ENZYMATIC MODIFICATION OF WHEAT FLOUR FOR PAPER SIZING[1]

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ABSTRACT

Soft white winter and soft red winter wheat flours were selected for modification studies. The flours were treated with amylolytic and proteolytic enzymes commercially available and also with the enzymes present in the flours for the preparation of suitable paper-sizing adhesives. Twenty percent wheat flour slurries were enzymatically modified under varying pH, time, and temperature conditions. The slurries were then heated at 95°C. for 30–45 min. to gel the starch and inactivate the enzyme. The resulting products, with viscosities from 1,000 to 5,000 cp., were applied to 7 by 8-in. sheets of unsized paper to determine their suitability as paper sizes. Conditions were established that gave adhesives which compared favorably with sizing materials now used industrially.

Enzymes have been employed for many years by the paper industry to convert raw starch into modified forms suitable for use as surface sizes or coating adhesives, but as yet, no enzymatically modified wheat flours have been used. Recently Rankin *et al.* (1,2) and Lancaster *et al.* (3,4) chemically modified whole flours to give products that compared favorably with tub-sizing agents commercially available. The studies reported here are on the enzymatic modification of wheat flour and wheat flour fractions for similar usage.

Materials and Methods

Flours. A soft white winter (SWW) and a soft red winter (SRW) wheat flour were selected. The commercial SWW flour was milled from a Genessee variety of Michigan wheat (1962 and 1963 crops) and contained 7–8% protein. The air-classified fractions from this Michigan wheat had 2–23% protein. The SRW flour was milled from a mixture of Knox, Vermillion, and Dual varieties of Indiana wheat, and the air-classified fractions of this mixture also had 2–23% protein.

Enzymes. Proteolytic and amylolytic enzymes of animal, plant, and microbiological origin were used to modify the flours. The second approach to modification was to use the "native" or indigenous enzymes present in the flour.

The commercially available enzymes used were: Nagarse,[2] ficin,

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[2]Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

and bromelain (Enzyme Development Corp., New York, N. Y.); crystalline pepsin (Wilson & Co., Chicago, Ill.); Pronase (California Corp. for Biochemical Research, Los Angeles, Calif.); trypsin (2x) salt-free, papain, and pan-protease (Worthington Biochemical Corp., Freehold, N. J.); pancreatic trypsin, trypsin 1-200, crystalline trypsin, crystalline alpha-chymotrypsin, and pancreatin (Novo Industri A/S, Copenhagen, Denmark); Vanzyme 31 (R. T. Vanderbilt Co., Inc., New York, N. Y.); Rhozymes P-11, A-4, J-25, H39, and 33 (Rohm & Haas, Philadelphia, Pa.); crystalline alpha-amylase (Biddle Sawyer Corp., New York, N. Y.).

For survey purposes most of the proteolytic enzymes were allowed to react with a 20% wheat slurry for a 24-hr. period near the optimum pH and temperature of each enzyme to ensure maximum action of the enzyme on the protein. The slurries were layered with toluene and shaken on a New Brunswick shaker bath for 24 hr.

The amylolytic enzymes were allowed to react with 20% wheat flour slurries in a 95°–100°C. bath with stirring for 30–45 min.

When both enzymatic treatments were used, the proteolytic reaction was followed by the amylolytic.

Modification of wheat flour with native or indigenous enzymes in flour used 20% wheat flour slurries that were incubated for 4 hr. with stirring at 62°C. with a pH of 6.0–6.5. They were then placed in a 95°–100°C. water bath and stirred for 30–45 min. The viscosity of the final product was 1,000–5,000 cp. as measured on a 20% paste with a Brookfield Synchro-Lectric Model LVF viscometer at 25°C. and with spindles 3 and 4 at 30 r.p.m. The 20% paste was used to make the necessary dispersions and the remainder of the paste was lyophilized.

Analytical Procedures. Dispersibility measurements were made on a 2% dispersion of the final product by the method of Rankin *et al.* (5).

Nitrogen was determined according to a modified method of Konchakh (6) with the Nessler reagent of Vanselow (7); proteins were determined according to Lowry *et al.* (8); reducing sugars and carbohydrates, by the micromethod of Somogyi (9); and sizing properties of the modified product, as directed in TAPPI Testing Methods (10).

In experiments on the tub-sizing of paper, 2.5 and 5.0 g. (d.b.) of modified wheat flour were added to enough distilled water to make 100 g. of slurry, which was then cooked in a hot water bath (95°–100°C.) with stirring for 30 min. Water was again added to the sample to make 100 g. The 2.5 and 5% dispersions were used to size 7 by 8-in. sheets of Kimberly-Clark magazine-grade raw stock paper by means of a jacketed pan attached to a washing machine wringer. The

temperature of the bath was maintained at 50°C. by circulating 50°C. water through the jacket. Unsized paper was drawn through the bath and then through the hand-wringer, the same pressure being applied on the rolls each time. The sized sheets were dried on a 100°C. heated drum dryer and conditioned in a physical testing room. The amount of size retained on the paper was determined by difference in dry weight of unsized and sized sheets.

Results and Discussion

Action of Proteolytic Enzymes on Wheat Flour. The enzyme preparations were examined for their ability to degrade the protein in wheat flours. The total protein dispersed is broken down into the dialyzable component (low molecular weight) and the nondialyzable component (high molecular weight). Table I shows that bromelain, pepsin, crystalline trypsin, and Pronase dispersed the protein in such

TABLE I
EFFECT OF PROTEOLYTIC ENZYME ON WHEAT FLOUR

Enzyme	Enzyme Concentration [a]	Protein Dispersed	Protein Dispersed	
			Dialyzable or LMW [b]	Nondialyzable or HMW [c]
	mg./100 g. flour	%	%	%
Papain, 30°C.	31.5	100	100	0
Nagarse, 40°C.	40.0	92	70	30
Pan-protease, 40°C.	300.0	100	55	45
Crystalline alpha-chymotrypsin, 40°C.	0.125	37	46	54
Trypsin, commercial, 30°C.	300.0	83	39	61
Pancreatin, 40°C.	2.0	30	37	63
Trypsin 1-200, 40°C.	1.0	36	31	69
Ficin, 40°C.	0.8	32	28	72
Crystalline pepsin, 40°C.	50.0	72	25	75
Pancreatin trypsin, 40°C.	0.5	30	23	77
Pronase, 40°C.	25.0	44	20	80
Bromelain, 40°C.	0.5	72	8	92
Crystalline trypsin, 40°C.	0.04	26	7	93

[a] Determined from proteolytic units to represent a slight excess of enzyme in relation to the amount of protein to be modified.
[b] LMW is the percentage of material of low molecular weight in protein dispersed.
[c] HMW is the percentage of material of high molecular weight in protein dispersed.

a manner that the greater portion was nondialyzable, or of high molecular weight. On the other hand, papain and Nagarse converted a high percentage of the protein into dialyzable material of low molecular weight.

Action of Amylolytic Enzymes on Wheat Flour. The enzymes of bacterial and fungal origin were examined for their ability to degrade the starch portion of wheat flour. Each enzyme preparation was first

assayed to determine the amount necessary for the conversion of starch to a desired viscosity of approximately 1,000–5,000 cp. as measured on a 20% paste with a Brookfield viscometer at 25°C. The dispersed carbohydrate was again analyzed for dialyzable and nondialyzable components.

TABLE II
EFFECT OF AMYLOLYTIC ENZYME ON WHEAT FLOUR

Enzyme	Carbohydrate Dispersed	Carbohydrate Dispersed	
		Dialyzable or LMW [a]	Nondialyzable or HMW [b]
	%	%	%
Crystalline bacterial alpha-amylase	97	14	86
Vanzyme 31	96	71	29
Rhozyme A-4	90	36	64
Rhozyme J-25	79	32	68
Rhozyme P-11	77	37	62
Protease 41	71	25	75
Rhozyme 33	74	42	58
Rhozyme H-39	80	30	70

[a] LMW is the percentage of material of low molecular weight in carbohydrate dispersed.
[b] HMW is the percentage of material of high molecular weight in carbohydrate dispersed.

Any one of the commercial enzymes listed in Table II is capable of modifying the starch portion to give the desired viscosity with a total dispersibility ranging from 71 to 97%, although the amount of material of high molecular weight varies from 29 to 86%. The modified starch should consist mostly of material of high and uniform molecular weight. Crystalline bacterial alpha-amylase converted 97% of the starch of which 86% was material of high molecular weight. It was chosen for our preliminary investigations because of its high purity and reproducibility of results.

Action of Indigenous Enzymes on Wheat Flour. Although the presence of amylolytic and proteolytic enzymes in wheat flour has been known for a long time, it was only recently that these enzymes were recognized as potentially useful in modifying flour for industrial use. Experiments on a series of air-classified flour fractions of Indiana SRW wheat showed that the higher the protein in a fraction the less added alpha-amylase was needed to reach a desired viscosity. It was postulated that the higher protein concentrations carried more native enzyme, which augmented the added enzyme and caused the reaction to proceed at a faster rate. Lancaster and Moulton (personal communication) of the Northern Laboratory also noted the effect of indigenous enzyme while working with a Michigan SWW wheat containing 7% protein.

Experiments were run on this SWW flour to determine the optimal time, temperature, and pH for modification. When a 20% flour slurry was held for 2–4 hr. at 60°–65°C. with a pH of 6.0–6.5, the modified materials had viscosities ranging from 1,000 to 5,000 cp., and their sizing values compared favorably with those of commercial sizing material.

Effect of Increased Protein on Sizing Properties. There was much interest in capitalizing on the protein in flour and gaining advantage from it, if possible. Air-classified fractions of SRW and SWW wheat flours were tested to see what effect increased protein had on strength. The main properties tested were: tensile strength, expressed as breaking length in meters; porosity, which measures the air resistance of paper; and bursting strength, which is the pressure required to rupture a sheet of paper.

The protein was first converted with pepsin, followed by treatment with crystalline alpha-amylase added in various amounts so that each fraction had a final viscosity of 1,000–5,000 cp. Sizing values were adjusted to a constant retention of 3% of the size by the paper to give more comparable results. Curves of sizing properties *vs.* retention were employed to make the adjustments. Figure 1 shows that, as the protein level increased above 5%, the sizing test values decreased. No advantage was realized with an increase in protein concentration.

The air-classified fractions on SWW wheat flour containing from 2 to 23% protein were reacted in two different ways (Fig. 2). In the first experiments, the protein was predigested with pepsin for 24 hr.

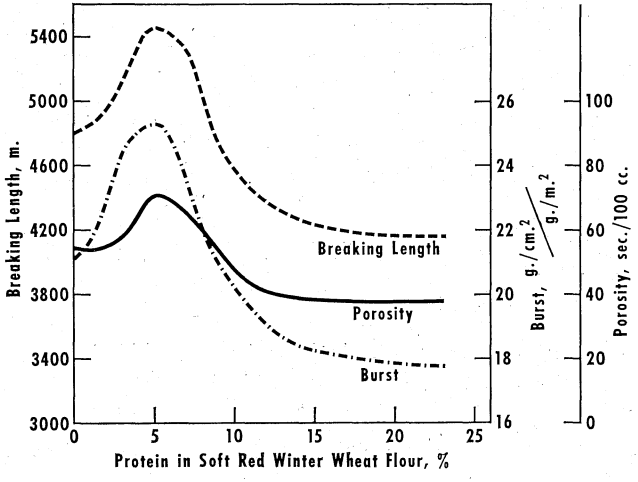

Fig. 1. Effect of protein in SRW wheat flour on sizing properties.

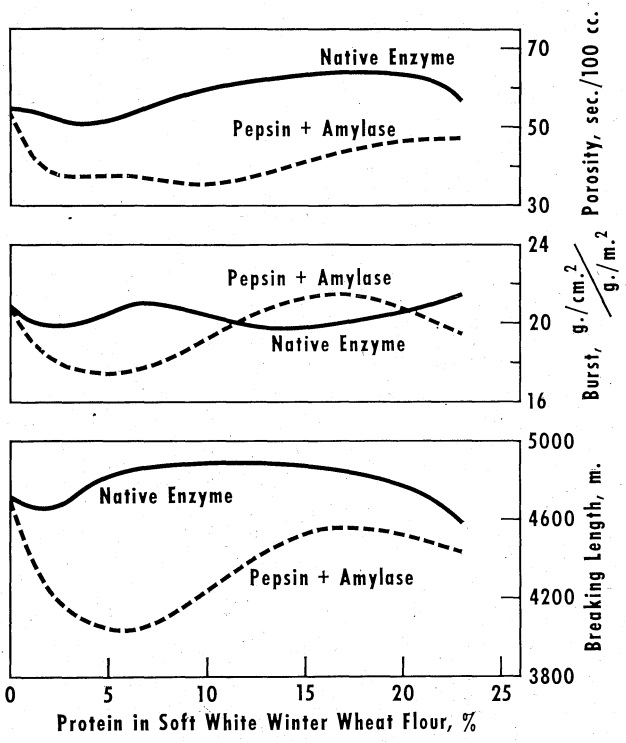

Fig. 2. Effect of protein in SWW wheat flour on sizing properties.

and then crystalline alpha-amylase was added in various amounts so that the final viscosity in all fractions dropped to 1,000–5,000 cp. A second series utilized the indigenous enzymes present in the flour. The series was allowed to incubate 4 hr. at 62°C. with a pH of 6.0. It was then placed in a 95°–100°C. bath for 30–45 min.

In the first experiments after the 5% level was reached, there was an increase in sizing value with higher protein level; however, when native enzyme alone was used, the results varied little and more closely resembled those obtained with commercial sizing materials.

A comparison of the two varieties of flour seemed to indicate that the protein in SWW wheat contributed more favorable characteristics than did the protein in SRW wheat.

Inhibition of Beta-Amylase Activity by Ascorbic Acid. The success of the conversion of wheat flour by native enzymes depends primarily on the action of alpha-amylase which produces a minimum of dextrin and no sugars. However, as beta-amylases are always present in great abundance, a large amount of reducing sugar was produced in the

2- to 4-hr. period necessary to reduce the flour to a workable viscosity. This problem was not apparent when crystalline alpha-amylase was added to wheat flour for modification, because the shorter time and the higher temperature (95°–100°C.) used inactivated the beta-amylase before excessive reducing sugar was formed (11).

TABLE III
INHIBITION OF BETA-AMYLASE BY ASCORBIC ACID DURING ENZYME MODIFICATION OF FLOUR

PROPERTY	ASCORBIC ACID CONCENTRATION, G./60 G. FLOUR			
	5.0	2.5	0.25	None
Total dispersion, %	73.5	66.2	75.3	78.4
Reducing sugars, %	4.9	8.0	20.6	21.7
Carbohydrate dispersed, %	67.8	70.3	80.6	93.7
Nondialyzable carbohydrate, %	87.9	87.3	46.8	40.9
Protein dispersed, %	30.0	31.7	36.8	40.8
Burst, (g./cm.^{2})/(g./cm.^{2})	22.2	20.8	20.6	20.4
Dry breaking length, m.	5,260	5,110	4,720	4,550
Tear factor, g./g.s.m.	72	70	71	81
Porosity, sec./100 cc.	53	65	47	60

Various amounts of ascorbic acid (see Table III) were added to 60 g. of flour and made to 300 g. with distilled water. The slurries were stirred 4 hr. at 62°C. and pH 5.9. Samples were then placed in a bath at 95°–100°C., with stirring, for 30–45 min. The incorporation of ascorbic acid markedly lowered the amount of reducing sugar produced and at the same time gave a 10–15% increase in strength as measured by burst and breaking length. This improvement was apparently due to the increase in dispersible nondialyzable material (high molecular weight). A decrease in protein dispersibility indicated that the proteinases had also been inhibited by the incorporation of ascorbic acid.

TABLE IV
TUB SIZE VALUES: MODIFIED WHEAT FLOUR

SIZE	TREATMENT	BURST	BREAKING LENGTH (DRY)	POROSITY	BRIGHTNESS
		$\frac{g./cm.^2}{g./m.^2}$	*m.*	*sec./100 cc.*	%
None (KC base paper)	None	16.0	4,350	40	70.9
Superfilm 40	None	22.3	5,241	49	69.7
Wheat starch	Amylase	23.8	5,418	66	69.1
Wheat flour	Amylase	21.6	5,206	80	69.8
Wheat flour	Native enzyme	20.2	4,780	60	70.6
Wheat flour	Bromelain	21.3	5,007	68	70.2
Wheat flour	Pronase-amylase	22.5	5,324	119	...

Tub-Sizing Evaluation. Table IV compares strength values for paper tub-sized with the more promising enzyme-treated flours and those of a commercial product, Superfilm 40 (hypochlorite-oxidized starch). The strength values of the standard untreated paper and a paper treated with a wheat starch modified with crystalline alpha-amylase are also included. All the values were adjusted to a retention of 3%. The 7% SWW wheat flours treated with Pronase and bromelain compared favorably with the commercial hypochlorite-oxidized starch. Seven-percent SWW wheat flour modified to give a workable viscosity, either with its indigenous enzymes or more rapidly with added crystalline bacterial alpha-amylase, also showed promise as an effective paper-sizing agent.

In laboratory-scale applications, a SWW wheat flour containing 7% protein was enzymatically converted to a paper-sizing product comparable to commercial surface-sizing agents. Tests of industrial equipment will be necessary to demonstrate that the product is satisfactory for commercial use.

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