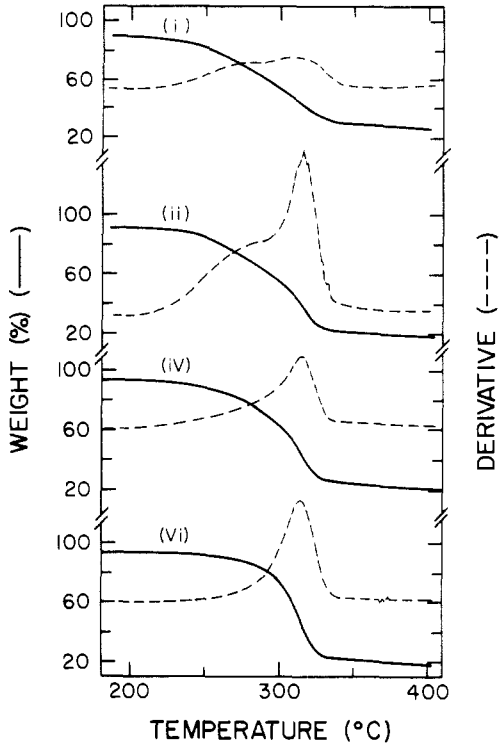

Figure 3. Typical TGA thermal curves for the pyrolytic degradation of corn husk residues, weight loss (%) and first-derivative, dx/dt (arbitrary units). Sample weights from top to bottom (mg): 15.99, 16.62, 15.25, 15.43. Samples i, ii, iv, and vi as designated in Table I. Heating rate 1.5 °C/min, flow rate of dry N_2 120 cm^3/min.

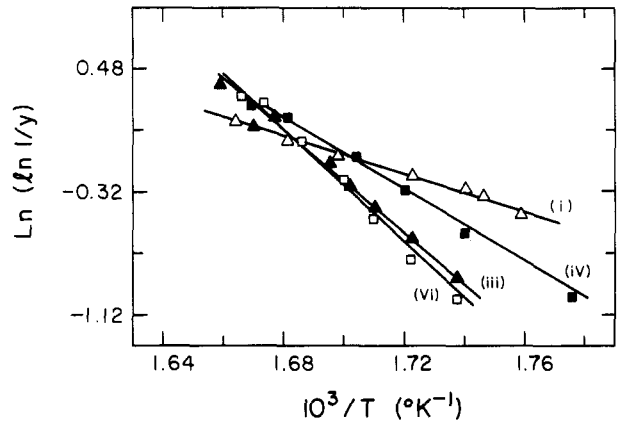

Figure 4. Plots of ln [(ln 1/y)] vs. $10^3/T$ (K^{-1}) using Broido's equation for the pyrolytic degradation of corn husk residues. Samples i, iv, iii, and vi as designated in Table I.

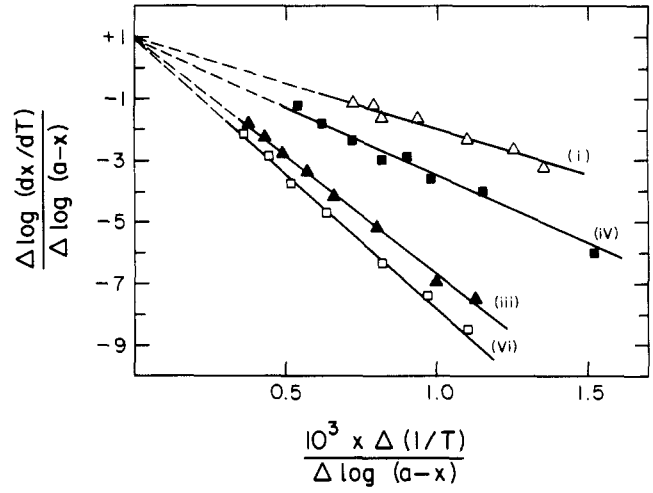

Figure 5. plots of $\Delta \log (dx/dT)/\Delta \log (a-x)$ vs. $10^3\Delta(1/T)/\Delta \log (a-x)$ using the Freeman and Carroll equation for the pyrolytic degradation of corn husk residues. Samples i, iii, iv, vi as designated in Table I.

of hemicellulose, substantial delignification, and intracrystalline swelling of the cellulose fibers (Chang et al., 1981; Avgerinos and Wang, 1983; Brown, 1983). The latter could result in changes in structure and size of the crystallites to yield a modified product of enhanced reactivity toward enzymatic or chemical reactions. Interestingly, the alkali-treated samples also exhibited increased crystallinities (Table I), as determined by X-ray, presumably due to removal of noncellulosic components. Under alkali conditions, however, cleavage of glycosidic bonds in the intercrystalline regions must be minimal; i.e., amorphous regions remain relatively intact. Since the cellulose fraction of these samples did not show any changes in thermal resistance (DSC peak temperature, Table II), it is reasonable to assume that the effect from the presence of the amorphous regions (decompose at lower temperatures) exerts considerable influence on the pyrolytic behavior of the entire cellulosic structure; i.e., they tend to reduce the thermal stability of the polymer.

The thermal properties of corn husk lignocellulosics were also investigated by TGA. Characteristic TGA thermal curves (both weight loss and first-derivative profiles) are shown in Figure 3. All samples exhibited a slight weight loss (7–10%) due to elimination of physically adsorbed water below 100 °C, a very slight gradual loss in weight between 100 and 250 °C, and major weight losses due to thermal decomposition between 280 and 340 °C. Finally, there was a slight weight loss in the region of 340–450 °C, indicative of further decomposition reactions involving the char. For native residues, the major weight loss appeared to take place in two distinctive consecutive stages, as shown by the two inflections in the rate of weight loss curves (i.e., derivative TGA). In view of the DSC data, it is suggested that this behavior mainly reflects the decomposition of hemicellulose and cellulose components occurring at two different temperature regions. As with the DSC curves, decreased proportions of the hemicellulose fraction, due to chemical pretreatments, affected the TGA profiles by shifting the onset of the pyrolytic events toward higher temperatures. Furthermore, the main stage of the process was confined within a narrower temperature range and thus yielded a sharper fall in weight loss in this step. These effects were particularly evident with samples iii and vi. In the case of these samples, the derivative TGA curve showed a single peak, as one would anticipate with one-component (cellulose) system.

Kinetics of thermal decomposition of corn husk cellulosics were determined from dynamic thermogravimetric data and employed the equations of Broido and Freeman and Carroll. Representative plots of the treated data according to these methods are shown in Figures 4 and 5. Table II presents estimates of the kinetic parameters (activation energy E_a and reaction order n) as well as the temperature range of the data used for kinetic treatments. As can be seen, plots for both equations gave significant linear relationships: $r = -0.94$ to -0.99, $p < 0.005$ (Freeman and Carroll, 1958; $r = -0.98$ to -0.99, $p < 0.001$ (Broido, 1969). Despite the complexity of all the reactions involved, the pyrolytic decomposition of all samples appeared to obey first-order kinetics as indicated by the values of n, which were between 0.85 and 0.99; note that the extrapolated intercepts of plots (n) in Figure 5 are near unity. The values for the apparent activation energy of α-cellulose

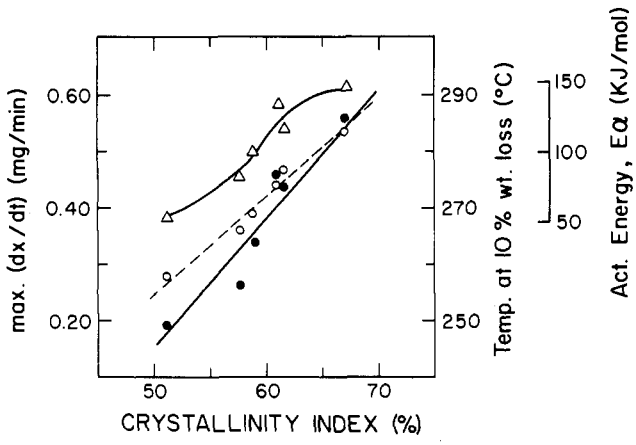

Figure 6. Relationships between crystallinity index (%) and TGA kinetic parameters: maximum rate of weight loss (dx/dt; ○); activation energy (Broido's equation; △); temperature at 10% weight loss (●).

were 178 (Freeman and Carroll, 1958) and 195 kJ/mol (Broido, 1969) and are generally within the range of values (140–240 kJ/mol) reported by various researchers (Dollimore and Holt, 1973; Basch and Lewin, 1973a; Jain et al., 1985). It should be noted here that the evaluation of kinetic parameters from dynamic measurements (e.g., TGA) is dependent on the temperature range of the data used as well as the kinetic equation applied for the analysis. Furthermore, the activation energy for solid-phase reactions, and in particular the thermal decomposition of complex materials like those of the present study, derived from a certain dynamic method is also affected by many factors such as heating rate, particle size, thermal lags of the thermoanalytical device, and pressure and atmosphere under which decomposition takes place (Chen, 1974; Jain et al., 1985). In this respect, comparisons between values given in the literature and those of Table II should be made with caution. Nevertheless, estimates of the kinetic parameters within a group of specimens tested similarly must reflect the structural/compositional differences of these materials. This has indeed been the approach undertaken in this study, and as such the kinetic data reported in Table II have only relative value. From Table II, it is also obvious that E_α increased considerably after chemical pretreatment. The higher values derived for the pretreated samples most likely reflect the changes in structure/composition of these materials; i.e. removal of hemicellulose, preferential hydrolysis of amorphous cellulose, and structural modifications of cellulose crystallites.

In view of the differences in pyrolytic behavior (as assessed by TGA) between amorphous and crystalline cellulose (Basch and Lewin, 1973a and 1973b; Cabradilla and Zeronian, 1976) as well as the observation that appreciable changes in crystallinity did occur upon acid or alkali pretreatments (Table I), it seemed of interest to explore any relationships between X-ray crystallinities and TGA thermal parameters. Plots of crystallinity (%) vs. maximum rate of weight loss, temperature at which the first 10% loss in weight of the dried cellulosic material occurs, and apparent activation energy (Broido, 1969) are shown in Figure 6. With regard to E_α, as the degree of crystallinity increases, there is a concomitant increase in activation energy. It is of interest here to note that literature data on activation energies for pyrolysis of pure cellulose (using various methods) lie generally in two groups of approximately 80–120 and 200–240 kJ/mol, as reviewed by Basch and Lewin (1973a). The former represents the activation energy of the less ordered regions of cellulose while the latter is characteristic of cellulose crystallites. These considerations, however, apply to pure cellulose. Impurities and other constituents naturally or artificially present can catalytically influence the pyrolysis of cellulose and thus overshadow the influence of the structural features on the pyrolytic behavior of this polysaccharide (Basch and Lewin, 1973b). With the above argument for cellulose, one would expect higher E_α with samples of greater crystallinity. Such a trend was indeed shown with the materials of the present study. Furthermore, similar relationships were also observed for the other two parameters investigated (Figure 6). Linear regression analysis for these plots yielded correlation coefficients of 0.98 and 0.93 for the maximum (dx/dt) and the temperature at 10% weight loss, respectively. High values for the latter two parameters are indicative of selective enrichment of the samples in cellulose (i.e., the system becomes more homogeneous) as a result of the chemical pretreatment(s).

ACKNOWLEDGMENT

The support of a research operating grant from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

Registry No. H_2SO_4, 7664-93-9; H_3PO_4, 7664-38-2; NaOH, 1310-73-2; cellulose, 9004-34-6; xylan, 9014-63-5; hemicellulose, 9034-32-6.

LITERATURE CITED

AOAC *Official Methods of Analysis of the AOAC*, 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1975.
Avgerinos, G. C.; Wang, D. I. C. *Biotechnol. Bioeng.* **1983**, *25*, 67.
Basch, A.; Lewin, M. *J. Polym. Sci. Polym. Chem. Ed.* **1973**, *11*, 3071.
Basch, A.; Lewin, M. *J. Polym. Sci. Polym. Chem. Ed.* **1973**, *11*, 3095.
Biliaderis, C. G. *Food Chem.* **1983**, *10*, 239.
Biliaderis, C. G.; Page, C. M.; Slade, L.; Sirett, R. R. *Carbohydr. Polym.* **1985**, *5*, 367.
Bouchard, J.; Leger, S.; Charnet, E. In *Proceedings of the 14th North American Thermal Analysis Society Conference*; Chowdhury, B. B., Ed.; NATAS: San Francisco, CA, 1985; p 553.
Broido, A. *J. Polym. Sci. Part A-2* **1969**, *7*, 1761.
Broido, A.; Javier-Son, A. C.; Ouano, A. C.; Barrall, E. M. *J. Appl. Polym. Sci.* **1973**, *17*, 3627.
Brown, D. E. *Philos. Trans. R. Soc. London, B* **1983**, *300*, 305.
Buleon, A.; Chanzy, H. *J. Polym. Sci. Polym. Phys. Ed.* **1982**, 20, 1081.
Cabradilla, K. E.; Zeronian, S. H. In *Thermal Uses and Properties of Carbohydrates and Lignin*; Shafizadeh, F., Sarkanen, K. V., Tillman, D. A., Eds.; Academic: New York, 1976; p 73.
Chang, M. M.; Chou, T. Y. C.; Tsao, G. T. *Adv. Biochem. Eng.* **1981**, *20*, 15.
Chen, D. T. Y. *J. Thermal Anal.* **1974**, *6*, 109.
Cowling, E. B.; Kirk, T. K. *Biotechnol. Bioeng. Symp.* **1976**, *6*, 95.
Dollimore, D.; Holt, B. *J. Polym. Sci. Polym. Phys. Ed.* **1973**, 11, 1703.
Freeman, E. S.; Carroll, B. *J. Phys. Chem.* **1958**, *62*, 394.
Furneaux, R. H.; Shafizadeh, F. *Carbohydr. Res.* **1979**, *74*, 354.
Gharpuray, M. M.; Lee, Y. H.; Fan, L. T. *Biotechnol. Bioeng.* **1983**, *25*, 157.
Goering, H. K.; Van Soest, P. J. *USDA Agriculture Handbook, No. 379*; Government Printing Office: Washington, DC, 1970; p 8–9.
Jain, R. K.; Lal, K.; Bhatnagar, H. L. *J. Appl. Polym. Sci.* **1985**, *30*, 897.
Liskowitz, J. W.; Weill, C. E.; Carroll, B. *Carbohydr. Res.* **1980**, *79*, 23.
MacDonald, D. G.; Bakhshi, N. N.; Mathews, J. F.; Roychowdhury, A. *Biotechnol. Bioeng.* **1983**, *25*, 2067.
Millet, M. A.; Baker, A. J.; Satter, L. D. *Biotechnol. Bioeng. Symp.* **1975**, *5*, 193.

Millet, M. A.; Baker, A. J.; Satter, L. D. *Biotechnol. Bioeng. Symp.* **1976**, *6*, 125.
Puri, V. P. *Biotechnol. Bioeng.* **1984**, *26*, 1219.
Reid, I. D. *Appl. Environ. Microbiol.* **1985**, *50*, 133.
Rodrig, H.; Basch, A.; Lewin, M. *J. Polym. Sci.* **1975**, *13*, 1921.
Segal, L.; Creely, J. J.; Martin, A. E., Jr.; Conrad, C. M. *Text. Res. J.* **1959**, *29*, 786.
Shafizadeh, F. *J. Polym. Sci. Part C* **1971**, *36*, 21.
Shafizadeh, F.; McGinnis, G. D.; Susott, R. A.; Tatton, H. W. *J. Org. Chem.* **1971**, *36*, 2813.
Shafizadeh, F.; McGinnis, G. D. *Carbohydr. Res.* **1971**, *16*, 273.
Shafizadeh, F.; Fu, Y. L. *Carbohydr. Res.* **1973**, *29*, 113.
Shafizadeh, F.; Lai, Y. Z. *Carbohydr. Res.* **1975**, *42*, 39.
Shafizadeh, F. *J. Appl. Polym. Sci. Appl. Polym. Symp.* **1983**, *37*, 723.
TAPPI Standard T22 05-74; TAPPI: Atlanta, 1974.
Vallander, L.; Eriksson, K. E. *Biotechnol. Bioeng.* **1985**, *27*, 650.
Yu, E. K. C.; Deschatelets, L.; Saddler, J. N. *Biotechnol. Bioeng. Symp.* **1984**, *14*, 341.

Received for review January 27, 1986. Accepted June 11, 1986.

Wax Components of *Asparagus officinalis* L. (Liliaceae)

Rainer W. Scora,* Edith Müller, and Paul.-G. Gülz

Chloroform-extracted epicuticular wax from cladophylls of *Asparagus officinalis* was investigated. Pentane, 2-chloropropane, and methanol fractions were eluted from a silica gel column. These fractions were separated by TLC and GC into individual alkane (31.5%), wax ester (20.1%), ketone (6.4%), aldehyde (13.5%), alcohol (16.4%), and fatty acid (8.5%) components. Catalytic hydrogenation, esterification, reduction, and acetylation were employed for component identification or derivatization for GC injection.

Asparagus officinalis L. has been cultivated since ancient times. According to Pliny, the Romans, as early as 200 B.C., wrote gardening instructions for the cultivation of asparagus and were well aware of quality differences in the spears (Hexamer, 1908). The Greeks, unlike the Romans, did not cultivate asparagus but used the short-branched, more or less prostrate plant parts collected from wild stands (Boswell, 1949). Today, this perennial, dioecious plant is grown in most parts of the world. The commercial lines that are cultivated today in the United States are improved forms of the indigenuous plants originally found along the seacoasts of Europe, North Africa, and Asia. Formerly used also for its diuretic properties and for heart afflictions (Hexamer, 1908), *A. officinalis* is now grown for consumption of its edible spring shoots. In some areas, however, like the mountains of Sabah, Borneo, it is harvested during the entire year.

While much effort was made to identify its gene resources, and to study its sex inheritance mechanisms and expressions, relatively little was done to investigate the phytochemical nature of this species which is considered an important commercial vegetable. Proteins and vitamins A and C contents were determined (USDA, 1981), as well as its steroid β-glycosides (Goryanu et al., 1976). Also determined were its steroid saponins (Goryanu and Kintya, 1976), the bitter principle and structure of furostanol saponin (Kawano et al., 1975), and the fructooligosaccharides (Shiomi et al., 1976). Foliar isozymes (Roux, 1980), as well as asparagusate dehydrogenase (Yanagawa, 1976), and the formation of sulfur-containing acids as flavor components were also investigated (Tressl et al., 1977).

Department of Botany & Plant Sciences, University of California, Riverside, California 92521 (R.W.S.), and Botanisches Institut der Universität zu Köln, D-5000, Köln 41, BRD (P.-G.G., E.M.).

We report in this paper on the individual components of the epicuticular wax, so important in the plant's natural defense mechanism to invasion by parasitic organisms, in the deposition of agricultural spray chemicals, and in the water economy of plants, especially those grown in semiarid climates.

EXPERIMENTAL SECTION

Fully grown cladophylls (fernlike leaves) were harvested in Aug 1985 at the Agricultural Experiment Station, University of California, Riverside. The soluble cuticular lipids were extracted by dipping the fresh material consecutively into three beakers of $CHCl_3$ (800 mL) for a total of 3 min. The extract was taken to dryness and the raw wax redissolved in 50 mL of warm n-C_5H_{12}. After cooling, the supernatant was fractionated on a Si gel CC (Type 60, Merck, 70–230 mesh). Hydrocarbons were eluted with pentane, esters and aldehydes with 2-chloropropane, and free alcohols and free acids with methanol. Yield and composition of the individual fractions are reported in Table I; fractionation and separation systems, in Figure 1 (Gülz, 1984).

The eluted individual fractions were streaked on TLC plates coated with silica gel 60 (Merck). The solvent system was benzene for hydrocarbons, aldehydes, wax esters, and methyl esters and 2-chloromethane–ethyl acetate (24:1) for fatty acids and alcohols. The detection reagent for both was bromothymol blue. The band extracts were purified and injected into a Hewlett-Packard 5750 gas chromatograph with fid and integrator 3380 S. The columns used were fused silica gel capillary OV-101, 25 m, temperature programmed from 160 to 280 °C with a 4 °C/min advance, and for wax esters a 12-m glass capillary column DUHT OV-101 temperature programmed from 160 °C to a maximum of 340 °C at a 4 °C/min advance was used. The components were characterized by comparing their retention times to those of standards.

0021-8561/86/1434-1024$01.50/0