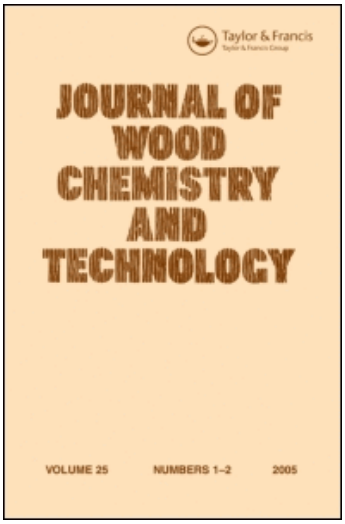

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EFFECT OF THE DEGREE OF ACETYLATION ON THE ENZYMATIC DIGESTION OF ACETYLATED XYLANS

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ABSTRACT

The effect of the degree of acetylation on the enzymatic digestibility of acetylated xylans has been investigated. Oatspelts xylans were reacetylated to degrees of 0.26 to 1.67 moles acetyl groups per mole of anhydroxylose units. These acetylated samples were then used to study the effect of acetylation on the xylanase (EC 3.2.1.8) and acetyl esterase (EC 3.1.1.6) activities of a commercial *Trichoderma reesei* cellulase preparation. The enzymatic digestibility was dramatically affected by the degree of acetylation. The onset of resistance is similar for both the xylanase and acetyl esterase enzymes, and both enzymes were completely inhibited by a degree of acetylation of 1.5 moles acetyl groups per mole anhydroxylose units at all enzyme loadings.

INTRODUCTION

Native xylan, due to its physical and chemical properties, may be partially responsible for the resistance to cellulolytic enzyme degradation exhibited by some plant tissue[1]. Xylan is a major component of hemicelluloses in many plants and can represent as much as 25 to 35% of the total dry weight[2,3]. The native xylan in grasses and hardwoods is extensively acetylated. Acetyl groups can

account for 70% of the substituents in some native xylans[4]. The xylans are also extensively acetylated during the chemical acetylation of wood. This chemical acetylation, used for dimensional stabilization, also confers to the wood a resistance to microbial decay[5-18]. Isolated xylans could also be acetylated and used in polymer applications[19]. Until recently, the effect of acetylation on the digestibility of native xylans has been overlooked, because most studies on xylan digestion have employed deacetylated xylan as the test substrate[20]. Most procedures that isolate xylan from native plant cell walls are harsh and disrupt the labile ester bonds of the acetyl groups[2,3]. Recent research, however, has shown that acetyl xylans isolated from various hardwoods with acetyl groups intact were more digestible by esterase-free xylanase preparations after they have been chemically deacetylated. The same study showed that acetyl xylans were also found more digestible once the xylanase was supplemented with acetyl esterase enzymes[21]. Other studies of the digestibility of dried grasses, carried out in ruminants, and by enzymatic hydrolysis, have shown that the degree of acetylation markedly effects their digestion[22,23]. Investigations of the effects of *in vivo* deacetylation on the cellulolytic digestion of aspen wood and wheat straw has indicated that as the acetyl xylan fraction becomes increasingly deacetylated, it becomes more digestible, which in turn makes the cellulose fraction more accessible to cellulase enzymes and therefore more digestible[1].

In the present study, xylan isolated from oatspelts was reacetylated to various degrees. Then, the effect of the degree of acetylation on the enzymatic digestibility of these acetylated xylans was investigated over the entire range of acetylation.

RESULTS AND DISCUSSION

Xylan Acetylation

Oatspelts xylans were chemically acetylated to various degrees, ranging from 0.26 to 1.67 mole acetyl group per mole

anhydroxylose units, by the pyridine and acetic anhydride reaction in formamide described below. The values for degree of acetylation are averages of the molar ratio of acetyl groups to anhydroxylose units (i.e., a value of 0.25 corresponds to 1 acetyl group per 4 anhydroxylose units). Since there are 2 sites for acetylation per anhydroxylose unit, the maximum theoretical value was 2. This sample with maximum acetylation was not prepared since it was determined that samples with degrees of acetylation higher than 1.4 were indigestible by mixtures of xylanases and acetyl esterases present in cellulase preparations.

Characterization of the Acetylated Xylan

The characteristics of the acetylated xylan samples followed the expected trends with degree of acetylation. The original oatspelts xylan had a composition by weight of 72% xylan, 19% ash (16% silica and 3% other), 5% moisture, 0.8% uronic acids, and 4% other sugars. The moisture content within the samples decreased with increasing degree of acetylation. This is expected since the acetyl groups are less hygroscopic than the hydroxyl groups they replaced. The anhydroxylose content apparently decreases with increasing degree of acetylation. This apparent loss is not a real loss of anhydroxylose, but an increase in the total dry weight of individual xylan chains upon the addition of acetyl groups. The ash content decreases slightly with an increase in acetylation. This will also include the same apparent loss as in the anhydroxylose content. Finally, the degree of acetylation was obtained by averaging the results from multiple gas chromatographic analyses on as many as eight separate experiments. Therefore, the values for degree of acetylation found for individual samples, and shown in Figure 1, deviate by no more than ±10% of the average value.

Enzyme Digestion

Figure 1 shows the effect of the degree of acetylation on enzymatic digestion for two enzyme loadings. It should be pointed

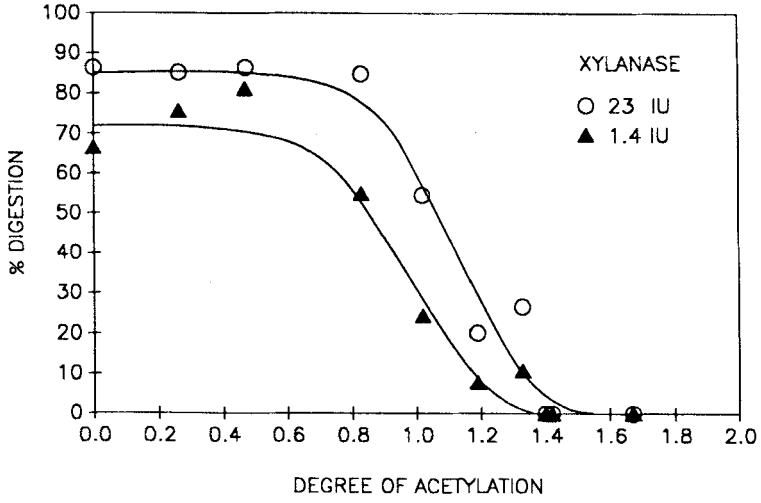

Figure 1. The effect of the degree of acetylation on the enzymatic digestion of acetylated xylans at two enzyme loadings.

out that the enzymatic solubilization is caused by the combined action of xylanases and acetyl esterases enzymes which are both present in the mixed cellulase-hemicellulase preparation produced by *T. reesei*. The results which could be obtained with purified xylanase preparations with no esterase activity could differ from the results obtained here. There is evidence that xylanases and acetyl esterase show synergistic effects in the degradation of acetylated xylans[21]. Unfortunately, there are no methods presently available that would cause the individual inhibition of either of these enzymes and thus allow a non-synergistic determination of each enzyme activity.

The results presented in Figure 1 are based on the gravimetric assay described below, and are precise to within ±5%. A dinitrosalicylic acid colorimetric assay produced similar results, but was less precise (results not shown). Therefore, it was decided that the gravimetric assay was an acceptable method for the digestion analysis. The most significant aspect of Figure 1 is that the onset of the resistance to enzymatic digestion is abrupt

and occurs near a degree of acetylation of 0.8. Above the 0.8 value, the increased acetylation of the xylans renders them completely indigestible by a degree of acetylation of 1.5. It may be difficult to isolate the exact mechanism(s), since there are several possibilities (e.g., surface area, hydrophobicity, and steric hindrance). It is most important to note; however, that irrespective of which mechanism(s) was the cause of increased resistance, it was brought about by increased acetylation. It was also significant that there was a substantial amount of resistance by the degree of acetylation of 1.0, since this is approximately the degree of substitution of native xylans. Therefore, acetylation of native xylans may play a role in the resistance of plant cell walls to cellulolytic enzyme degradation and definitely plays a role in resistance of acetylated wood to microbial decay.

Deacetylation during Digestion

The effect of the degree of acetylation on the relative deacetylation during digestion is shown in Figure 2. This figure is a graphical representation of the effect of the degree of acetylation on the acetyl esterase activity towards the various substrates. Recent research has shown the importance of acetyl esterase activity in the digestion of acetyl xylan[21]. It would be expected that the xylanase and acetyl xylan esterase enzymes would work cooperatively to completely digest the biomass, but apparently the acetyl xylan esterase is inhibited in the same way that the xylanase is at high degrees of acetylation.

It should be noted that this resistance to both xylanase and acetyl esterase enzyme must be considered when determining their respective activities, especially when the degree of acetylation of the substrate is 0.8 or higher.

In conclusion, the enzymatic digestion of xylans is dramatically affected by their degree of acetylation. The onset of resistance to both xylanase and acetyl esterase is a function of enzyme loading, but both enzymes are completely inhibited by a

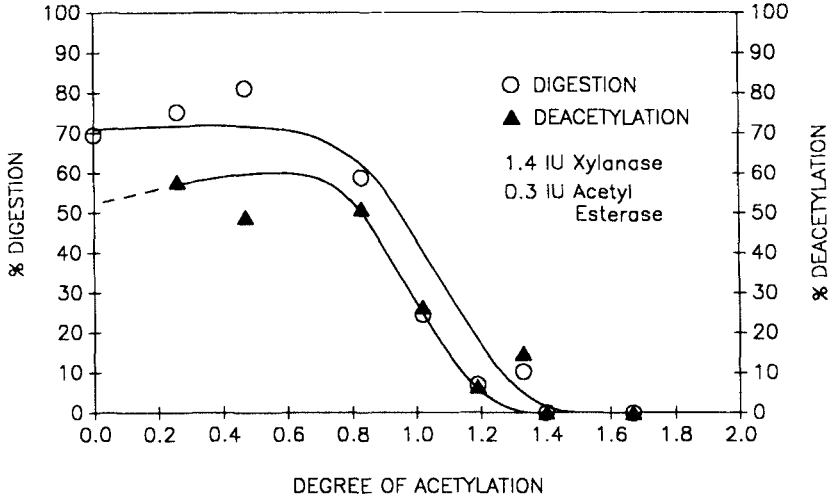

Figure 2. The effect of the degree of acetylation on the degree of deacetylation during enzymatic digestion.

degree of acetylation of 1.5. There is a marked resistance to digestion by a degree of acetylation of 1.0, the average degree of substitution of native xylans. Since these acetyl groups are the main substituent of native xylans, the acetylation of native xylans plays a role in some plant cell walls' resistance to combined cellulolytic enzyme degradation.

EXPERIMENTAL

Materials

Xylan from oatspelts, formamide, pyridine, acetic anhydride, and all reagent grade chemicals were obtained from the Sigma Chemical Co. A cellulase preparation (Celluclast 1.5L) and a β-glucosidase preparation (Novozym SP188) were gifts of NOVO Industries, Inc.

Xylan Acetylation

Xylans to be acetylated were swollen by constantly stirring a 10% w/v xylan slurry in formamide (100 mL total volume) for 18 to 24 hrs[24]. Once sufficiently disrupted, required volumes of pyridine, then acetic anhydride, were added drop-wise from a buret to the xylan suspension (e.g., 15 mL pyridine and 14.6 mL acetic anhydride were added to produce a degree of acetylation of 1.33). These reaction mixtures were left to react at ambient temperatures for 4 to 6 hrs. Then to quench the reaction and precipitate the water-insoluble, acetylated xylan fraction, the mixtures were poured directly into 1 L of deionized water. These samples were stirred for 1/2 to 1 hr more, then filtered through a Whatman GF/C filter. The resulting filter cakes were washed with 2 L of deionized water and then freeze-dried. Alternatively, the xylans with lower levels of acetylation were dialyzed against deionized water to remove formamide and other low molecular weight compounds. Insoluble xylan fractions were collected by centrifugation and freeze-dried. The dried, acetylated xylans were stored at 25°C. Also, for comparative reasons, the original oatspelts xylan was water washed exhaustively and the water insoluble fraction (approximately 60%) was used for all subsequent assays.

Acetylated Xylan Characterization

Dry weights were determined by standard methods[25]. Ash analyses were performed by gravimetric analysis according to A.O.A.C. methods[26].

Anhydrosugars were determined by a procedure slightly modified from a procedure developed at the U.S. Forest Products Laboratory[25]. Rationale and details of the modifications have been discussed previously[27].

The degree of acetylation of the acetylated-xylans was obtained by conversion of the esterified acetate to free acetic acid by hydrolyzing the xylans in 64% w/w H_2SO_4 for two hours at ambient temperature. This procedure assured complete

solubilization of the sample. The xylan hydrolyzate was then diluted with deionized water to 3% w/w H_2SO_4 and subjected to a moderate temperature cook (121°C for 1 hr) to free the acetate and to complete xylan hydrolysis. Several saponifications using 1 N NaOH at 121°C for 1 hr, followed by acidification to pH 2.5, were included as controls[28]. The free acetic acid in the subsequent hydrolyzates were analyzed by gas chromatography using a Hewlett-Packard model 5840A gas chromatograph equipped with a flame ionization detector and a Carbopack C/0.3% CW 20M/0.1% H_3PO_4 pack column (2mm x 2m, Supelco). Column, injection port, and detector temperatures were maintained at 175, 190, 190°C, respectively. Nitrogen served as the carrier gas. The column was calibrated with C-2 to C-5 volatile fatty acids standards (0.1%, Supelco). Once the amount of free acetic acid, the percentage of anhydroxylose in the sample, and the respective dry weights were determined, the degree of acetylation (the molar ratio of acetyl groups to anhydroxylose units) could be calculated.

Enzyme Digestion

The acetylated-xylan samples were assayed for enzyme digestibility using combined NOVO Celluclast 1.5L/Novozym SP188 (cellulase/β-glucosidase) preparation. Enzyme aliquots were made from dilutions of a stock 11/1 (v/v) mixture of NOVO Celluclast 1.5L/Novozym SP188. The stock cellulase preparation was found to have a xylanase activity of 55 IU/mL (1 International Unit (IU) defined as that amount of enzyme required to release 1 μmole xylose equivalent per min from a 1% w/v suspension of oatspelts xylan at 50°C). The xylanase activity assays were performed in a 50 mM citrate buffer pH 4.9. The stock cellulase solution was also found to have an acetyl xylan esterase activity of 12 IU/mL. Here, 1 IU is defined as that amount of enzyme required to release 1 μmole of acetic acid per min from a 2% w/v slurry of acetylated xylan (degree of acetylation 0.26) at 50°C. The acetyl-xylan esterase activity was also determined in 50 mM citrate buffer pH 4.9. Enzyme digestions of acetylated-xylan samples were performed by

gentle agitation of 10 mg/mL substrate suspensions in 50 mM citrate buffer pH 4.9 at 50°C. Various enzyme loadings, ranging from 1.4 to 23 IU xylanase, were employed. Initially, the extent of digestion was determined gravimetrically by dry weight recovery from Whatman GF/F (pore size 0.7 μm) filtrations of the digestion-slurries. As a check of this method, several samples of the post-digestion-slurries were saponified (1 N NaOH at ambient temperature for 4 hours) to release acetate from the substrate. The number of free reducing groups were then determined colorimetrically using dinitrosalicylic acid[29].

Deacetylation during Digestion

In order to determine the release of acetate during digestion, aliquots of the digestion filtrates were acidified (e.g., pH < 2.0 using 0.03 M oxalic acid) and analyzed using gas chromatography. Propionic acid was included as an internal standard. The results from these assays, combined with the previously determined degree of acetylation for each sample, permitted the calculation of the relative degree of deacetylation during digestion.

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REFERENCES

1. K. Grohmann, D.J. Mitchell, M.E. Himmel, B.E. Dale, and H. A. Schroeder, Biotech. Bioeng. Symp., (1989) In press.

2. T.E. Timell, Adv. Carbohydr. Chem., 19, 247 (1964).

3. T.E. Timell, Adv. Carbohydr. Chem., 20, 409 (1965).

4. J.S.D. Bacon, A.H. Gordon, and E.J. Morris, Biochem. J., 149, 485 (1975).

5. P.C. Arni, J.D. Gray, and R.K. Scougall, J. Appl. Chem., 11, 157 (1961).

6. B.R. Baird, Wood Fiber, 1, 54 (1969).

7. H.J. Callow, J. Ind. Chem. Soc., 28, 605 (1951).

8. L.P. Clermont and F. Bender, For. Prod. J., 7, 167 (1957).

9. Cotopa Ltd., British Patent No. 579,255, July 29, 1946.

10. W.A. Dreher, I.S. Goldstein, and J.R. Cramer, For. Prod. J., 14, 66 (1964).

11. I.S. Goldstein, E.B. Jeroski, A.E. Lund, J.F. Nielson, and J.W. Weaver, For. Prod. J., 11, 363 (1961).

12. I.S. Goldstein and J.W. Weaver, U.S. Patent No. 3,094,431, June 18, 1963.

13. J. Risi and D.F. Arseneau, For. Prod. J., 7, 210 (1957).

14. S.P. Singh, I. Dev, and S. Kumar, Wood Science, 11, 268 (1979).

15. A.J. Stamm and H. Torkow, U.S. Patent No. 2,417,995, March 25, 1947.

16. A.J. Stamm and R. H. Baechler, For. Prod. J., 10, 22 (1960).

17. A.M. Tillman, R. Simonson, and R.M. Rowell, In The 4th International Symposium on Wood and Pulping Chemistry, Vol. 1, p. 125-130, Paris, France, 1987.

18. H. Tarkow, A.J. Stamm, and E.C.O. Erickson, Forest Products Laboratory Report No. 1593, p. 29, U.S. Forest Service, U.S. Department of Agriculture, Madison, WI, 1950.

19. H.H. Nimz, In The 4th International Symposium on Wood and Pulping Chemistry, Vol. 1, p. IIIA - IIIK, Paris, France, 1987.

20. P. Biely, J. Puls, and H. Schneider, FEBS Lett., 186, 80 (1985).

21. P. Biely, C.R. MacKenzie, J. Puls, and H. Schneider, Bio/Technology, 4, 731 (1986).

22. E.J. Morris and J.S.D Bacon, J. Agric. Sci. Camb., 85, 327 (1977).

23. T.M. Wood and S.I. McCrae, Phytochem., 25, 1053 (1986).

24. J.F. Carson and W.D. McClay, J. Amer. Chem. Soc., 68, 1015 (1946).

25. W.E. Moore and D.B. Johnson, In Procedures for the Chemical Analysis of Wood and Wood Products, report no. 67-045, Forest Products Lab., U.S. Department of Agriculture, Madison, WI, 1967.

26. In Official Methods of Analysis of the Association of Official Analytical Chemists, 13th Edition, method no. 14,064, W. Horwitz, ed., Association of Official Analytical Chemists, Washington, D.C., 1980.

27. K. Grohmann, R. Torget, and M.E. Himmel, Biotech. Bioeng. Symp., 15, 59 (1985).

28. Tentative Methods of Testing Cellulose Acetate, ASTM Designation D-871-61T, sections 15-17.

29. G.L. Miller, Anal. Chem., 31, 426 (1959).