Enzymolysis of Pentosans of Wheat Flour[1]

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ABSTRACT

A water-soluble araboxylan was isolated from baker's patent flour. This polysaccharide was used as substrate in viscometric estimation of pentosanase activities of commercial enzyme preparations. Two cellulases derived from aspergilli and one from *Trichoderma viride* were found to be high in activity against araboxylan. The solubilizing effect of these enzymes on the pentosans of tailings was not correlated with the assay pentosanase activity. The *T. viride* cellulase solubilized the insoluble pentosan fraction to a greater extent than the enzymes of aspergilli. This cellulase also modified the properties of tailings. A gradual enzymolysis of pentosans of tailings caused a decrease of water-absorption of this fraction. The modified tailings were superior in baking quality to those isolated from flour. At the optimum level of treatment their baking quality was comparable to that of prime starch. The aspergilli cellulases were less effective than that of *Trichoderma*. The pH, temperature optima, and heat-stabilities of the enzymes are reported.

Pentosans that occur in wheat endosperm belong to the araboxylan series of polysaccharides. The backbone of this polymer is a xylan chain of 1,4-linked D-xylopyranose units with L-arabinofuranose side-chain residues attached to certain xylose units, principally through position 3 (1). These polysaccharides exist in flour endosperm in free form and in association with proteins and other polysaccharides. They are differentiated also by their solubilities in water. The average baker's-patent flour contains 2–3% total pentosans, of which approximately half is extractable with water. The residue remains insoluble, associated with tailings. The polysaccharide fractions of the water-soluble and water-insoluble pentosans are considered structurally similar. The insoluble fraction was reported to be more branched than the soluble one. The differences in solubilities reflect also variations in molecular shapes, degrees of polymerization, physical entanglement, and association with other nonpentosan polymers.

The functional properties of the pentosan fractions were also found to be varied. The water-soluble pentosans were found slightly beneficial or without effect in bread preparation. Experiments reported by Pence *et al.* (2) showed a slight improving effect on bread volume which could be attributed to the protein impurities of their preparations. More recently Tracey (3), by degrading pentosans *in situ* with an enzyme from digestive juice of starved garden snails *(Helix aspersa),* showed that destruction of pentosans caused a slight reduction of bread volume (10% or less).

The effects of water-insoluble pentosans on bread preparation were reported in previous publications from this laboratory (4). Increased water-absorption without change of rheological characteristics of doughs was observed. Further, higher pentosan levels tended to reduce loaf volume and coarsen its grain. The pentosan fraction was also shown to be the adverse component of tailings (5).

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The possibilities of modifying the water-insoluble pentosans present in tailings by enzymatic means were investigated with the objective of improving the baking properties of flours. The results of this study are reported herein.

MATERIALS AND METHODS

Flours

The prime starch, tailings, and water-soluble and water-insoluble pentosans were isolated from commercial untreated baker's-patent flours. The flours used in baking studies were of patent grade (12.0% protein, 0.40% ash at 14% m.b.) and received all normal treatments.

Substrates for Assays and Enzymatic Studies

Water-Soluble Pentosans. It was necessary to prepare a pure pentosan as substrate, preferably from wheat, for determination of pentosanase activity. After an investigation of published procedures for isolation of pentosans, the method of Holme (6) was modified and used as follows: 1,200 g. of patent flour was dispersed in 2,400 ml. water in a Hobart A-120 mixer operated at low speed for 10 min. The slurry was then centrifuged; the supernatant extract was made 0.8*M* in ammonium sulfate, kept overnight at 4° C., and then centrifuged. The supernatant extract was made 1.74*M* in ammonium sulfate. The precipitate was removed by centrifugation and the supernatant extract was heated at 95°C. for 10 min. The coagulated proteins were removed by filtration, and the pentosans were precipitated from the filtrate by making it 3.0*M* in ammonium sulfate. The pentosans were then redissolved in water and reprecipitated as before. The residual proteins were removed with Filtrol according to the procedure of Pence *et al.* (7) after the last precipitation. Pentosans were dissolved in 500 ml. water, 30 g. Filtrol was added (Super Filtrol grade 4 from Filtrol Corp., Los Angeles, Calif.), and pH was adjusted to 5.5; the mixture was stirred for 30 min. and filtered through asbestos. The salts were removed by dialysis against distilled water at 4°C. The pentosans were then recovered by freeze-drying. The yields were 1.4–2.0 g. of solids which contained 95–100% pentosans. Arabinose and xylose were the only sugars detected in acid hydrolysates by paper chromatography. They were present in a ratio of 0.58:1.00 (arabinose:xylose). The viscosity of a 1% water solution at 30°C. was 18.1 centistokes and its intrinsic viscosity was 3.4. From the analytical data it is evident that the preparation was an araboxylan rather than the entire water-soluble pentosan fraction, which contains in addition to the araboxylan a series of glycoproteins.

Carboxymethylcellulose. CMC of 0.42, 0.77, and 1.24 degree of substitution was received from Hercules Powder Co.

Flour Fractions. Tailings, prime starch, and water-insoluble pentosans were prepared according to procedures reported in preceding publications (4,5). The tailings contained 10% pentosans (14% m.b.) and the water-insoluble pentosan fraction, 60%. The ratio of arabinose to xylose in the pentosan preparation was 0.92:1.00.

Enzymes

Commercial enzyme preparations were tested for activity against ara-

boxylan and CMC. On the basis of these assays, three preparations were selected for systematic study. Cellulase NBC was a preparation of Nutritional Biochemical Corp. derived from *Aspergillus niger*. Cellzyme, also a cellulase of selected strains of *A. niger*, was supplied by the Wallerstein Co. Meicelase was a cellulase of *Trichoderma viride* manufactured by Meiji Seika Kaisha Co., Japan. All preparations were used without further purification.

Assay Procedures

Pentosanase Activity Against Water-Soluble Araboxylan. This activity was estimated by a viscometric procedure similar to that of Simpson (8). An aqueous 1.1% (w./v.) solution of araboxylan was prepared and 9 ml. of this solution was placed in a capillary Ubbelohde viscometer. One milliliter of suitable enzyme solution, containing buffer salt to make the final dilution 0.05*M* in respect to acetate buffer and pH 5.0, was used. The reaction temperature was maintained at 30°C. The zero-time viscosity was established with a control to which buffer solution without enzyme was added. The viscosity change was then determined after a reaction time of 30 min. and expressed in percent of the original viscosity. When the logarithm of the enzyme concentration was plotted against the viscosity changes, a straight-line relation was obtained when the change did not exceed 60%. (To keep below this limit the enzyme was suitably diluted.) The viscosity changes were then converted to units of activity. One unit was defined as the amount of enzyme necessary to lower the viscosity by 50% under conditions of this assay.

Carboxymethylcellulase (CMC-ase) Activity. The assay was the same as for pentosanase, except that CMC of 0.77 degree of substitution was used in the place of araboxylan. The unit of activity was defined as in the paragraph above.

Estimation of Reducing Groups. Release of reducing values when used as an index of pentosanase activity was determined by the Somogyi method (9). Xylose was used as a standard.

Methods Based on Solubilizing Power of Enzymes. The enzymatic activity on insoluble substrates was estimated by using changes of solubility of pentosans as an index. Water-insoluble pentosans (0.3 g.) were dispersed in 20 ml. of acetate buffer (0.05*M*, pH 5.0) and incubated with 9 units of pentosanase at 30°C. for 4 hr. under toluene. The insoluble residue was recovered and analyzed for pentosans. From these values the percent of solubilized pentosans was reported.

Assay of Secondary Enzymes. Proteolytic activity was estimated by the hemoglobin procedure (10). Amylolytic activity was tested with the amylograph (10).

Treatment of Tailings with Enzymes

One hundred and fifty grams of tailings was dispersed in 700 ml. water; 1,400 units of pentosanase was added (Cellulase NBC, Cellzyme, or Meicelase used), and this was incubated at 40°C. for various digestion times. Toluene was added to control microbial growth. The slurries of treated

tailings were dehydrated by freeze-drying and tested by farinography and baking tests. Changes in the solubility of pentosans were followed by the procedure given above for pentosans.

Farinography

Changes of absorptive power of tailings were evaluated by preparing mixtures of 85% flour and 15% of treated or untreated tailings. According to the constant flour weight procedure (10), the weight of flour or flour-tailings was kept constant. The water used in each case corresponded to the absorption of flour, which was the amount necessary to bring the flour-dough consistency to 500 B.U. The farinogram maximum is reported as an index of the absorptive power.

Baking Experiments

Mixtures of 85% flour and 15% tailings were used in these tests. Prime starch was used as a control instead of tailings. The test breads were prepared by sponge (4 hr.) and dough procedure as described before (4,5).

Chromatographic Methods

Sugars were determined after acid hydrolysis of pentosans in evacuated tubes (0.1 g. pentosans, 5.0 ml. 1*N* sulfuric acid, 24 hr. at 100°C.). The hydrolysates were neutralized with ion-exchange resins and the residue was concentrated by freeze-drying. When enzyme hydrolysates were studied, the reaction was arrested by 10 min. of heating in a boiling-water bath. The salts were removed with an electrolytic desalter. Simple sugars were separated on Whatman No. 1 or 4 chromatographic paper when low concentrations were encountered, by moving the sample with solvent system 1 (ethyl acetate: pyridine:water in proportions of 8:2:1). Higher sugars were studied with solvent system 2 (ethyl acetate:pyridine:water in proportions of 10:4:1) (11). The sugars were detected with silver nitrate or aniline oxalate to differentiate between hexose and pentose sugars (12). Quantitative estimations of sugar ratios were made by the phenol-sulfuric acid method of Dubois *et al.* (13).

RESULTS AND DISCUSSION

Enzymatic Activity of Enzyme Preparations

All three enzymes used in this study had a high activity against the water-soluble araboxylan. This is evident from the values reported in the table below. This activity is undoubtedly the result of an action of at least

Activities of Enzyme Preparations

Enzyme Preparation	*Pentosanase* *units/g.*	*CMC-ase* *units/g.*	*Protease* *H.U./g.*
Cellulase NBC	28,500	23,500	15,912
Cellzyme	3,030	55,000	1,479
Meicelase	28,600	9,000	1,044

two enzymes, xylanase and arabanase, but for convenience it will be referred to as pentosanase. Besides the ability to attack the pentosan substrate, high activity on CMC was observed. Special attention was given to this activity, in

view of reports (14) that the enzymolysis of pentosans may be due to the broad specificity of some cellulases rather than to specific pentosanases. Both cellulose and pentosans contain beta-1,4 glycosidic linkages, and CMC is generally considered a suitable substrate for estimation of cellulases. The values of CMC-ase were obtained by the viscometric procedure with the CMC of 0.77 D.S. This was done after an examination of the activities with CMC of D.S. 0.42 and 1.24. The selected substrate showed the highest susceptibility to the enzyme attack. Less activity was observed with the lower- and higher-substituted CMC.

The activities of pentosanase and CMC-ase were expressed in arbitrary units and thus no direct comparison between them is possible. From the data it is evident, however, that there was no consistent relationship of these two activities in the enzyme preparations. Cellulase NBC was high in pentosanase and CMC-ase, Meicelase was low in CMC-ase and high in pentosanase. On the other hand, Cellzyme was high in CMC-ase and relatively low in pentosanase. This lack of proportionality between the two activities suggests that each substrate was attacked by a different enzyme system. Further evidence in this respect was obtained from the heat-stability studies given below.

The presence of high levels of proteolytic enzymes interferes with the evaluation of pentosanases in baking studies. Measurements of this activity show that Cellzyme and Meicelase were rather low and Cellulase NBC high in proteolytic activity. These values were used in selecting experimental conditions in baking studies to keep the levels of enzymes used below critical levels of proteases.

The effect of enzyme preparations on the amylograms showed low amylolytic activity, so that in the baking studies no serious complications from this activity were expected.

Pentosanase Activity of Cereals and Other Materials of Interest

No pentosanase activity could be demonstrated in water extracts of cereals by the viscosity changes of the araboxylan. Therefore, acetone powders were prepared according to the procedure of Preece and Hobkirk (15), and from their activities the enzyme levels of cereals were estimated. These results are shown in the table below.

Enzyme Activities of Cereals and Other Materials

Sample	*Pentosanase units/g.*	*CMC-ase units/g.*
Whole wheat	0.3	0.3
Dark rye flour	0.6	0.6
Patent flour	0.8	0.4
Malted barley flour	25	9
Fungal amylase	215	220
Fungal protease	570	310

The values show very low activities of pentosanases and CMC-ase in wheat, rye, and flour. This observation was further confirmed by paper chromatography. No observable amounts of pentoses or oligosaccharides of pentoses were found in digests (24 hr., 40°) of araboxylan (10 mg./ml.)

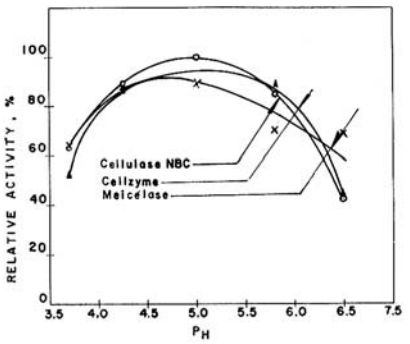
with acetone powders (1 mg./ml.). This was in general agreement with the study of Preece and Hobkirk (15), who observed a decrease of viscosity of rye araboxylan but very slow release of sugars.

Of other results in this table, it is worth noting that the enzymatic preparations generally used in baking, such as barley malt flour, fungal amylase, and protease, contain significant pentosanase activity.

Conditions of Enzymolysis of Araboxylan

The optimum conditions of pH and temperature for the three enzyme preparations were tested. Increase of reducing values was used as an index of enzymolysis rather than viscosity, because viscosity of the substrates (araboxylan, and CMC) is affected by conditions of pH and temperature.

The effect of pH on the pentosanase activity of Cellulase NBC, Cellzyme, and Meicelase is shown in Fig. 1. The enzymolysis was conducted at 30°C. for 3 hr.; the 1% araboxylan solution used contained 0.3 units/ml.



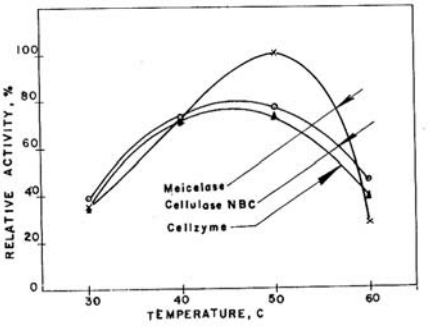


Fig. 1 (left). Effect of pH on pentosanase activity.
Fig. 2 (right). Effect of temperature on pentosanase activity.

of Meicelase, Cellulase NBC, and 0.15 units/ml. of Cellzyme. The acetate buffers were used to give final concentrations of 0.05*M*. Optimum pH range under these conditions was found to be 4.5–5.4 for Cellulase NBC, 4.5–5.7 for Cellzyme, and 4.2–5.4 for Meicelase. Similar data were obtained for CMC-ase. These showed optimum ranges of 4.0–5.0 for all three enzyme preparations.

The effect of temperature on the activity was determined at pH 5.0 by the same procedures as used in the study of the pH effect. The temperature for the range from 30° to 60°C. was studied. These results are given in Fig. 2. The temperature-activity curve of Meicelase showed a sharp peak around 50°C. The optima of Cellulase NBC and Cellzyme were broader than that of Meicelase. The optimum temperature ranges (optimum ± 5%) were: for Cellulase NBC, 40°–51°C.; for Cellzyme, 40°–51°C., and for Meicelase, 47°–53°C.

The dependence of CMC-ase on temperature was tested similarly. This activity was found to be less susceptible to heat-deactivation than that of pentosanase. The optimum temperature under the conditions of the assay was 60°C. or above (no higher temperatures were tested). The same results

were obtained with the three enzymes. The differences between the temperature-activities suggest that the actions against araboxylan and CMC are catalyzed by different enzyme systems.

Heat-Stability of Pentosanases and CMC-ases

Heat-stability of the enzymes was measured by exposing the buffered enzyme solution (1 mg. enzyme/1 ml.) in test tubes to 60°C. water-bath temperature for various time intervals. The heating was interrupted by placing the test tubes in an ice bath. The residual activity was determined by the viscometric procedure. The heat-deactivation curves are shown in Fig. 3.

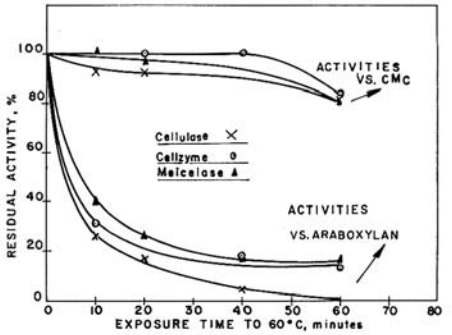


Fig. 3. Heat stability curves of pentosanases and CMC-ases.

It is evident that pentosanase was heat-labile and that CMC-ase showed a remarkable stability. This is a further evidence that these two activities are due to different enzyme systems.

The differences in heat-stability of the enzymes on araboxylan were slight only. Meicelase was somewhat less heat-resistant than the pentosanases of aspergilli. The differences in the stabilities of the CMC-ases were insignificant.

Mode of Action of Pentosanases

Figure 4 illustrates the viscosity changes (A) and progress of hydrolysis

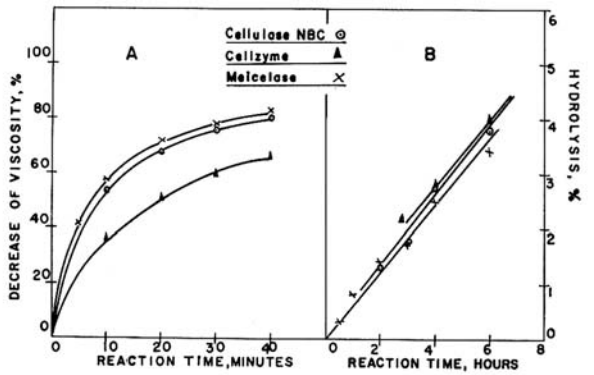


Fig. 4. A, change of viscosity of araboxylan by pentosanases. B, release of reducing groups by pentosanases.

(B) as affected by the action of pentosanases. The enzyme concentrations and other experimental conditions were kept comparable for each enzyme in A and B. The degree of hydrolysis was estimated from the release of reducing groups.

The viscosity of the araboxylan solution was reduced rapidly by each enzyme, whereas the release of reducing groups was extremely slow. Thus, after 30 min., when the viscosity of the substrate was lowered by 60–80% of its original value, less than 0.3% of the total hydrolyzable groups was released. This type of reaction indicates that the xylan chain was cleaved by a predominantly endo- or random-splitting action. All three enzymes acted by this pattern.

Enzymolysis of Water-Insoluble Pentosans

Action of the enzymes on the insoluble substrates was established by measuring the solubilization of pentosans present (a) in the water-insoluble pentosan fraction (60% pentosans) and (b) in tailings (10%).

The results given in the table below were obtained with the water-insoluble pentosan fraction. The enzymes were added at equal pentosanase levels. Digestion was carried out at 40°C. for 4 hr. at pH 5.0 (acetate buffer). A similar solubilization of the pentosans was expected, but contrary to this, Meicelase solubilized 33 and 66.4% more than Cellzyme and Cellulase NBC, respectively. The increase of soluble pentosans above the control was 80%.

Enzymolysis of Water-Insoluble Pentosans

Enzyme	*Pentosanase Added* *units/g. pentosan*	*Pentosan Solubilized* *%*
Meicelase	31	45.8
Cellzyme	31	27.8
Cellulase NBC	31	15.4
Control	....	9.1

Analogous results were obtained when tailings were used as the substrate for enzymolysis. These data are shown in Fig. 5.

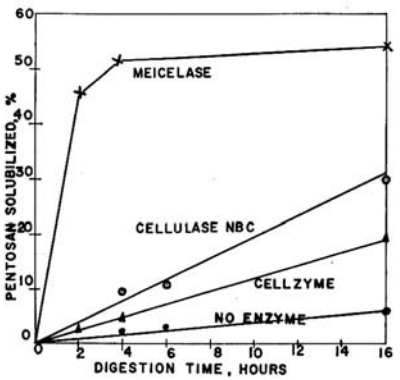


Fig. 5. Solubilization of pentosans of tailings by pentosanases.

Tailings were treated in these experiments with 10 units of pentosanase per 1 g. tailings at 40°C. It is evident again that Meicelase was most powerful in solubilizing pentosans. It solubilized 55% of total pentosans of tailings, Cellulase NBC 29%, and Cellzyme 15% after 16 hr. of incubation. In a qualitative respect the activities on tailings were comparable with those on the purified pentosan fraction. This suggests that the susceptibility of the pentosan complex in both substrates was similar and unaltered by the purification procedure. The tailings used in the solubility measurements were also used in farinography and baking studies.

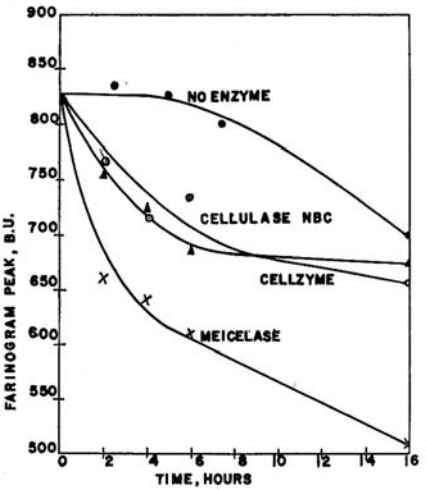
Effect of Enzyme Treatment on Properties of Tailings

The main measurable properties of tailings include high absorption and deleterious effects in bread-baking (4,5).

Effect on Absorption. Absorption is expected to be lowered by depolymerization of the pentosans by enzymolysis. This was established by farinography (Fig. 6).

The peaks of farinograms (85% flour and 15% tailings) were plotted against the time the tailings were treated with enzyme (Fig. 6). The higher the peak, the higher absorptive power of tailings is indicated. The control tailings were treated in the same way as experimental tailings, except that no enzyme was used.

The control tailings showed little change in absorption for the first 6 hr. of incubation. A slight decrease of absorption occurred after 16 hr. This change may be due not only to the action of residual pentosanase but also to that of amylases present in tailings. Of the three enzymes, Meicelase was most effective. After 16 hr. of digestion, the treated tailings had an absorption comparable to that of flour. The peak of the farinogram was reduced by this treatment from 825 to 510 B.U. The other two enzymes,



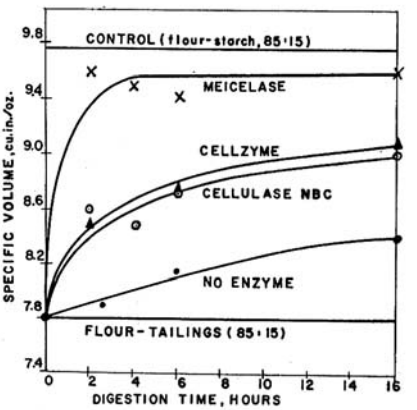


Fig. 6 (left). Effect of pentosanases on absorption of tailings.
Fig. 7 (right). Effect of treatment of tailings by pentosanases on loaf volume.

Cellulase NBC and Cellzyme, lowered the absorption much less. Cellulase reduced the peak to 655 B.U. and Cellzyme to 675 B.U. only.

The reduction of absorption did not correlate with pentosanase activity established by the assay in which the water-soluble araboxylan was used. On the other hand, it was in accordance with the solubilizing effects on the water-insoluble pentosan complex.

Effect on Baking Properties. The same mixtures of flour (85%) and tailings (15%) as used in farinography were used in baking tests. As a control, flour and prime starch mixture was used in the same proportions. Prior to these tests, an investigation was made to determine if the level of enzymes introduced into the doughs by addition of treated tailings had any effect (a) on the flour, (b) on the prime starch-flour mixture (15:85). In accordance with the level used in treatment of tailings, 1,400 pentosanase units were used per 1,000 g. flour in these preliminary experiments. The addition of enzymes at this level of pentosanase activity was without significant effect on bread volume and quality of either the flour or the flour-prime starch mixtures. Thus any change observed with the flour-tailings mixtures could be attributed to the modifying action of enzymes on the tailings. Results of these experiments are shown in Fig. 7.

The tailings reduced the bread specific volume from 9.7 to 7.8 cu. in./oz. The endogenous activity had a slight improving effect; it raised the volume to 8.4. All three enzymes significantly improved the baking properties of tailings which were positively correlated with their solubilizing power of pentosans and their ability to lower the absorption of tailings.

Cellzyme, which showed relatively low activity in solubilizing water-insoluble pentosans and reduced the absorption of tailings only slightly, was also slightly effective in reversing the ill effects of tailings. The baking properties of tailings were changed to a similar degree by Cellulase NBC.

Meicelase, which showed high activities both in solubilizing of water-insoluble pentosans and in restoring a normal absorption of flour-tailings mixture, removed almost completely the adverse effect present in tailings. The breads made with tailings which were treated for 16 hr. with Meicelase were comparable to the controls in volume and grain quality.

These results show clearly that the pentosan fraction is the quality-determining factor of tailings and that it can be modified with suitable enzymes. These findings are in accordance with previously published observations (4,5).

The reasons for the dissimilar activity of the enzyme preparations on the water-insoluble pentosan fraction are not clear. To gain insight into this situation, several possibilities were investigated.

The water-insoluble pentosans were reported to have more arabinose side-chains than the water-soluble araboxylan (1). The preparations studied herein showed a ratio of arabinose/xylose = 0.58/1.00 in the water-soluble araboxylan, and 0.92/1.00 in the water-insoluble pentosan fraction.

On the basis of these data, it was speculated that the enzymatic attack on the araboxylan required removal of some arabinose side-chains to

render the xylan backbone accessible to depolymerization. Thus the lower solubilization of the water-insoluble substrate by the cellulases from aspergilli than by Meicelase might have been due to the low level of the enzyme component (e.g. arabinase) which debranched the polysaccharide. Therefore the rates of release of arabinose by the three enzyme preparations during digestion of araboxylan were tested by paper chromatography. Enzymatic hydrolysates of araboxylan after 6 and 24 hr. of enzymolysis were investigated. In the 6-hr. digest by Cellulase NBC and Cellzyme, arabinose and oligosaccharides of pentoses were detected. Xylose was found in addition to all the other sugars in the 24-hr. digests. In the 6-hr. digests with Meicelase, xylose and oligosaccharides of pentoses were detected; arabinose was released later and it was present in the 24-hr. digest. The ratio of monosaccharides in 24-hr. digests was similar, regardless of the enzyme used. It was 1.5/1.0 (arabinose/xylose). From visual observation of the chromatograms it was apparent that the digests with Meicelase contained more oligosaccharides of pentoses than those with the other cellulases. These results indicate that there was sufficient activity in the preparations from aspergilli to remove the arabinose side-chains, and that this step cannot account for the differences in the effect of the enzyme preparations on insoluble pentosans. Chromatograms with water-insoluble pentosans were complicated by the presence of glucose and its oligosaccharides. The pattern of pentose sugars was similar to that from the water-soluble pentosans. In the 24-hr. digest mannose was detected, which confirmed the finding of Perlin and Suzuki (16) that a mannan is present in this fraction and also that all three enzymes contained mannanase. The ratio of monosaccharides in the 24-hr. digest was 0.24/1.1/1.0 (mannose/arabinose/xylose). Similar oligosaccharides of pentoses were observed in all three enzyme digests. The lower amount of arabinose in respect to xylose in the digests from water-insoluble pentosans than in those of the water-soluble araboxylan indicated lower enzymatic accessibility of the arabinose branches in the insoluble substrate than in the soluble one.

It is most likely that Meicelase contains a secondary enzyme system not present in the other cellulases. Thus far this factor has not been identified. The heat-stability properties of pentosanase were used to study the heat-stability of this factor. Meicelase was heated at 60° for 60 min. This lowered its solubilizing power on water-insoluble pentosans to 10% of the original. When a pentosanase from another source (Cellzyme or Cellulase NBC) was added to replace the deactivated pentosanase of Meicelase, the increase of solubilizing power was only that which could be expected from the activity of each component. There was no synergistic increase. This shows the heat-lability of the factor in Meicelase. It has been established that cellulase is a multicomponent enzyme system and that its components vary in activity on native cellulose according to the origin of the enzyme. The cellulases from aspergilli are generally lacking in the ability to degrade native cellulose. Cellulase from *Trichoderma viride,* on the other hand, contains a component which is active in this respect, as was shown by Li *et al.* (17). The presence of cellulose in the pentosan fraction was reported

by Perlin and Suzuki (16). Thus one may look for explanation in the close association of pentosans and cellulose in the insoluble fraction. If these two polysaccharides are chemically or even physically entangled, solubilization of this complex may require a synergistic attack of pentosanases and cellulases.

Acknowledgment

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