NONSTARCHY POLYSACCHARIDES AND PROTEINS OF SOFT WHEAT FLOUR TAILINGS[1]

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ABSTRACT

The composition of nonstarchy polysaccharides and proteins of subfractions of soft wheat tailings was determined. The subfractions were: (a) the supernatant solids (5.5% pentosans, 52% protein); (b) mucilaginous material (18% pentosans, 16% protein); and (c) a polysaccharide-protein complex (95% pentosans, 5% protein) purified by enzyme digestion of the mucilage. The polysaccharides of the starch-free supernatant solids contained 1.5% L-arabinose, 0.8% D-xylose, and 6.6% D-glucose; the glucose was believed to be derived from a nonstarchy polysaccharide. The mucilage contained 4.5% L-arabinose, 5.8% D-xylose, and 17.2% D-glucose; glucose was largely the result of starch degradation. The purified polysaccharide-protein complex was an arabinoxylan consisting of 27.4% L-arabinose and 43.5% D-xylose. Starch-gel electrophoresis and amino acid analyses showed similarities between the proteins of the tailings supernatant solids and gluten and between the proteins of the tailings mucilage and the water-solubles of the same flour. The resistance of the polysaccharide-protein complex to complete hydrolysis prevented total analyses of amino acids associated with the arabinoxylan. Undigested material, suggestive of cell-wall debris, appeared to remain structurally intact in the hydrolysate, attesting to the strength of bonding of the glycoproteins of the tailings of soft wheat flour.

The water-insoluble, nonstarchy polysaccharides of wheat flour, generally referred to as hemicelluloses (1, p. 304), are largely endosperm cell-wall material primarily found in the tailings fraction of flour (2,3,4). The crude tailings fraction is obtained as an upper layer of a centrifuged flour residue from which water-soluble constituents and gluten proteins have been removed. This removal can be accomplished by various procedures: e.g. a dough method (5), a batter method (6), or water-extraction of flour followed by dispersion of the residual dough in dilute acetic acid to remove the gluten proteins (7).

"Purified" tailings, obtained by wet-sieving or centrifugation of crude tailings, has been shown by Yamazaki (3) to consist of 30 to 65% pentosans and 0.5 to 3% nitrogen. Kulp and Bechtel (8) prepared tailings subfractions consisting of 54 to 60% pentosans and 5.3% protein; with further purification by pancreatin digestion and extraction with alkali they obtained a precipitate of 69% pentosans and 2.2% protein. The amino acid composition of a water-insoluble pentosan

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fraction from hard wheat flour was reported by Kulp and Bechtel (9) to be similar to that of water-soluble proteins of wheat. They also suggested that the protein associated with the pentosans is an integral component of water-insoluble glycoproteins.

"Purified" tailings or pentosan-rich fractions have been known to decrease cookie spread (3); increase flour absorption, lower the specific volume of bread, and increase the coarseness of grain (8); and improve the volume, grain, and texture of cakes (10). The deleterious or beneficial effects are generally ascribed to the insoluble pentosans or to a polysaccharide-protein complex, but the exact role or nature of these tailings components is not clear.

In an attempt to elucidate further the nature of the nonstarchy polysaccharides, proteins, or polysaccharide-protein complex of wheat tailings, two subfractions of a centrifuged water-suspension of crude tailings were investigated: (a) the starch-free solids of the supernatant and (b) the mucilaginous layer mechanically separated from a small-granule starch layer. The supernatant of tailings is generally discarded after centrifugation, and the composition of the solids present has not been previously reported.

Materials and Methods

Preparation of Samples. A commercial bleached cake flour (pH 5.1, 8.6% protein and 0.3% ash on 14% moisture basis) was the source of the tailings. The flour fractionation method was that of Sollars (7) for bleached flour; the tailings fraction was lyophilized and ground in a Wiley mill to pass a 40-mesh sieve.

Fractionation of the crude tailings and purification of the nonstarchy polysaccharide and protein components was carried out as illustrated in Fig. 1. A composite of crude tailings, 14.4% of the flour on a dry basis, was subfractionated by suspension in 5 parts water, shearing in a Waring Blendor for 5 min., and centrifuging for 10 min. at 15,000 r.p.m. in a Servall refrigerated centrifuge at 5°C. The three subfractions obtained were a clear supernatant containing suspended matter of very low density, a loosely packed gray-to-tan mucilaginous layer, and a lower layer of tightly packed small starch granules. The latter fraction was resuspended in water and centrifuged to remove any remaining mucilage which was added to the mucilaginous fraction. The supernatant and mucilage were lyophilized, the starchy fraction was air-dried, and the latter two were ground in a Wiley mill to pass a 40-mesh sieve. The hygroscopic solids of the supernatant were pulverized quickly with mortar and pestle before use.

The mucilage, being relatively high in both protein and pentosans,

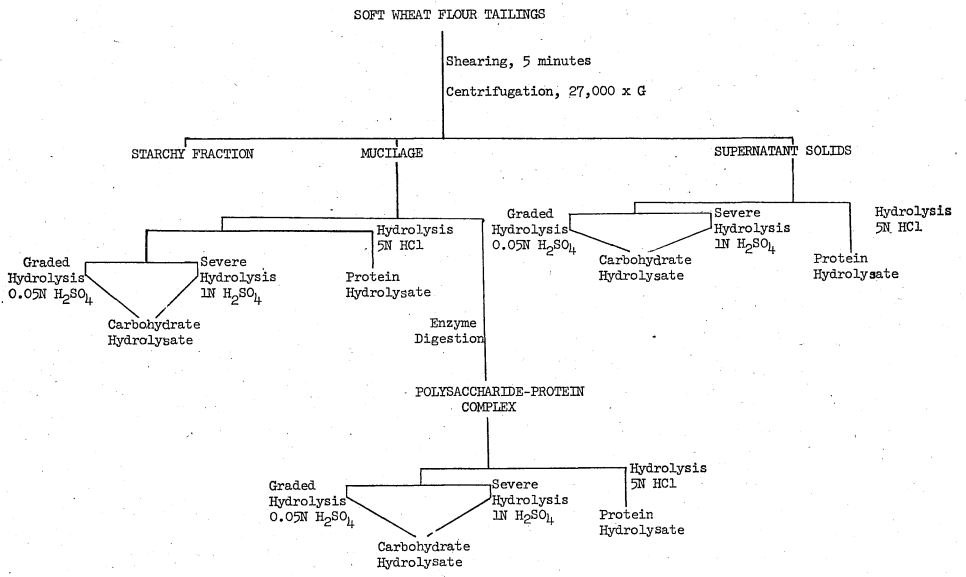

Fig. 1. Scheme for the fractionation of the crude tailings, purification of the nonstarchy polysaccharide and protein components, and preparation of hydrolysates.

was further purified by enzymatic digestion. Pancreatin[3] digestion was carried out for 5 days at 37°C. by the procedure of Simpson (11); the enzyme preparation was 1% of the mucilage by weight and the pH was maintained at 7.3 with 0.06*M* phosphate buffer. The undigested residue was lyophilized and some samples were subjected to further digestion by 1% protease of *Streptomyces griseus*.[4] Digestion periods were 1 and 3 days at 42°C. and the pH was maintained at 8.0 with 0.02*M* borate buffer which was 0.01*M* in calcium chloride (12). For bactericidal treatment, ethanol was added to a concentration of approximately 3% in the buffered dispersion. Digestion was followed by dialysis and the residues were lyophilized.

The enzymes were used as obtained and were not tested for purity.

Analytical Methods. Moisture, ash, and protein were determined by standard methods. The starch content of the crude tailings and three subfractions was measured by dispersion in calcium chloride (13) followed by spectrophotometric measurement of the color produced by iodine-potassium iodide (14).

Pentosans were calculated after determination of furfural by the procedure of Fraser *et al.* (15). Total carbohydrate of the supernatant fraction was determined colorimetrically by the phenol-sulfuric acid

[3] Fisher Scientific Co.
[4] California Corp. for Biochemical Research.

method of Dubois *et al.* (16). The total carbohydrate was calculated from a standard curve for glucose.

To identify the components of the polysaccharides of the supernatant solids and the mucilage, 50 to 500 mg. of sample (depending upon the pentosan concentration) was dispersed in 5 ml. of 0.05*N* sulfuric acid in test-tubes fitted with Teflon-lined screw caps. Tubes were immersed in a boiling water bath and subjected to graded hydrolysis for 3, 6, 12, and 24 hr. Under similar conditions and with 1*N* sulfuric acid, samples were subjected to severe hydrolysis for 24 hr. Each hydrolyzed dispersion was centrifuged and the supernatant decanted and tested for neutrality; Congo red was used as indicator. Barium carbonate was added when necessary to neutralize the supernatant, and further centrifugation removed precipitated barium sulfate. The supernatant was decanted and used for analysis. Descending chromatography was carried out for 18 hr. with Whatman No. 1 paper and a solvent of ethyl acetate, pyridine, and water in a volume ratio of 8:2:1. Aniline hydrogen phthalate was used for color development, and quantitative determination of the sugars was made by the method of Wilson (17).

The proteins of the supernatant solids and the mucilage were studied by starch-gel electrophoresis; buffer was aluminum lactate, containing 3*M* urea; the method was that of Woychik, Boundy, and Dimler (18). Electrophoresis patterns of the gluten and water-soluble proteins of the same flour were also obtained for comparison.

Analyses were made of the amino acids of the hydrolyzed proteins of the supernatant and mucilaginous fractions, of the mucilage that had been digested by pancreatin followed by digestion by *S. griseus* protease for 1 and 3 days, and, for comparison, the gluten and water-soluble fractions of the same flour. Samples were hydrolyzed in 5*N* hydrochloric acid in sealed vials in an autoclave at 119°C. at 18 p.s.i. for 24 hr. The dispersions were cooled to room temperature, filtered through Whatman No. 2 paper, evaporated almost to dryness on a rotary evaporator under reduced pressure at 40°C., and brought to a known volume. The amino acids were determined quantitatively[5] by the colorimetric ninhydrin method of Moore and Stein (19). No enzyme blank was run.

Results and Discussion

The relative percentage distribution of the tailings subfractions and the pentosan, protein, and starch content are given in Table I.

[5] Technicon Auto Analyzer.

TABLE I

PENTOSAN, PROTEIN, AND STARCH CONTENT OF THE SUBFRACTIONS OF CRUDE TAILINGS OF A SOFT WHEAT FLOUR

FLOUR FRACTION	FRACTION YIELDS	PENTOSANS	PROTEIN (N × 5.7)	STARCH
	%[a]	%[b]	%[b]	%[b]
Crude tailings		7.4	6.4	87.5
Tailings subfractions				
Supernatant	3.7	5.5	52.1	Nil
Mucilage	13.9	17.8	15.9	72.5
Starchy	82.5	6.2	2.6	90.6

[a] Adjusted to 100%; actual recovery was 95.2%.
[b] Dry weight basis; average of two samples.

The ash content of the crude tailings was 0.14%, of the mucilage 0.16%, and of the supernatant solids 0.56%.

The supernatant was initially decanted from a centrifuged tailings suspension and discarded until the clear liquid, when view microscopically, was found to contain considerable suspended matter. When lyophilized, the low-density solids of the supernatant represented 3.7% of the tailings. The solids contained 52% protein and 5.5% pentosans; the remaining 42.5% of the supernatant solids was thought to be largely cellulose owing to the fibrous characteristics observed macro- and microscopically. Qualitative tests for cellulose, however, were negative; these included solubility in hydrochloric acid-zinc chloride and color reaction in iodine-zinc chloride. Total carbohydrate was determined quantitatively as 40.3% including pentosans. Except for the pentosans, the exact nature of the carbohydrate was not confirmed; but since the supernatant solids were found to be starch-free, it may be assumed to be nonstarchy polysaccharide material not readily hydrolyzed.

The mucilaginous fraction represented 14% of the crude tailings and the concentration of both pentosan and protein was approximately two and one-half times greater than in the crude material. The pentosan content of the mucilage was approximately three times greater than that of the two other subfractions, but it was considerably less than that of the "purified" tailings prepared by Yamazaki (3) and Kulp and Bechtel (8), by other methods. The protein content of the mucilage was markedly less than that of the supernatant solids; it was three times greater than the pentosan-rich fraction investigated by Kulp and Bechtel (8).

The starchy fraction was the major part of the crude tailings (82.5%). The 6.2% pentosans and 2.6% proteins in this fraction may have been closely associated with the starch, or simply mechanically entrapped in the tightly packed starch granules during centrifugation;

this fraction, however, had been rewashed to remove residual mucilage. The starchy fraction was not used for further investigation of the polysaccharides and proteins.

After enzymatic hydrolysis of starch and protein in the mucilage, the residues were lyophilized, weighed, and analyzed for total nitrogen and pentosans. These data are presented in Table II. Pancreatin was used primarily to remove starch, but it also markedly decreased the protein and increased the amount of measurable pentosan. Digestion by *S. griseus* protease, after pancreatin treatment, further increased the measurable pentosans and only slightly decreased the total protein. Digestion for 1 day by the protease was almost as effective in protein hydrolysis as for a 3-day period. Increasing the digestion time to 7 days did not effect further protein hydrolysis. The residue remaining after pancreatin-protease digestion appeared to be a purified polysaccharide-protein complex, as evidenced by its composition of 95.4% pentosans and 4.9% protein and by its resistance to further enzyme digestion. This purified complex accounted for approximately 22% of the original mucilage or 3% of the crude tailings. Considering the various treatments the mucilaginous fraction had received, it is unlikely that the residue would represent structurally entangled molecules of polysaccharides and proteins; more likely the residue is a covalently bonded polysaccharide-protein complex.

Polysaccharides. The constituent sugars of the tailings subfractions and of the more purified polysaccharide-protein complex were separated by paper chromatography after both graded and severe hydrolysis (see Fig. 1). Every sample contained some insoluble precipitate after hydrolysis; this appeared greatest in the supernatant fraction. The sugars of the hydrolyzable portions of the fractions were L-arabinose, D-xylose, and D-glucose; galactose and nitrogen-containing sugars were not detected.

TABLE II

PENTOSAN AND PROTEIN CONTENT OF THE MUCILAGE SUBFRACTION OF SOFT WHEAT FLOUR TAILINGS AFTER ENZYME DIGESTION[a]

ENZYME TREATMENT	TIME OF DIGESTION	AMOUNT OF ORIGINAL MUCILAGE REMAINING	PENTOSAN	PROTEIN
	days	%	%	%
None	0	100.0	16.9	15.2
Pancreatin, 1%	5	23.8	80.2	5.3
Pancreatin, 1%	5			
+ protease, 1%	1	21.8	95.4	4.9
Pancreatin, 1%	5			
+ protease, 1%	3	21.4	95.0	4.4

[a] Average of two samples; percentages based on weight of samples after lyophilization.

After 24 hr. of hydrolysis in 0.05*N* sulfuric acid, the supernatant solids contained 1.5% arabinose, 0.8% xylose, and 6.6% glucose. The glucose was believed to be largely derived from unidentified polysaccharide material resistant to hydrolysis. Following the same conditions of hydrolysis, the mucilage contained 4.5% arabinose, 5.8% xylose, and 17.2% glucose. The relatively large amount of glucose in the hydrolysate of the mucilage was probably the result of starch degradation; the mucilage contained 72.5% starch. The purified polysaccharide-protein complex, derived from enzyme digestion of the mucilage, was an arabinoxylan containing 27.4% arabinose, 43.5% xylose, and no detectable glucose or amino sugars.

Within each series of samples subjected to graded hydrolysis, the amount of L-arabinose released from the polymer appeared to be independent of the time of hydrolysis, whereas the amount of D-xylose increased with time. More severe hydrolysis using 1*N* sulfuric acid for 24 hr. generally resulted in a diminution in quantity of sugars. Since degradation of the sugars might well occur under these conditions, the data were discounted. As in Perlin's work dealing with soluble pentosans (20), different ratios of arabinose to xylose were found among the different tailings fractions of the same flour; e.g., 24-hr. hydrolysis in 0.05*N* sulfuric acid resulted in arabinose-to-xylose ratio of 2:1 and 1:1.3 respectively for the supernatant and mucilaginous fractions.

That arabinose was released more readily from the polysaccharides than xylose suggests that arabinose might be (a) more exposed in the polymer structure than xylose, (b) bound to the polysaccharide polymer by a less stable bond than xylose, and/or (c) present in a more labile structural form than xylose. These conclusions are generally in keeping with the proposed structure of the water-soluble polysaccharides of wheat endosperm in which the main chain was envisioned as xylopyranose units to which single arabinofuranose units were appended as side chains (20,21,22). Montgomery and Smith (2) found the same general type of structure to be present in the insoluble polysaccharides of the "squeegee" or tailings fraction of flour; in addition, these workers found the insoluble polysaccharides to possess a more branched structure than the soluble polysaccharides.

Proteins. The proteins associated with the supernatant and mucilaginous subfractions of the tailings varied in number and rate of migration when separated by starch-gel electrophoresis; the patterns were reproducible in three replications. The supernatant solids (52% protein) gave four bands in the stained gel, whereas the mucilage (16% protein) gave seven bands as shown in Fig. 2. The electrophoresis

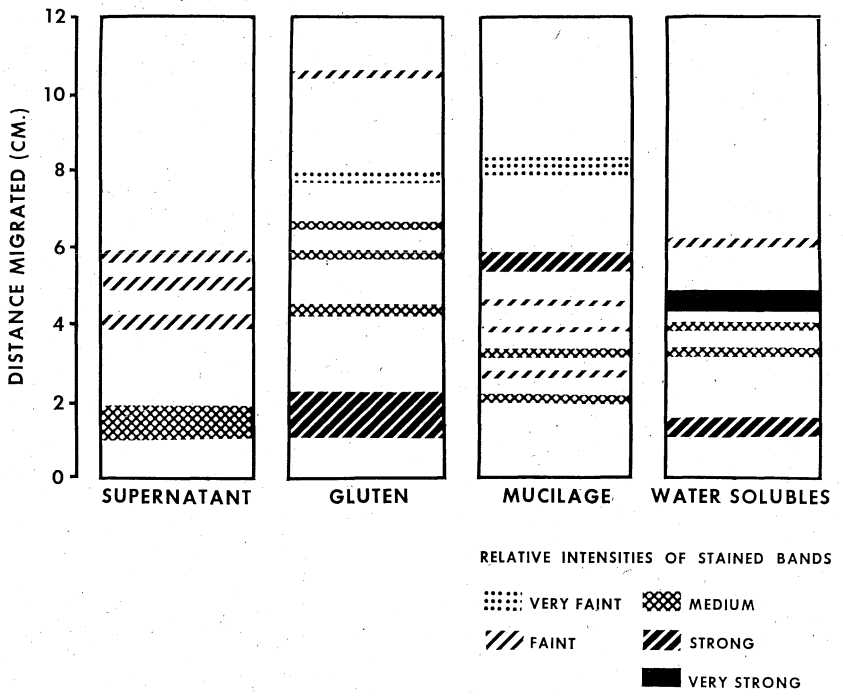

Fig. 2. Electrophoresis patterns of the proteins of the supernatant and mucilaginous subfractions of tailings and the gluten and water-solubles of soft wheat flour.

patterns of the proteins of the gluten and water-solubles from the same flour as the tailings are also shown in Fig. 2.

The electrophoresis patterns of the proteins of the tailings supernatant and the gluten were similar in some respects; both showed a broad band of medium to strong intensity which remained within 2 cm. of the origin; both had three more-rapidly moving components giving narrow bands of less intensity. The gluten (83% protein) showed two additional faint bands which had migrated more rapidly to approximately 8 to 10 cm. from the origin.

The proteins of the tailings mucilage were more similar in electrophoresis patterns to the water-soluble proteins of the flour than to gluten. Both the mucilage and the water-solubles gave a strong or very strong band about 5 to 6 cm. from the origin. Unlike the mucilage, however, the water-solubles (23% protein) gave a fairly broad band in the stained gel within 2 cm. of the origin.

The polysaccharide-protein complex, obtained by pancreatin-protease digestion of the mucilage, showed doubtful, if any, migration through the starch gel. There was possibly one very faint and narrow

band about 4 cm. from the origin when a 12.5% dispersion of the complex in the buffer containing urea was applied to the starch gel. Lack of detection of proteins of the complex may be accounted for in one or more ways. The concentration of protein, only 5% of the complex, may have been too dilute for positive detection of stained bands; however, higher concentrations of the low-density and cottonlike material could not be dispersed in the buffer. The predominating polysaccharide portion of the complex, coupled with strong bonding of the protein to the polysaccharide, may have minimized solubilization of the protein in the buffer and subsequent migration through the gel matrix. The evidence given by Kuendig *et al.* (23) that the behavior of wheat polysaccharide-protein complexes is dominated by the polysaccharide portion and that such complexes do not give typical protein reactions would support the latter reasoning.

The amino acid compositions of the hydrolysates (see Fig. 1) of the supernatant solids and mucilage of the tailings and of the gluten and water-solubles of the flour are given in Table III. Approximately 20%

TABLE III

AMINO ACID COMPOSITION OF THE PROTEINS OF TWO TAILINGS SUBFRACTIONS, GLUTEN, AND WATER-SOLUBLES OF SOFT WHEAT FLOUR

AMINO ACID	GRAMS/16 GRAMS TOTAL NITROGEN IN THE HYDROLYSATE[a]			
	Tailings Supernatant	Gluten	Tailings Mucilage	Flour Water-Solubles
Alanine	2.9	2.1	5.2	4.7
Arginine	3.5	3.2	6.0	5.1
Aspartic acid	3.3	2.8	7.6	7.2
Cystine	0.5	0.0	0.2	1.4
Glutamic acid	30.2	32.6	19.9	21.4
Glycine	4.4	2.7	5.2	4.0
Histidine	2.2	3.2	6.0	5.1
Isoleucine	4.2	4.0	4.8	3.8
Leucine	7.8	7.1	4.8	7.0
Lysine	2.0	1.5	6.0	5.1
Methionine	0.2	0.0	0.6	0.7
Phenylalanine	5.1	5.5	5.0	4.1
Proline	13.6	14.2	8.1	10.3
Serine	4.3	3.5	4.4	3.6
Threonine	2.6	1.9	3.5	3.0
Tyrosine	3.3	2.0	3.5	2.4
Valine	4.6	3.9	6.1	5.4
Ammonia	5.1	4.7	3.2	3.2

[a] Average of two samples.

of the total nitrogen recovered from the mucilage and water-solubles and 30% from the supernatant solids and gluten was ammonia nitrogen. This might be accounted for by partial destruction of amino acids upon acid hydrolysis of the proteins and/or by the presence of a relatively large proportion of the dicarboxylic acids as amides. A general

similarity of amino acid composition can be seen between the tailings supernatant solids and gluten proteins and between the mucilage and water-soluble proteins. The proteins of supernatant solids and gluten contained more glutamic acid and proline than did the mucilage and water-soluble proteins. Aspartic acid is greater in the mucilage and water-solubles than in the supernatant solids and gluten.

The presence of glutenlike proteins in the solids of the supernatant of tailings might be explained on the basis that these proteins were initially physically entrapped or chemically complexed with large carbohydrate molecules in the endosperm cell wall, hence were not soluble in dilute acetic acid used to extract gluten from the flour. Mecham *et al.* (24) demonstrated that protein initially insoluble in dilute acetic acid was converted to an extractable form by dough mixing. In the hydration and manipulation of dough, such protein or polysaccharide-protein complex might physically adhere to the gluten complex. Thus the protein of the supernatant solids is probably the acetic acid-insoluble portion of the total gluten complex.

The mucilage is very similar in amino acid composition to the pentosan-rich tailings subfraction reported by Kulp and Bechtel (9). These investigators also reported that the proteins closely associated with the polysaccharides were similar in amino acid composition to the water-soluble proteins. Some of the simple proteins, albumins and globulins, are likely complexed with large carbohydrate polymers; this would render them insoluble and prevent their extraction from flour with water.

To determine the composition of the proteins closely associated with the insoluble polysaccharides, amino acid analyses were made on hydrolysates of the polysaccharide-protein complex derived by enzymatic digestion of the mucilage. It was believed that the protein left after digestion by pancreatin and *S. griseus* protease would be the protein most closely associated with the polysaccharides. When this material was subjected to standard conditions for hydrolysis for amino acid analysis, a charred debris formed and was removed by filtration. When this was viewed microscopically, some transparent particles were suggestive of cell-wall material.

The protein content of the lyophilized mucilage after digestion by pancreatin and *S. griseus* protease for 1 day was 4.9%. Only 64.5% of this protein was subsequently hydrolyzed by acid and thus was available for amino acid analysis. Similarly, the protein content of the mucilage after digestion by pancreatin and the protease for 3 days was 4.4%, and only 34.6% of this protein was in the acid hydrolysate for amino acid analysis. In comparison, 80.5% of the protein of the

nonenzyme-treated mucilage was present in the acid hydrolysate. Although the total amount of protein in each acid hydrolysate decreased, approximately the same weight of undigested protein remained resistant to acid hydrolysis regardless of enzyme treatment used. These results indicate that about 20% of the total protein of the mucilage and most of the protein remaining after protease digestion for 3 days could not be split from the polysaccharide under the conditions used and remained as an integral part of the charred cell-wall debris.

The efficient protein hydrolysis of the enzymes can be seen from the data in Table IV by comparing the concentration of the amino acids of the original and digested fractions. Based on the total γ of amino acids recovered in the original mucilage as 100%, the total γ of amino acids of the residue recovered after 1 day of protease digestion was 14.5% and after 3 days, 8.3%. In a digestion period of 7 days by *S. griseus*, no more protein was hydrolyzed than in 3 days. Since the original mucilage was very high in starch, which was almost completely hydrolyzed by pancreatin, the large reduction in hydrolyzable protein after enzyme treatment may indicate that the enzymes preferentially digested protein formerly associated with starch rather than with the nonstarchy polysaccharide which remained. Upon hydrolysis of starch, any protein previously associated with this polymer would be more available for enzyme attack.

TABLE IV

AMINO ACID COMPOSITION OF THE PROTEINS OF THE ORIGINAL AND ENZYME-DIGESTED MUCILAGE OF SOFT WHEAT FLOUR TAILINGS[a]

AMINO ACID	ORIGINAL MUCILAGE	PANCREATIN-DIGESTED FOR 5 DAYS FOLLOWED BY PROTEASE DIGESTION FOR:	
		1 Day	3 Days
	γ	γ	γ
Alanine	7.1	1.6	0.9
Arginine	8.1	1.0	0.5
Aspartic acid	10.4	1.0	1.1
Cystine	0.3	0.0	0.0
Glutamic acid	27.2	2.0	1.1
Glycine	7.1	1.8	0.9
Histidine	4.0	0.6	0.3
Isoleucine	6.5	1.4	0.9
Leucine	11.3	1.6	0.9
Lysine	8.1	0.9	0.5
Methionine	0.9	0.1	0.1
Phenylalanine	6.9	1.1	0.6
Proline	11.0	1.3	0.5
Serine	6.0	1.1	0.6
Threonine	4.8	1.1	0.6
Tyrosine	4.7	0.9	0.5
Valine	8.3	1.4	0.8
Ammonia	4.4	1.0	0.6

[a] γ per mg. of lyophilized samples; average of two samples.

Although the amounts of all the amino acids were significantly reduced in the pancreatin-protease-digested material compared to the original mucilage, the reduction in the amounts of aspartic acid, glutamic acid, leucine, and proline was most striking. This would seem to suggest that the large majority of these amino acid residues are not involved in close proximity to the site of polysaccharide-protein bonding. Because approximately 65% of the protein of the enzyme-digested material was not hydrolyzed by the acid, the amino acids found cannot be identified as those in close association with the polysaccharides. The especially broad specificity of *S. griseus* protease suggested its use in this work. Kuendig (21), however, found this enzyme ineffective in completely removing residual amino acids from the carbohydrate portion of the glycoprotein of soluble polysaccharides because of the inability of the proteolytic enzyme to hydrolyze peptide bonds in close proximity to the carbohydrate. Kuendig (21) presented evidence that polypeptide chains are associated with polysaccharides in wheat flour and tentatively suggested that an ester linkage may exist between the carboxyl group of alanine of a peptide chain and a hydroxyl group of the arabinoxylan.

The results of the study attest to the structural integrity and resistance to hydrolysis of the proteins complexed with polysaccharides and structurally involved with cell-wall material. As a result of incomplete hydrolysis, both by enzymes and acid, the amino acid residues still associated with the polysaccharides could not be determined. The amino acids recovered after acid hydrolysis of the enzyme-digested samples, however, were probably part of glycoproteins present in a purified polysaccharide-protein complex of the mucilage. Kuendig *et al.* (23) also reported finding all the amino acids normally present in protein in the form of glycoproteins associated with the soluble polysaccharides of hard wheat flour.

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