THE EFFECT OF TAILINGS OF WHEAT FLOUR AND ITS SUBFRACTIONS ON THE QUALITY OF BREAD[1]

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ABSTRACT

When 5, 10, and 15% of flour was replaced with flour tailings isolated from untreated patent flour, a progressive decrease in loaf volume and quality of grain was observed. Prime starch used in the controls did not show a similar adverse effect. Components of the tailings fraction were studied with the objective of localizing the factor responsible for its adverse baking performance. Damaged starch, small-granule starch, lipids, enzymes, and pentosans were evaluated. Analytical data and results of baking tests with model systems show that the pentosan-rich fraction accounted for the poor baking characteristics of tailings. The protein (5.6–7.0% of the pentosan) associated with this fraction has an amino acid composition similar to that of water-soluble wheat proteins. It appears not to affect the functional properties of pentosans.

Tailings is a fraction which is obtained as an upper layer in recovering starch from the wash-water during preparation of gluten from wheat flour. Sandstedt *et al.* (16) called this fraction "amylodextrin," and Sandstedt reported (15) that it was composed of 20–25% small starch granules, undamaged fragments of injured granules suspended in a gelatinous mixture of residual dextrins (60–70%), small amounts of insoluble pentosans, proteins, and inorganic salts. This component increased flour absorption and the absorption tolerance, but lowered

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bread volume. MacMasters and Hilbert (10) analyzed similarly prepared tailings and reported that they contained 87–94% starch (undamaged small granules and damaged large granules), 1–2% protein, 4% pentosans, 0.7% fatty material, 0.3% ash, and approximately 3% cellulosic material. It was later suggested that pentosanase activity during preparation was responsible for the low pentosan value (14). Others have reported 14% (2), 7.4% (3), and 8% (13) pentosans in tailings. In this laboratory a pentosan fraction (55–60% pentosans) was separated from tailings and tested for baking performance. It increased the flour absorption but reduced the loaf volume. No measurable effects on gas production or retention, mixing characteristics, or extensigraph properties of doughs were observed (8). Its effect on the breadmaking process was strikingly similar to that of tailings as reported by Sandstedt *et al.* (16).

A systematic study of the tailings was therefore undertaken to determine if the pentosan fraction was the sole factor responsible for the adverse properties of tailings or if other factors were involved.

Materials and Methods

Flour. Flour fractions were prepared from a commercial hard spring wheat flour, unbleached and untreated (protein 13.3%, ash 0.48%, pentosan 2.7%, farinograph absorption 63%, amylogram over 1,000 B.U. at 14% moisture). For evaluation of the baking properties of the fractions they were added to a commercial hard winter wheat bakers' patent flour which was bleached, bromated, and malted (protein 12.5%, ash 0.40% at 14% moisture).

Flour Fractions. Prime starch, tailings, and pentosans were prepared from flour by methods described previously (8). Tailings contained 2.3% proteins, 2.2% lipids, and 8.6% pentosans, all on the dry basis. The purified pentosans contained 58% pentosans, dry basis.

Ball-Milled Starch. Prime starch was ball-milled 72 hr. in a laboratory mill. Preliminary experiments showed that after this period most of the granules stained with Congo red (5).

Deactivation of Enzymes of Tailings. Two methods were used. The first was essentially that of Lee and Geddes (9). One hundred grams of tailings were dispersed in 250 ml. of 95% ethanol with stirring and the solvent removed by evaporation on a steam bath. The dry product was hammer-milled and rehydrated prior to baking. Sullivan *et al.* (19) reported that this procedure produces only partial inactivation of amylases and probably of other enzymes. They obtained complete inactivation with a solution of trichloroacetic acid in 90%

n-butanol (30 g. of the acid made up to 1,000 ml. with n-butanol). Forty grams of tailings were treated with 150 ml. of this agent for 30 min. at room temperature. The dispersion was filtered and washed with n-butanol (two 50-ml. portions) followed by equal amounts of ethyl ether. The solvents were evaporated on a water bath. To determine if these treatments altered the starch component, prime starch was subjected to similar deactivation and used as a control.

Preparation of Defatted Tailings. Tailings and prime starch, 50 g., were refluxed with 500 ml. 85% methanol following the procedure of Schoch (18). The solvent was decanted and fresh methanol added for another extraction. This was repeated three additional times. These five extractions reduced the lipid content of tailings from 2.2 to 0.4%, dry basis. The lipids were recovered from the methanol by evaporation of the solvent under vacuum, stored under refrigeration, and used within 1 week.

Preparation of Small-Granule Starch. A thin slurry of tailings was passed through a 400-mesh sieve mounted on a laboratory shaker as described before (8). The majority of pentosans remained on the sieve. The slurry which passed through the sieve was allowed to settle in a refrigerator overnight and the top layer decanted from the sediment. Microscopic examination showed that the sediment was contaminated with pentosans and other cell-wall materials, but the water layer contained relatively pure starch. Starch was separated from the water layer by centrifugation and further purified by two additional resuspensions and sedimentations under the same conditions as the first. After the final purification step the recovered starch was freeze-dried. This preparation is called "purified small-granule starch." The sediments from the purification steps were also collected and freeze-dried and will be referred to as "crude small-granule starch." The crude preparation contained 5.6% pentosans, 2.12% protein, and 1.34% lipids. The pentosan content of the purified preparation was 1.4% and the protein 0.8%.

Digestion of the Pentosan Fraction with Enzymes. Ten grams of the pentosan fraction were rehydrated overnight in the refrigerator, and 50 mg. of bromelin (Mann) or trypsin (2 × crystallized Sigma) added. The pH for bromelin digestion was adjusted to 6.0 and for tryptic digestion to 7.2 with phosphate buffers. After incubation at 30°C. for 24 hr. under toluene the digest was centrifuged; the pentosan fraction was washed with distilled water to remove the soluble materials and enzymes, and freeze-dried. The preparation was used for baking tests after rehydration.

Analytical Methods. Moisture, protein, ash, and lipids by acid hydrolysis were determined by AACC methods (1). Pentosans were estimated by the phloroglucide method after redistillation in order to eliminate interference of hydroxymethylfurfural (10). Damaged starch was determined by the Hampel (4), Sullivan *et al.* (19), and Sandstedt-Mattern (17) methods with some modifications. In accordance with the Hampel procedure a sample was extracted for 10 min. at 40°C. with ammonium sulfate-formamide solution containing sulfosalicylic acid. After filtration an aliquot was transferred to a test tube, hydrogen peroxide added, and the solution heated in boiling water. After cooling, starch was determined colorimetrically with iodine. The heating time with hydrogen peroxide, 5 min. according to the original method, was found excessive as no color, or a fading color, was obtained. Reduction of the heating period to 2 min. was found essential. Results are given in Hampel numbers. The Sullivan *et al.* method was found satisfactory for determination of flour and tailings without modification. It was not necessary to deactivate prime starch, as negligible amylolytic activity was observed. For the ball-milled starches the sample weight was reduced to 0.5 or 0.25 g. while the enzyme-to-sample-weight ratio was kept constant. The Sandstedt-Mattern method was followed for prime starch, flour, and tailings. For ball-milled flour, sample weight was reduced to 0.1 g. and the enzyme activity of the medium kept at 160 SKB units. This sample weight was optimum for this enzyme level. Higher sample weights gave lower results.

The amino acids of the pentosan-fraction protein were determined after acid hydrolysis (4*N* hydrochloric acid in autoclave for 24 hr.) by the Moore, Spackman, and Stein ion exchange method (11). The eluted amino acids were estimated by the colorimetric ninhydrin method (12).

Determination of the Size of Starch Granules. The sample was dispersed in water (50 mg./100 ml.) and a specimen was transferred into a melted gelatin jelly; as soon as this solidified, the size of starch granules was measured microscopically with a stage micrometer at a magnification of 700. At least 600 granules were measured from each sample.

Baking Experiments. The effects of fractions and treatments on doughs and bread were studied, by the sponge and dough method. The formula based on total flour was 2.5% yeast, 0.5% yeast food, 2% salt, 6% sucrose, 4% nonfat dry milk, and 2% lard. Loaf volume was determined by rapeseed displacement (1), and bread was scored by an expert baker. Baking results are based on duplicate two-loaf batches, baked on different days.

Results and Discussion

Effect of Starch Damage. To establish whether damaged starch causes the adverse effects of tailings in baking, mixtures were prepared in which 5, 10, and 15% of the flour was replaced by equal amounts of prime starch, tailings, and ball-milled starch. These were analyzed for damaged starch and were used in place of part of the flour in baking bread. Analytical data by the three methods for damaged starch (4,17,19) and results of the baking tests are given in Table I. There was no quantitative relationship between the analytical values by the three methods which would permit an interconversion, but the general trend of the values was consistent. Starch damage of all the flour-prime starch and flour-tailings mixtures was in the range of that in commercial hard wheat bakers' patent flours (17,19). The values did not differ greatly from those of the flour itself. Starch damage of the flour-ball-milled starch mixtures was 136 to 210% of that of the flour by the Sandstedt-Mattern method, or more by the other methods.

TABLE I

EFFECT OF STARCH DAMAGE AND TAILINGS ON QUALITY OF WHITE BREAD

Composition		Bread Quality		Starch Damage		
		Specific Volume	Grain	Maltose [a]	Damaged Starch Index [b]	Amylose [c]
		cu. in./oz.		%		*mg./100 g.*
Flour		10.2	very good	6.75	134	198
Prime starch	Flour			4.16	23.3	19
5%	95%	10.3	good+	6.62	128.5	189.1
10%	90%	10.4	good+	6.50	122.9	180.1
15%	85%	10.1	good+	6.37	117.4	171.5
Tailings				7.90	182	270
5%	95%	9.8	very good	6.81	136	202
10%	90%	9.5	fair	6.87	139	205
15%	85%	9.0	fair	6.93	141	210
Ball-milled prime starch				56.10	1,400	2,950
5%	95%	9.6	good	9.22	197	336
10%	90%	9.3	fair	11.69	261	473
15%	85%	8.5	poor+	14.16	324	612

[a] Sandstedt-Mattern method.
[b] Sullivan *et al.* method.
[c] Hampel method.

Replacement of up to 15% of the flour with prime starch had no appreciable effect on dough absorption, whereas each increment of damaged starch or tailings increased absorption by approximately 2.2%. Doughs with prime starch were normal, while those with tailings or damaged starch were somewhat sticky at the mixer but recovered almost completely during floor time. There was no evidence of loss of absorptive capacity due to amylolytic degradation, even at the highest level of ball-milled starch. Doughs with tailings or ball-milled starch were deficient in oven spring, as were those with pentosan fraction (8), which resulted in reduced loaf volume.

Prime starch addition to flour had no appreciable effect on loaf volume, and reduced the score for grain very slightly. With increasing amounts, the texture became slightly harsh. With increasing amounts of tailings or ball-milled starch there was progressive decrease in volume and deterioration in grain. However, the decrease in volume and deterioration in grain of bread with tailings occurred with only a slight increase in damaged starch. The volume and grain of bread with 10% tailings were comparable to those of bread with 10% ball-milled starch, yet the latter had approximately twice the level of damaged starch. Bread with 5% ball-milled starch had good grain, yet the level of damaged starch was approximately 50% greater than that of bread with 10% tailings, which had only fair grain. Thus the amount of damaged starch contributed by tailings was insufficient to account for the adverse effects of this fraction.

Effect of Enzyme Deactivation. The tailings were isolated under conditions which were expected to preserve any enzyme activity that might be present. Although there is no indication in the literature of involvement of enzymes in the baking properties of tailings, the scarcity of knowledge in this area required evaluation of this possibility. Tailings deactivated with ethanol (9) and with a solution of trichloroacetic acid in n-butanol (19) were evaluated by baking tests using mixtures of 85% flour and 15% deactivated tailings. As a control, bread was made from the same proportion of flour and untreated tailings. Prime wheat starch is essentially free from enzymes. To test the effect of the deactivating processes on the baking properties of starch, bread was also made with the same proportions of flour and prime starch and flour with deactivated prime starch. Table II(B) shows that the deactivation process had a relatively small effect on the bread made with prime starch. Loaf volume was lowered slightly and the grain was somewhat coarser, but still good, while the texture was slightly harsher. If the enzymes of tailings were responsible for deleterious effects of this fraction in baking, one might expect bread

TABLE II
EFFECT OF SUBFRACTIONS OF TAILINGS ON BREAD QUALITY

Composition	Bread Quality: Specific Volume	Grain	Texture
	cu. in./oz.		
	A. Controls		
15% Prime starch, 85% flour	10.2	very good	very good
15% Tailings, 85% flour	8.4	poor	fair
	B. Tailings with deactivated enzymes		
85% Flour, 15% prime starch[a]	10.0	good	very good
85% Flour, 15% prime starch[b]	9.9	good	good
85% Flour, 15% tailings[a]	8.1	very poor	poor
85% Flour, 15% tailings[b]	8.0	poor	poor
	C. Defatted tailings		
85% Flour, 15% defatted prime starch	9.6	good	very good
85% Flour, 15% defatted tailings	7.6	poor	poor
85% Flour, 15% prime starch, lipids from tailings	10.2	very good	excellent

[a] Deactivated with ethanol.
[b] Deactivated with butanol-trichloroacetic acid.

made from deactivated tailings to show some improvement over that made from the original tailings. Table II(B) shows that the deactivation process had the same effect on bread made with tailings as on that made of the prime starch. Loaf volumes were lowered to the same degree, and grain and texture were somewhat poorer. Thus there is no evidence that enzymes possibly present in tailings are responsible for the poor baking properties of this fraction.

Effect of Lipids of Tailings. The effect of lipids from tailings on baking properties was determined by defatting the tailings fraction and adding the recovered lipids to a dough made of 85% flour and 15% prime starch. Bread was also made from the defatted tailings and defatted prime starch. Table II(C) shows that the defatted fractions gave bread of lower volume and reduced the score for grain. These effects might be the result of either the process of defatting or of removal of lipids. Therefore, the lipids removed from the amount of tailings used in the flour-tailings mixture were added to a dough of 85% flour and 15% prime starch. As shown in Table II(C) the volume and grain of the resulting bread were equal to those of the control, while the texture was improved. Thus the lipid component of tailings is not associated with its adverse properties.

Effect of Small-Granule Starch of Tailings. Table III(A) shows that the purified small-granule starch consisted almost entirely of

TABLE III

EFFECT OF SMALL-GRANULE STARCH RECOVERED FROM TAILINGS ON THE QUALITY OF BREAD

A. DISTRIBUTION OF STARCH GRANULES IN THE PURIFIED SMALL-GRANULE FRACTION AND IN PRIME STARCH

SAMPLE	SIZE OF STARCH GRANULES			
	5μ and Below	6 to 10μ	11 to 15μ	Over 15μ
	%	%	%	%
Prime starch	38.87	17.20	13.33	30.60
Purified small-granule starch	98.50	1.50	0.00	0.00

B. BAKING QUALITY

COMPOSITION	SPECIFIC VOLUME	GRAIN	TEXTURE	PENTOSANS IN STARCH-FLOUR MIXTURE
	cu. in./oz.			
15% Prime starch, 85% flour	10.1	very good	fair+	2.30
15% Crude small-granule starch, 85% flour	8.5	poor	poor	3.23
15% Purified small-granule starch, 85% flour	9.8	excellent	excellent	2.72

granules of 5μ or less in diameter, whereas in the prime starch 60% of the granules, by number, were above this size and 44% were above 10μ. This difference in size is much greater than would be found in the starch of normal flours. The effect of granule size on baking quality was tested by baking bread of mixtures of 85% flour and 15% starch. There were no differences in the doughs made with added prime starch or small-granule starch. Scores of the resulting bread are given in Table III(B), which shows also that the pentosan content of the flour and small-granule starch mixtures was somewhat greater than that of the flour-prime starch mixture. This is due to the difficulty of complete separation of the small-granule starch from the pentosan gel. While the volume of bread with purified small-granule starch was slightly below that of bread with prime starch, its grain and texture were better. Tailings, when used at 15% of the flour-tailings mixture, lowered bread volume on the average 1.1 to 1.4 cu. in. per oz. and affected both grain and texture adversely (Tables I and IV), compared with prime starch. Bread made with crude small-granule starch also had reduced volume and poor grain and texture, showing that the factor responsible for the deleterious baking effects of tailings was present in the crude small-granule starch. These experiments indicate that the poor baking characteristics of the tailings fraction cannot be attributed to its content of small-granule starch.

Effect of Pentosans of Tailings. The adverse effect of the pentosan fraction of tailings on bread was reported previously (8). Since none of the subfractions studied thus far could account for the baking characteristics of tailings, the pentosan fraction was evaluated on a quantitative basis. Two series of bake tests were made, one with tailings and one with "synthetic tailings" which were prepared by adding pentosan fraction to prime starch to bring the pentosan level to that of the tailings. Absorptions of these two fractions were the same. Thus, under ideal conditions both series would be expected to yield breads of almost equal characteristics as none of the other components of tailings was found to be of appreciable importance in determining its baking performance. Experimental results, given in Table IV, are in good agreement with this expectation, as both volume and internal

TABLE IV
EFFECT OF TAILINGS AND "SYNTHETIC TAILINGS" ON QUALITY OF BREAD

Composition [a]		Bread Quality			Pentosan
		Specific Volume	Grain	Texture	
		cu. in./oz.			%
Flour		10.30	very good	very good	2.70
Prime starch	Flour				
5%	95%	10.33	very good	very good	2.57
10%	90%	10.15	very good	good	2.43
15%	85%	10.45	very good	good−	2.29
Tailings					
5%	95%	9.90	good	good−	3.07
10%	90%	9.85	fair−	fair+	3.43
15%	85%	8.95	poor	poor	3.80
Synthetic tailings					
5%	95%	9.50	good	good	3.07
10%	90%	9.35	good−	good−	3.43
15%	85%	8.60	poor	poor	3.80

[a] Flour absorptions: flour = 65.5%; flour and prime starch = 65.4, 65.2, 65%; flour and tailings or synthetic tailings = 67.7, 70.3, 73.4% at 5, 10, and 15% level of substitution, respectively.

characteristics were similar, although the synthetic tailings were somewhat more effective in lowering volume. This may be due to the absence of some minor component present in native tailings, e.g., lipids; or, as indicated previously (8), to the fact that the purified pentosan fraction was not entirely representative of the pentosans of tailings. Thus, the experimental evidence indicates that the pentosan fraction is the quality-determining factor of tailings.

Effect of Proteins Associated with the Pentosan Fraction. The isolated pentosan fraction contained 5.6–7.2% proteins. In view of the evidence that a part of the water-soluble pentosans of wheat flour exists as glycoproteins (6,7) and that these proteins determine some of its functional properties, e.g., the ability to form gels with oxidizing agents, special attention was given to the protein subfraction of the water-insoluble pentosans. To establish whether this protein resulted from an interaction of the pentosans with flour proteins in the course of the separation process or if it is a protein specifically associated with this fraction, the amino acid composition was determined. From the results given in Table V it is clear that the composition of the protein differed greatly from that of gluten, but was similar to that of water-soluble protein of wheat (20). These data suggest the possibility that an interaction between the water-soluble proteins and pentosans occurred, but eliminate the possibility of a reaction of pentosans with gluten proteins. In view of the demonstrated existence of glycoproteins in wheat, it is likely that this protein is an integral component of water-insoluble glycoproteins. To determine if the baking properties of the pentosan fraction were affected by the protein moiety, it was treated with trypsin and bromelin. Tryptic digestion lowered the protein content by 50% while the bromelin reduced it by only 10%. Since bromelin was not effective, the trypsin-treated pentosans were used in baking tests. Results in Table VI show that tryptic digestion did not appreciably alter the functional properties of the pentosan

TABLE V
AMINO ACID COMPOSITION OF THE PROTEIN ASSOCIATED WITH THE WATER-INSOLUBLE PENTOSAN FRACTION

AMINO ACID		AMINO ACID	
	g./16 g. N		*g./16 g. N*
Alanine	5.15	Lysine	5.21
Arginine	6.92	Phenylalanine	4.29
Aspartic acid	7.38	Proline	4.46
Cystine	3.65	Serine	4.69
Glutamic acid	14.06	Threonine	3.72
Glycine	5.72	Tyrosine	3.58
Histidine	2.48	Valine	4.88
Isoleucine	3.23	Ammonia	2.31
Leucine	6.92		

TABLE VI
EFFECT OF PARTIALLY DEPROTEINIZED PENTOSAN FRACTION ON QUALITY OF BREAD

COMPOSITION	BREAD QUALITY		
	Specific Volume	Grain	Texture
	cu. in./oz.		
Flour, 99%; prime starch, 1%	9.5	very good	good +
Flour, 99%; pentosan, 1%	9.0	good−	good
Flour, 99%; pentosan treated with trypsin, 1% [a]	8.8	good−	good

[a] Protein content of the pentosan fraction was reduced by trypsin digestion from 5.6 to 2.7%.

fraction. Thus it appears that the adverse effects of water-insoluble pentosans are more likely due to the pentosans than to the pentosan-protein complex.

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