

Reconstitution Studies with Sound and Sprouted Wheat Flour¹

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ABSTRACT

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Sound and sprouted wheat flours were fractionated into gluten, starch, and solubles using either distilled water or a 2% (w/v) NaCl solution. Starch with adhering matter (AM) was isolated by a nonaqueous procedure after the wheat flours were pin milled. Enzymatic activity was highest in the soluble fraction. Salt solution was more effective in extracting proteolytic enzymes than was distilled water. Amylolytic activity was high in the starch-AM fraction from the sprouted wheat flour. Farinograph and baking data of starch-AM/gluten blends indicated that sprouting had detrimental effects on gluten properties and that NaCl in some way prevented gluten deterioration. The starch-AM had a definite effect on the mixing and baking properties of wheat flour doughs. However, whether the

differences between starch-AM samples isolated from sound and sprouted wheats were caused by sprouting or by the type of wheat itself could not be established. Flours were reconstituted with starch-AM from sound wheat flour and gluten that had been isolated from sound flour with distilled water and then incubated with different dialyzed salt solubles. Physical dough and baking data from the reconstituted flours revealed that mixing properties of the doughs were not affected by the presence of α -amylase during incubation. However, a gluten weakening effect was noted, even if the dialyzed salt solubles had previously been boiled. These results indicate that the enzymes associated with gluten, but not extracted with distilled water, had a considerable effect on gluten properties.

One of the major problems related to sprout damage in wheat is the degradation of the protein or carbohydrate components in the endosperm. Although the detrimental effect of sprouted wheat on baking quality is attributed to high amylase activity, other biochemical changes may alter the ability of flours to produce normal doughs.

Hwang and Bushuk (1973), working with laboratory-sprouted wheat, found a decrease in the amount of residue protein with sprouting. Similar results were found by Preston et al (1978). Redman (1971) and Hanford (1967) found that gluteins isolated from flours to which certain levels of malt had been added or from flours derived from sprouted wheat were softer than gluteins isolated from sound wheat flour.

Several workers have reported the effect of NaCl on wheat flour proteases. According to Miller and Johnson (1948), NaCl inhibits wheat flour proteases, and the presence of salt during fermentation permits the use of high levels of malt with no harmful effects on baking properties. McDonald and Chen (1964) found a reduction in proteolytic activity in the presence of salt. Redman (1971) indicated that 90% of the proteolytic activity was extracted with NaCl solution. The dialyzed saline extracts caused a softening of gluteins isolated from sound wheat flour. Bean et al (1974), working with Japanese-type noodles, found that the addition of NaCl to sprout-damaged wheat flour strengthened the dough and reduced its stickiness.

The present study investigated the rheological and baking properties of gluteins extracted with distilled water and salt solution from sprouted wheat flour. Concomitantly, the proteolytic and amylolytic activity in the flour fractions and the effect of salt-extracted enzymes on gluten properties were investigated.

MATERIALS AND METHODS

Wheat Flour Samples

The sound wheat flour used in the present study was derived from a composite sample of Waldron, a hard red spring wheat, grown at several locations in North Dakota during the 1978 crop year. The sprouted wheat flour was milled from a sample of hard red spring wheat obtained from a commercial mill. Pertinent information on the sound and sprouted wheat flours is given in Table I.

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Fractionation of Wheat Flour

Two aqueous fractionation procedures were used to isolate gluten, starch, and solubles. Flour was dispersed with distilled water or with a 2% (w/v) NaCl solution in a 1:2 ratio (w/v) at low speed in a Waring Blendor and centrifuged. When distilled water was used, the supernatant, which represented the water solubles, was shell-frozen and freeze-dried. The salt-soluble supernatant was dialyzed for three days against distilled water at 2°C with two water changes per day, then centrifuged, and the remaining solubles were shell-frozen and freeze-dried. This fraction is referred to as "salt solubles." In both procedures, the sludge layer, including the gluten located on top of the prime starch, was removed and washed with distilled water to recover the gluten. The gluten from the distilled water dispersion was then chilled, freeze-dried, and ground to pass through a U.S. standard No. 70 mesh sieve. Gluten isolated from flour dispersed with salt solution was washed with 12 250-ml portions of 2% salt solution (v/w) and then with six 250-ml portions of distilled water before being freeze-dried. For additional purification, the starch was reslurried in distilled water and centrifuged at 2,000 × g for 10 min. This process was repeated twice. The recovered starch was air dried and ground to pass through a U.S. standard No. 70 mesh sieve.

Isolation of Starch with Adhering Matter

Starch with adhering matter (starch-AM) was isolated by a procedure analogous to that of Simmonds et al (1973) adopted to centrifugation by Kulp et al (1976). The sprouted and sound wheat flours were pin milled on an Alpine Kolloplex laboratory model 160Z mill (Alpine American Corp., Natick, MA) at 18,000 rpm. The resultant pin-milled flour (1.0 kg) was dispersed in a chloroform/benzene solution (800 ml, density 1.45 g/cc) and centrifuged at 2,000 × g for 10 min. The material containing the starch-AM at the bottom of the centrifuge cup was collected, and

TABLE I
Characteristics of Sprouted and Sound
Hard Red Spring Wheat Flour Samples

	Sound	Sprouted
Moisture (%)	15.0	15.1
Nitrogen (%) ^a	3.02	2.61
Ash (%) ^a	0.49	0.45
Falling number (sec)	458	74
Farinograph absorption ^b (%)	63.6	59.7
Dough development time (min)	6.0	3.5
Stability (min)	8.2	6.1
Mechanical tolerance index (BU)	30	45

^a Dry basis.

^b 14.0% moisture basis.

the starch-AM was isolated from it by successive dispersion and centrifugation with benzene/chloroform solutions of increasing densities (800 ml, densities 1.47 and 1.49 g/cc). Finally the residue was dispersed in chloroform/tetrachloroethylene solution (800 ml, density 1.52 g/cc) and centrifuged at $2,000 \times g$ for 10 min. The supernatant containing the starch-AM was allowed to air dry and ground to pass through a U.S. standard No. 70 mesh sieve.

Amylase Activity

Amylase activity was measured with the grain amylase analyzer (Perkin-Elmer Corp., Coleman Instruments Div., Oak Brook, IL), according to the Perkin-Elmer model 191 grain amylase analyzer instruction manual (1979). Extraction solution (20 ml) containing 0.5% NaCl and 0.2% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was added to a sample of flour (4.0 g). The suspension was mechanically shaken for 5 min, followed by filtration through Whatman No. 2v filter paper. Three milliliters of the substrate solution (β -limit dextrin) was pipetted into cuvettes and allowed to equilibrate. After equilibration, 200 μl of the flour extract was added; the cuvette was capped, rotated by hand, and inserted into the measurement well of the instrument. The value on the instrument was recorded using the two-amylase mode of the instrument, indicating a 2-min reaction time. The results are expressed as model 191 grain amylase analyzer units per 200 μl of sample extract.

Because of the high level of amylase activity in the sprouted

TABLE II
Blends of Gluten and Starch-AM^a

Source of Starch-AM	Source of Gluten	
	Wheat Flour/ Extracting Medium	Incubated with
Sprouted wheat flour	Sprouted/distilled water	...
	Sprouted/salt solution	...
	Sound/distilled water	...
	Sound/salt solution	...
Sound wheat flour	Sprouted/distilled water	...
	Sprouted/salt solution	...
	Sound/distilled water	...
	Sound/salt solution	...
Sound wheat flour	Sound/distilled water	Salt-solubles from sound wheat
		Salt-solubles from sprouted wheat
		α -Amylase inactivated salt-solubles from sprouted wheat
		Previously boiled salt-solubles from sprouted wheat

^a Starch with adhering matter.

TABLE III
Baking Formulas for Loaves Containing Flour Reconstituted from Gluten/Starch-AM^a Blends

Ingredient	Baking Method		
	Straight Dough	Sponge and Dough	
		Sponge	Dough
Reconstituted flour (g)	25.0	15.0	10.0
Salt ^b (%)	2.0	...	2.0
Sugar ^b (%)	5.0	...	5.0
Yeast ^b (%)	3.0	2.5	...
Shortening (%)	3.0	...	3.0
Water	Variable	Variable (60%)	Variable (40%)
Potassium bromate (ppm)	...	10	...

^a Starch with adhering matter.

^b Dissolved in water before being added.

wheat flour, dilution of the extracts was necessary. Values reported correspond to the meter reading on the instrument times the dilution factor.

Protease Activity

Hemoglobinase activity was determined according to the method of Bushuk et al (1971). Each of the isolated flour fractions (100–1,000 mg) was incubated at 37°C with 5.0 ml of 0.2M acetate buffer, pH 3.8, and 50.0 mg of bacto-hemoglobin (DIFCO). After 5 hr of incubation, the reaction was stopped by adding 5.0 ml of 5% trichloroacetic acid. The solution was allowed to stand for 15 min, centrifuged at $20,000 \times g$ for 20 min, and then filtered through Whatman No. 2 filter paper. The soluble nitrogen was determined by the indanetrione hydrate (ninhydrin) method specified by Hanford (1967). Blanks were determined for every sample by adding 5 ml of 5% trichloroacetic acid to the reaction mixture at zero time. Activity was expressed as the increase in absorbance per 100 mg per hour. Azocaseinase was determined according to Preston et al (1978) without modification. Azocaseinase activity was expressed as milligrams of hydrolyzed azocasein per 100 mg per hour.

Incubation of Gluten with the Salt-Soluble Fraction

Gluten isolated from the sound wheat flour dispersed with distilled water was used in this experiment. Gluten (30.0 g) was incubated with the salt-soluble fraction dissolved in 75.0 ml of 0.2M acetate buffer, pH 4.5, for 3 hr at 37°C. After incubation, the treated gluten was washed with three 250-ml portions of distilled water, frozen, and freeze-dried. The following systems were used for gluten incubation: 1) gluten and the salt-soluble fraction from sound wheat flour, 2) gluten and the salt-soluble fraction from sprouted wheat flour, 3) gluten and the salt-soluble fraction, α -amylase inactivated, from sprouted wheat flour, and 4) gluten and previously boiled salt-soluble fraction from sprouted wheat flour. All the protease-active salt-soluble fractions had the same hemoglobinase activity (230.18 absorbance units per 100 mg per hour).

α -Amylase was inactivated by dissolving the salt-soluble fraction (2.0 g) in 35.0 ml of 0.2M acetate buffer, pH 3.6, and keeping the solution at 50°C for 30 min. The resulting solution was centrifuged at $2,000 \times g$ for 10 min; the supernatant was collected, dialyzed against distilled water, and then shell frozen and freeze-dried. Inactivation of α -amylase had no effect on hemoglobinase activity.

Reconstitution Studies

Reconstitution studies were conducted with gluten/starch-AM systems. Blends contained 13.0 g (db) of gluten protein and 87.0 g (db) of starch-AM, so that the protein content in the blends was constant. Attempts to use a gluten/starch/soluble system failed; the farinograms were abnormal and no conclusions could be drawn. The gluten/starch/soluble system showed extremely rapid hydration with the farinograph, and the resultant curve was irregular in shape compared to that of a farinogram obtained with flour. The better results with the starch-AM fraction suggest that the adhering matter controls the rate of water hydration, thus resulting in a more normal type of farinogram.

The gluten/starch-AM blends used for this study are shown in Table II.

Physical Dough Testing

Mixing properties of 50 g of each of the various gluten/starch-AM blends (14.0% mb) were studied with the farinograph. The curve was centered on the 500-BU line.

Baking Study

The gluten/starch-AM blends used for the physical dough testing studies were baked into 25.0-g pup loaves by both the straight and sponge and dough baking procedures. Baking formulas are shown in Table III. Baking absorption was estimated from the farinograph absorption. A 3-hr fermentation time was used for the straight dough baking procedure. During fermentation, the doughs were punched at 105 and 150 min,

TABLE IV
Yield (%), Nitrogen Content (%), and Grain Amylase Analyzer (GAA) Values (GAA units) of Flour Fractions Isolated from Wheat Flour Dispersed with Distilled Water or Salt Solution^a

Flour Fraction	Yield		Nitrogen		GAA Value	
	Sprouted Flour	Sound Flour	Sprouted Flour	Sound Flour	Sprouted Flour	Sound Flour
Flour	2.61	3.02	5,512	0
Fractions from distilled water						
Starch	41.5	35.7	0.07	0.05	750	0
Gluten	17.4	21.4	12.25	12.37	1,926	24
Water-soluble	6.2	4.0	3.02	4.89	55,000	292
Fractions from salt solution						
Starch	42.9	45.2	0.05	0.05	286	8
Gluten	12.4	16.0	14.3	13.60	210	18
Salt-soluble ^b	1.8	1.2	7.28	8.61	8,375	293
Starch-AM ^c	45.6	31.7	0.77	0.75	11,040	162

^aYield and nitrogen content expressed on a dry basis.

^bDialyzed against distilled water.

^cStarch with adhering matter.

respectively, after mixing. After 180 min, the doughs were sheeted, molded, panned, and proofed at 30°C for 55 min and baked at 230°C for 20 min. A 4-hr sponge and 30-min dough floor time were used for the sponge and dough baking procedure. The proofing and baking conditions were the same as for the straight dough baking method.

Mixing time varied for the different blends and was determined by visual examination of the dough. Loaf volume was measured by rapeseed displacement after cooling. Specific volume was calculated by dividing the loaf volume (in cubic centimeters) by the loaf weight (in grams).

RESULTS AND DISCUSSION

Amylolytic and Proteolytic Activity

Yield of flour fractions obtained after dispersion with distilled water and with salt solution are shown in Table IV. Gluten isolated from sprouted wheat flour dispersed with distilled water was very difficult to handle. Thus those glutes were not completely washed free of starch, which explains their higher yields. When the sprouted wheat flour was dispersed with salt solution, the resultant gluten was firm and could be readily isolated. Sprouted wheat flour yielded higher amounts of water-soluble and salt-soluble fractions than sound wheat did.

Nitrogen contents of the sprouted and sound wheat flours and fractions are shown in Table IV. Glutes isolated from flours dispersed with salt solution had higher nitrogen content than those obtained from flours dispersed with distilled water. The lower nitrogen content of the water-soluble and salt-soluble fractions isolated from sprouted wheat flour compared to the nitrogen content of these fractions from the sound wheat flour suggested that higher amounts of soluble polysaccharides were present in the sprouted wheat flour. After dialysis, the water-soluble and salt-soluble extracts were free of low molecular weight compounds; therefore, the main polymers present in these extracts were proteins and polysaccharides. Nitrogen contents of the starch-AM fractions from sprouted and sound wheat flours were similar to those reported by Kulp et al (1976).

Table IV also shows the amylase activity associated with the wheat flour fractions. As expected, the sprouted wheat flour fractions had higher activity than the corresponding sound wheat flour fractions. Although some of the amylase activity remained associated with the starch, most of the enzyme was extracted with distilled water. Flour fractions obtained by dispersion of the flour with salt solution had lower activities than those isolated with distilled water. Although these results indicate that salt, at the concentration used, had an inhibitory effect on α -amylase activity, some loss of activity may have occurred during dialysis. The relatively high amylase activity associated with the starch-AM agreed with studies reported by Kulp et al (1976).

Protease activities of the flours and fractions are shown in Table V. In general, sprouted wheat flour fractions had levels of

TABLE V
Protease Activity of Flour Fractions Isolated from Wheat Flour Dispersed with Distilled Water or Salt Solution

Flour Fraction	Sprouted Flour		Sound Flour	
	Hemoglo- binase ^a	Azocase- inase ^b	Hemoglo- binase	Azocase- inase
Flour	2.391	0.149	1.952	0.122
Fractions from distilled water				
Starch	0.084	0.054	0.022	0.022
Gluten	5.294	0.131	4.758	0.090
Water-soluble	7.032	0.558	6.829	0.590
Fractions from salt solution				
Starch	0.056	0.000	0.011	0.000
Gluten	3.301	0.023	2.666	0.036
Salt-soluble ^c	23.018	1.697	10.377	1.287
Starch-AM ^d	1.516	0.108	1.389	0.054

^aHemoglobinase activity is expressed as: absorbance per 100 mg per hour.

^bAzocaseinase activity is expressed as: mg of azocasein per 100 mg per hour.

^cDialyzed against distilled water.

^dStarch with adhering matter.

hemoglobinase and azocaseinase activity higher than those from sound wheat flours. Like the α -amylase activity, some of the proteolytic activity was associated with the starch and gluten. However, contrary to what was observed with the α -amylase activity, the salt-soluble fraction had the highest activity, indicating that in this case considerable amounts of the proteases were extracted by salt solution.

Physical Dough Testing

Table VI shows pertinent farinograph data obtained with the gluten/starch-AM blends. The blend containing gluten from sprouted wheat flour dispersed with distilled water had the weakest farinograph characteristics among all blends investigated. Using salt solution to isolate gluten from the sprouted wheat flour caused a marked improvement in its farinograph properties. Blends containing glutes from sound flour had similar farinograph characteristics regardless of the method used for isolation. This result, in conjunction with the high protease activity found in the salt-soluble fraction, indicated that NaCl may have prevented gluten proteolysis from occurring. Blends containing starch-AM from sound wheat flour had stronger farinograph characteristics than those with starch-AM from sprouted flour. This result indicated that the starch-AM had an important effect on the mixing properties of the doughs. However, because the starch-AM was isolated from flour derived from different wheats, definite conclusions on the effect of sprouting on the starch-AM properties are difficult to make.

The effect of salt-extracted enzymes on gluten properties is shown in Table VII. Incubation of gluten caused a general

weakening in the dough, which was reflected by a marked decrease in absorption, a less pronounced decrease in the peak time and stability, and an increase in the mechanical tolerance index. The gluten-weakening effect noted upon incubation, even when the gluten was incubated with previously boiled salt-soluble fractions, suggested that the enzymes associated with sound gluten and not extracted with distilled water had a considerable effect on gluten properties, particularly on absorption. Blends containing gluten incubated with the active salt-soluble fraction from sprouted wheat flour had similar farinograph characteristics, regardless of the presence of α -amylase enzymes. These results indicate that, in the systems studied, α -amylase did not affect the mixing properties of the dough. Gluten incubated with the salt-soluble fraction from sound wheat flour produced stronger doughs than did the corresponding gluten incubated with sprouted salt-soluble fractions. Considering that at the same hemoglobinase activity, the sound salt-soluble extract had higher azocaseinase activity than the sprouted one, the results indicate that the enzymes in these extracts are distinct with respect to gluten breakdown. Preston et al (1978) found that endoproteolytic enzymes in ungerminated seeds are apparently unable to hydrolyze significant amounts of storage proteins.

Baking Studies

The gluten/starch-AM blends used for physical dough studies were baked by the straight and sponge and dough baking procedures. Figure 1 illustrates the baking results for the various

blends. Although the same general profile was obtained with both baking procedures, the specific volume for breads produced by the sponge and dough method were higher than those produced by the straight dough. Blends containing wheat gluten isolated with salt solution from sprouted wheat had higher volumes than those extracted from sprouted wheat with distilled water. This result again suggests the beneficial effect of salt on preventing gluten breakdown. Similarly to what was observed with the farinograph, incubation of gluten with α -amylase had no effect on gluten baking properties. However, gluten was washed out after incubation with α -amylase, and therefore, a gluten free of amylase was used for blending. The presence of α -amylase during baking possibly would alter the results. Regardless of the baking procedure, the highest specific volumes among all blends containing enzyme-active incubated glutes were obtained with glutes incubated with salt-soluble fractions extracted from sound wheat flour. This result, again, indicated differences in protease enzymes in the salt solubles from sound and sprouted wheat flours.

SUMMARY

The results of this study show that NaCl solution was more effective in extracting proteolytic enzymes from wheat flour than distilled water was.

Physical dough testing and baking results indicated that sprouted wheat has a detrimental effect on gluten properties. The

TABLE VI
Farinograph Data for Blends of Starch-AM^a and Gluten

Starch-AM Source	Gluten Source and Extracting Medium	Farinograph characteristics			
		Absorption ^b (%)	Peak Time (min)	Stability (min)	MTI ^c (BU)
Sprouted wheat flour	Sprouted wheat flour				
	Distilled water	60.0	1.0	1.6	120
	Salt solution	59.8	5.5	9.0	80
	Sound wheat flour				
	Distilled water	60.5	6.5	11.3	45
	Salt solution	62.2	5.5	7.2	85
Sound wheat flour	Sprouted wheat flour				
	Distilled water	63.0	3.0	4.0	50
	Salt solution	65.4	7.5	9.1	50
	Sound wheat flour				
	Distilled water	68.4	8.0	12.6	30
	Salt solution	65.2	8.0	9.1	50

^a Starch with adhering matter.

^b Expressed on a 14.0% moisture basis.

^c Mechanical tolerance index.

TABLE VII
Farinograph Data for Blends of Starch-AM^a and Enzyme-Treated Gluten^b

Farinograph Characteristic	Unincubated Gluten	Sound Wheat Flour	Sprouted Wheat Flour	Gluten Incubated ^c with Enzyme ^d from	
				Sprouted Wheat Flour	Protease and Amylase Inactivated
Absorption ^e (%)	68.8	61.4	61.4	61.2	63.2
Peak time (min)	8.0	5.5	5.5	5.5	7.0
Stability (min)	12.6	9.5	7.5	8.0	9.4
Mechanical tolerance index (BU)	30.0	40.0	65.0	65.0	60.0

^a Starch with adhering matter.

^b Hemoglobinase activity = 230.18 absorbance units per 100 mg per hour.

^c For 3 hr.

^d Salt-extracted.

^e Expressed on a 14.0% moisture basis.

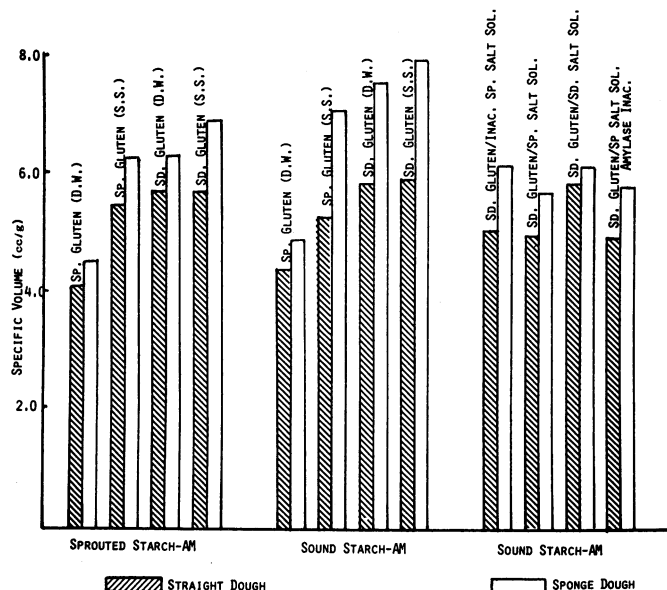


Fig. 1. Specific volume of blends of gluten and starch with adhering matter. SP = sprouted, SD = sound, S.S. = salt solution, D.W. = distilled water.

results suggest that NaCl prevents gluten deterioration, which could be of importance in determining the baking procedure to employ if one is using sprouted wheat flour. Wheat flour starch-AM has a definite effect on the mixing and baking properties of wheat flour doughs. However, the present study could not establish whether the difference between the sound and sprouted starch-AM was due to sprouting or to the type of wheat. Studies involving wheat varieties and their starch-AM properties may be rewarding.

In the model systems used, the mixing properties of the doughs were not affected by the presence of α -amylase. A qualitative difference, in terms of gluten breakdown, between salt solubles from sound and sprouted wheat flour was indicated. The presence of enzymes that lack the required specificity to break down gluten and/or the presence of a high molecular weight protease inhibitor in the salt solubles from sound wheat flour may account for this result.

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