Hydrolytic Conversion of Sawdust into Metabolizable Sugars

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The hydrolytic conversion of sawdust and pure cellulose powder was studied by sulfuric acid–enzymatic and sodium hydroxide–enzymatic treatments. Sugars were identified by paper chromatography and quantitated colorimetrically. Cellulose powder yielded only dextrose in concentrations ranging from 2.2 to 2.9 grams per 100 grams, while sawdust yielded dextrose and xylose in concentrations ranging from 3.0 to 4.1 and 1.0 to 6.7 grams per 100 grams, respectively. Under optimum conditions, the total concentration of sugars was 10.8 grams per 100 grams. Since sawdust is extremely inexpensive, and both sugars are available to the human digestive tract, further work is needed to commercialize the process.

In the search for new sources of nutrients to feed a rapidly increasing world population, appraisal of nonutilized agricultural by-products demands careful attention. Of primary concern is the hydrolysis of natural cellulosic materials, such as sawdust, into their component sugars which would be available to the human digestive system. As early as 1922, Kressman hydrolyzed wood products into sugars for the microbiological production of alcohol. Saeman *et al.* (1963) have shown that pure cellulose may be totally hydrolyzed by primary treatment with a strong mineral acid followed by hydrolysis in dilute acids. Weak alkalies, such as 0.05*N* sodium hydroxide (Machell and Richards, 1957), have also been studied. Enzymatic hydrolysis, because of the specificity of action, has presented a more favorable approach to the conversion of cellulose into component sugars (Reese *et al.*, 1959). However, very little emphasis has been placed on the utilization of hydrolytic breakdown products of cellulosic materials for use directly in human nutrition.

Combining the speed and ease of acid or alkaline hydrolysis with the specificity of enzymatic action seemed therefore a logical approach to the conversion of natural cellulosic materials into nutrients suitable for human consumption.

MATERIALS AND METHODS

Sources of cellulose studied were Whatman standard grade cellulose powder and sawdust from Southern longleaf pine. Sawdust was prepared for treatment by washing in distilled water to remove soluble impurities, followed by grinding in a laboratory Wiley mill, and drying in a convection-type oven at 105° C.

Cellulolytic enzymes were a commercial preparation (Rohm and Haas) derived from *Aspergillus oryzae*.

Acid hydrolytic treatments were carried out in a water bath under reflux conditions at 50° C. for 1, 2, and 3 hours. In each case, 100 ml. of 15, 20, or 25% sulfuric acid was added to 5 grams of sample. Following hydrolysis, the solution was filtered through a Gooch filter-crucible and neutralized with barium hydroxide. Alkaline treatments differed only in concentration. Sodium hydroxide at concentrations of 1.0 and 1.5% were used. Neutralization was accomplished by elution through an MB3 resin column.

Where enzymatic treatment was superimposed on acid or alkaline hydrolysis, the substrates were again washed to remove soluble impurities and hydrochloric acid was used to adjust the pH of the solutions to 5.0. After enzymatic hydrolysis, inactivation was accomplished by elevating the temperature to 80° C. for 15 minutes. Enzyme concentration in each case was 1.25% by weight.

Cellulose determinations were carried out by the Crampton-Maynard method (1938) as modified by Matrone (1944). This modification consisted of adding 20 ml. of cold 95% ethanol to the weighed sample and drying at 105° C. to constant weight. Ignition at 600° C. for 6 hours followed to achieve complete destruction of the cellulose. Differences in weight, before and after treatments, were taken as the amount of cellulose hydrolyzed. Sugars were separated by standard methods of paper chromatography by spotting on Whatman No. 1 paper and using a solvent system of butanol–acetic acid–water (4:1:1). Chromatograms were developed with aniline phthalate. Quantitation by routine colorimetric procedures utilizing phenol–sulfuric acid reagent (1 ml. of 5% phenol and 5 ml. of concd. H_2SO_4) added to 1 ml. of unknown. Colorimeter readings were taken at 490 mμ for hexoses and 480 mμ for pentoses. All values listed are averages of triplicate determinations on duplicate samples.

RESULTS AND DISCUSSION

The hydrolysis of pure cellulose powder and sawdust as a result of sulfuric acid treatment is shown in Table I. At an acid concentration of 15%, hydrolysis of pure cellulose increased slightly as a function of time, while that for sawdust exhibited no pronounced variations between 1 and 3 hours of treatment. Sulfuric acid concentration of 20% exhibited a similar trend, but values for sawdust were

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Table I. Acid Hydrolysis of Pure Cellulose and Sawdust as a Function of Time

Substrate	Time, Hr.	Acid Concn., %	Cellulose Hydrolyzed,[a] %
Cellulose	1	15	10.62
	2	15	11.02
	3	15	14.14
	1	20	10.07
	2	20	13.51
	3	20	12.21
	1	25	1.85
	2	25	2.48
	3	25	1.75
Sawdust	1	15	14.82
	2	15	14.38
	3	15	13.71
	1	20	12.20
	2	20	12.40
	3	20	10.32
	1	25	9.60
	2	25	11.75
	3	25	12.03

[a] Determined by difference from weight of cellulose recovered.

Table II. Acid-Enzymatic Hydrolysis of Pure Cellulose and Sawdust as a Function of Time

Substrate	Time, Hr.	Acid Concn., %	Cellulose Hydrolyzed,[a] %
Cellulose	1	15	11.43
	2	15	13.85
	3	15	10.92
	1	20	20.96
	2	20	21.43
	3	20	21.95
	1	25	12.97
	2	25	10.79
	3	25	10.46
Sawdust	1	15	14.87
	2	15	19.43
	3	15	16.97
	1	20	12.58
	2	20	12.18
	3	20	21.29
	1	25	16.72
	2	25	15.05
	3	25	14.29

[a] Determined by difference from weight of cellulose recovered.

quantitatively reduced from those observed at the 15% acid concentration. Of particular interest was the pronounced reduction in hydrolytic breakdown of pure cellulose at 25% acid concentration. Sawdust samples also reflected reduced hydrolytic activity at these levels although to a lesser magnitude. Increasing the time of exposure of samples to acid of increasing concentration appeared to result in improved hydrolysis until a concentration of approximately 20% was attained. However, laboratory data indicated 25% acid treatment as the most favorable condition to precede enzymatic action, from the standpoint of sugar yields. A major drawback to acid hydrolysis of cellulosic products for human consumption is that breakdown occurs randomly along the entire chain length. To be of use in human diets, there must be a considerable degree of certainty as to where this cleavage will occur. For this reason, the specificity of enzymatic treatment is a necessity in human application in order to assure metabolizable products.

Samples subjected to acid hydrolysis were given an enzymatic treatment for from 1 to 3 hours (Table II). A comparison of Tables I and II illustrates the fact that commercial cellulolytic enzymes of *A. oryzae* origin improve the hydrolytic breakdown of both pure cellulose and sawdust over sulfuric acid treatment alone. Enzymatic action very nearly doubled hydrolysis at the 20% acid level, and increased from five- to six-fold at the 25% acid concentration in the case of pure cellulose powder. Increases at other concentrations and with the sawdust substrate were not nearly so pronounced.

Alkaline hydrolysis of either substrate was clearly less effective than sulfuric acid treatment (Table III). Increasing both time and sodium hydroxide concentration resulted in improved hydrolytic breakdown of cellulose and sawdust. Alkali concentrations greater than 1.5% were shown in the laboratory to be even less effective than the 1.5% level.

Table IV lists the results of superimposing enzymatic treatment on the alkaline hydrolysis. Increase in cellulose breakdown was three-fold at the 1.0% sodium hydroxide concentration at all time intervals measured. Aside from this effect, no significant tendencies were observed for enzymatic treatment to raise the yields of cellulose hydrolysis in alkali-treated samples of sawdust or cellulose powder.

Samples of the hydrolyzed solutions were spotted on chromatographic paper and separated according to accepted practices. Each spot was then eluted and subjected to colorimetric analysis for quantitative evaluation (Tables V and VI). Only dextrose and xylose were detectable on any of the paper chromatograms. In the case of pure cellulose powder, dextrose was the only sugar yielded on hydrolysis, as expected. Yields of dextrose were not appreciably different between acid-enzymatic and alkaline-enzymatic treatments. However, xylose yields were markedly greater after acid hydrolysis than after alkaline treatment. In addition, yield of xylose from sawdust increased substantially as a function of time. It is apparent from Table VI that if dextrose and xylose yields were combined, from 7 to 10 grams of total sugars per 100 grams of sample would result from superimposing acid and enzymatic hydrolysis of sawdust. By comparison, using a cellulose from *Streptomyces* sp. QMB814, Reese obtained a 32% hydrolysis of Walseth cellulose into

Table III. Alkaline Hydrolysis of Pure Cellulose and Sawdust as a Function of Time

Substrate	Time, Hr.	Alkali Concn., %	Cellulose Hydrolyzed,[a] %
Cellulose	1	1.0	2.54
	2	1.0	1.28
	3	1.0	2.94
	1	1.5	2.04
	2	1.5	6.37
	3	1.5	6.80
Sawdust	1	1.0	6.50
	2	1.0	8.62
	3	1.0	9.88
	1	1.5	6.05
	2	1.5	4.40
	3	1.5	7.23

[a] Determined by difference from weight of cellulose recovered.

Table IV. Alkaline-Enzymatic Hydrolysis of Pure Cellulose and Sawdust as a Function of Time

Substrate	Time, Hr.	Alkali Concn., %	Cellulose Hydrolyzed,[a] %
Cellulose	1	1.0	7.09
	2	1.0	6.70
	3	1.0	7.66
	1	1.5	7.17
	2	1.5	7.86
	3	1.5	6.52
Sawdust	1	1.0	11.88
	2	1.0	9.86
	3	1.0	10.20
	1	1.5	10.38
	2	1.5	8.35
	3	1.5	8.00

[a] Determined by difference from weight of cellulose recovered.

cellobiose and cellotriose, recovering only trace amounts of glucose. An enzyme of *A. niger* was incorporated by Whistler and Smart (1953) to hydrolyze cellulose into cellobiose and glucose. These workers reported on optimum conditions for hydrolysis but made little reference to quantitative recovery. The differences in hydrolyses between the present study and those reported earlier can be attributed to differences in enzymes and substrates.

It was concluded that the optimum conditions for the hydrolysis of sawdust in these studies were 3 hours of treatment with 25% sulfuric acid, followed by 3 hours of enzymatic hydrolysis. These treatments resulted in yields of 10.8% sugars from sawdust substrates. Concentration and evaporation techniques could be used to obtain these sugars in pure crystalline form following filtration. At this stage, unhydrolyzed cellulose could be recycled for further breakdown.

Table V. Yield of Sugars from 25% Acid-Enzymatic Hydrolysis of Pure Cellulose and Sawdust

Substrate	Time, Hr.	Dextrose, G./100 G.	Xylose, G./100 G.
Cellulose	1	2.92	...
	2	2.60	...
	3	2.43	...
Sawdust	1	3.17	3.40
	2	3.58	4.50
	3	4.10	6.70

Table VI. Yield of Sugars from 1.5% Alkaline-Enzymatic Hydrolysis of Pure Cellulose and Sawdust

Substrate	Time, Hr.	Dextrose, G./100 G.	Xylose, G./100 G.
Cellulose	1	2.97	...
	2	2.29	...
	3	2.31	...
Sawdust	1	3.07	1.21
	2	3.64	1.74
	3	3.07	1.06

Yields of metabolizable sugars, dextrose and xylose, from pine sawdust suggested further work to develop this procedure. Sugar yields of approximately 11% cannot be taken as a commercial possibility, per se. However, sawdust is a by-product of virtually no value to the sawmill, therefore, yields of this nature become more appealing. Also, sulfuric acid and commercial enzyme preparations from *A. oryzae* are not overly expensive, and when populations begin to outrun food supplies, present economic considerations may have to be revised.

Metabolizable carbohydrates rank as one of mankind's most serious nutrient deficiencies. While protein deficits are receiving increased attention from researchers, much of the world's population suffers from an equally severe caloric deficiency.

Sugars from sawdust and other cellulosic by-products may become tremendously important in human nutrition in the future.

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