Wheat Protein–Starch Interaction. I. Some Starch-Binding Effects of Wheat-Flour Proteins

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ABSTRACT

Amylose and amylopectin in a gelatinized wheat-starch solution are separated by centrifugation. Differences of amylopectin content in starch solutions are indicated when the iodine-starch colorimetric procedure is employed at two wave lengths. These procedures enable the detection of wheat protein-wheat starch interaction in aqueous systems. Association of gelatinized wheat starch and wheat protein occurs at acidic and neutral pH, but is diminished at alkaline pH. Modification of the wheat protein by heat denaturation results in loss of the starch-binding properties of the wheat protein. Viscosity of wheat protein-wheat starch systems is altered by heat denaturation of the protein.

A procedure for separating amylose and amylopectin in a gelatinized wheat-starch solution by centrifugation was described by Baum et al. (1). The higher-molecular-weight amylopectin sedimented as a gel during centrifugation, while the amylose remained in the supernatant liquid. The "blue value" of the amylose measured 1.4 and the "blue value" of amylopectin measured 0.2 in a typical experiment. Amylose was examined for viscometric properties by these workers, following complexing with butanol.

Spectrophotometric studies of iodine-starch complexes reveal a peak absorbance near 550 nm. for amylopectin and a peak absorbance near 650 nm. for amylose (2). These differences of maxima suggested a possible means for showing differences of amylopectin content in starch solutions by measuring the iodine-starch complex at these two wave lengths. Two starch solutions absorbing equally at 650 nm. could be expected to absorb differently at 550 nm. if they differed in amylopectin content, since an appreciable amount of absorbance at 550 nm. (the amylopectin peak) must be contributed by the amylopectin component.

The centrifugation procedure of Baum et al. (1) provided a means for testing the above idea. Wheat-starch solutions are lowered in amylopectin by centrifugation. Absorbance of iodine-starch complexes at 650 and 550 nm. of gelatinized wheat-starch solutions were compared before and after centrifugation and are reported here.

An interaction of gelatinized wheat starch and wheat protein occurs which interferes with the blue color associated with iodine-starch complexing. Use of the procedures described above has enabled certain tests and experiments which provide data that offer some insight into the nature of starch-protein interaction. Results of such experiments are reported here and are interpreted on the basis of published research on starch–protein interaction. Also, some interpretation is made from literature which reports the effects of changes of pH and heat denaturation of flour on its functional behavior.

MATERIALS AND METHODS

Samples of amylose (Superlose) and amylopectin (Ramalin-G) were obtained from Stein, Hall & Co., Inc. High-protein air-classified flour (Beevo, 22.3% protein) was obtained from Pillsbury Co.

Preparation of Starch Solutions

Gelatinized wheat-starch dispersions were prepared by dispersing the starch in water, transferring hot water (90° to 100°C.) to it, and holding at 90° to 100°C. for 15 min. Appropriate aliquots were taken for starch-protein aggregation experiments.

Amylose and amylopectin were dispersed in 1.0N NaOH at room temperature to form clear solutions which were then neutralized with 0.5N HCl (Methyl Orange, indicator). These solutions were then diluted to give volumes, and aliquots of each were combined in various proportions to give blends of amylose and amylopectin.

Preparation of Protein Extracts

Wheat-protein extracts were prepared by dispersing flour in 0.1N acetic acid (1:5), allowing to stand an hour, and then centrifuging. The supernatant was recovered and used as protein extract.

Separation of Amylose and Amylopectin by Centrifugation

The procedure used was that reported by Baum et al. (1). Gelatinized wheat-starch dispersions were centrifuged, resulting in the bulk of high-molecular-weight amylopectin sedimenting to the bottom as a gel. Most of the amylose remained in solution in the supernatant.

Starch Determination

An adaptation of the method of McCready and Hassid was used (3). A 1-ml. aliquot of starch solution was combined with 5 ml. 1.0N NaOH. After standing at room temperature 15 min., the solution was neutralized with 0.5N HCl (Methyl Orange, indicator) and made to 100 ml. volume, including the addition of 1 ml. iodine reagent (0.2% I_2; 2.0% KI). The resulting blue color was read at 550 and 650 nm.

Starch–Protein Interaction Experiments

The following procedure was followed in tests for starch–protein interaction. Ten grams of gelatinized wheat-starch solution (5%) were combined with an aliquot of protein extract. Additional water was added when necessary to give a final added volume of 10 ml. After complete mixing of ingredients, the mixture was centrifuged.

Heat Denaturation of Protein Extract

Protein extracts were heat-denatured by immersing the flask in a boiling-water bath for 15 min. The flask and contents were weighed before and after to insure against loss by evaporation.

EXPERIMENTAL

Effect of Centrifugation on Iodine-Starch Complex Absorbance

A gelatinized wheat-starch solution (2%) was prepared and a portion of it was centrifuged. Aliquots of the wheat-starch solutions, before and after centrifuging, were determined spectrophotometrically at 550 and 650 nm. Values at both wave lengths were obtained and plotted. Absorbance at 550 nm. accompanying given absorbance at 650 nm. were determined from the plots for the wheat-starch solutions before and after centrifuging.

Effect of Amylose:Amylopectin Ratio Changes on Starch-Iodine Complex Absorbance

Amylose (Superlose) and amylopectin (Ramalin G) were combined in various ratios. Equal weights of these starches were determined spectrophotometrically at 550 and 650 nm. Values at both wave lengths were obtained and plotted.

Effect of Wheat Starch–Wheat Protein Interaction on Starch-Iodine Complex Absorbance

A wheat-protein extract was prepared from the high-protein air-classified flour as previously described. The extract was refined by adjusting the extract to pH 6.5 with 1.0N NaOH, centrifuging to sediment protein, and then discarding the supernatant liquid. The sedimented protein was resuspended in pH 6.5 buffer. This extractible precipitable protein was expressed on the basis of the amount of flour from which it was removed, assuming one had diluted the high-protein flour by one-half to give a flour of 11.15% protein.

Increments of the protein preparation were added to gelatinized wheat-starch solutions with additions of buffer to maintain constant volume. These mixtures were centrifuged. Supernatant liquid was determined spectrophotometrically for starch.

Effect of pH on Wheat Starch–Wheat Protein Interaction

Wheat-protein extracts were adjusted to various pH values by careful addition of increments of 1.0N NaOH. These extracts were combined with gelatinized wheat-starch solutions, centrifuged, and the supernatant liquid determined for starch spectrophotometrically.

Effect of Heat Denaturation on Wheat Starch–Wheat Protein Interaction

The procedure was the same as that used for the pH effect. Heat-denatured

protein extracts were prepared and compared with native protein extracts which had not been subjected to heat.

Effect of Heat Denaturation on Viscosity of Gelatinized Wheat Starch–Wheat Protein Mixtures

Gelatinized wheat-starch solutions and protein extracts were combined in varying proportions. Viscosity was determined by the MacMichael viscometer, using a No. 30 wire.

RESULTS

The effect on absorbance at 550 nm. of removal of amylopectin from wheat-starch solution is seen in Table I. The contribution to absorbance by amylopectin at this wave length is measurable. This effect is also seen in the data of Williams et al. (4), who determined that a correction was necessary for measurement of amylose at 650 nm. in starch solutions of varying amylopectin contents. An even larger factor could be expected at 550 nm because this is the peak absorbance of amylopectin.

Blends of pure amylose and pure amylopectin form iodine complexes which absorb linearly with changing concentration of amylose at both 650 and 550 nm., as is shown in Fig. 1. The amount of starch (amylose plus amylopectin) does not vary; the diminishing absorbance at both wave lengths with decreasing amylose content is evident. With increasing amylopectin content, the difference of absorbances at 650 and 550 nm. diminish owing to the maximum of absorbance of amylopectin at 550 nm. Thus these blue-iodine values change, not only on the basis of quantity of starch, but also on the basis of amylose:amylopectin ratio changes.

The ability of wheat protein to bind starch (amylose and amylopectin) is evident in Fig. 2. The amount of starch bound relates to the amount of protein (flour extract) introduced. Also, the decreasing difference of absorbances at 650 and 550 nm. indicates that starch remaining in the supernatant liquid, which complexes with iodine, is diminishing in amylose content. In the absence of protein, amylose remains in the supernatant and complexes with iodine, as can be seen by the large differences of absorbance at 650 and 550 nm.

The pH sensitivity of starch–protein interaction is shown in Table II. At acidic pH, starch-iodine complexing is inhibited. This inhibition at acidic pH occurs also in the absence of centrifugation; any starch-protein complex which forms may remain

TABLE I. COMPARISON OF ABSORBANCE (550 nm.) OF WHEAT-STARCH SOLUTIONS OF DIFFERING AMYLOPECTIN CONTENTS DUE TO CENTRIFUGATION

Absorbance (650 nm.)	Absorbance (550 nm.) Wheat starch (complete)	Absorbance (550 nm.) Wheat starch (amylopectin removed)
.6	.52	.37
.5	.43	.31
.4	.34	.25
.3	.26	.19
.2	.17	.13
.1	.09	.06

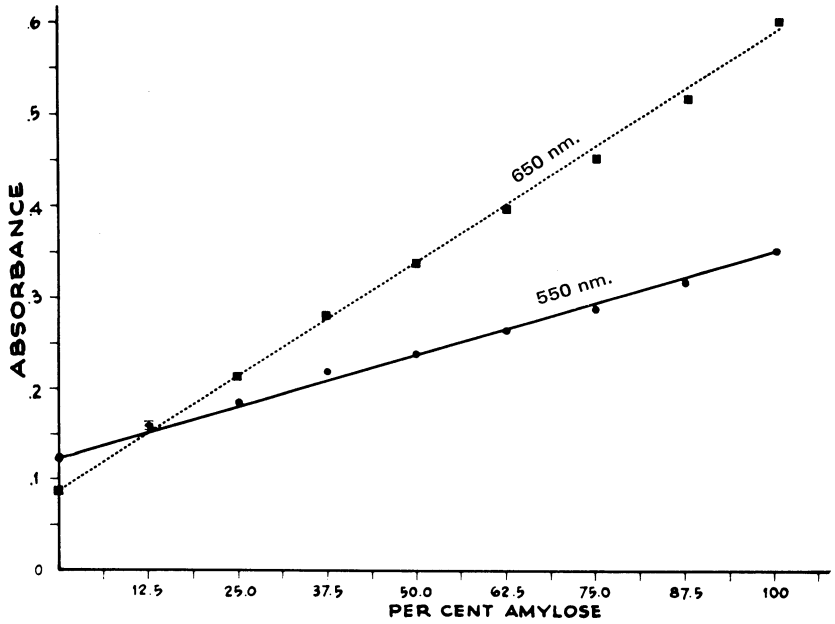

Fig. 1. The effect on absorbance at 650 and 550 nm. of varying amylose:amylopectin ratios of a fixed weight of starch (0.02 mg. per ml.).

TABLE II. EFFECT OF pH ON ABILITY OF WHEAT PROTEIN TO INTERACT WITH STARCH

pH	Absorbance (650 nm.) of supernatant following centrifugation of wheat starch-wheat protein mixture[a]
3.6	.29
4.5	.27
5.2	.24
6.5	.17
7.0	.26
8.3	.48
6.0 (no protein)	.55

[a]3 ml. protein extract added to a system containing 10 g. gelatinized wheat-starch solution (5%) plus water to give added volume of 10 ml.

suspended even after centrifugation as a consequence of the soluble, positively charged protein. At neutral pH, starch-iodine complexing is inhibited. Because protein sediments at this pH, it is likely that a starch-protein complex sediments at neutral pH. The absence of inhibition of starch-iodine complexing due to starch-protein interaction at alkaline pH is evident.

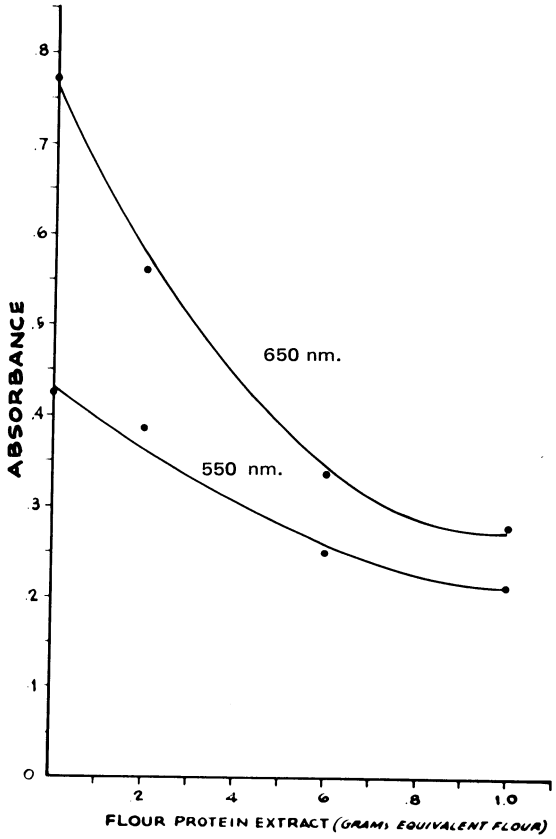

Fig. 2. Interaction of starch and flour protein extract as shown by diminution of measurable iodine-starch complex. Aliquots of 1.5 ml. starch solution were taken for spectrophotometric determination from the system which contained 10 g. gelatinized wheat-starch solution (5%) plus added volume (protein extract and water) of 10 ml. Total starch in system was the equivalent of 0.65 g. flour.

Effect of heat denaturation on ability of wheat protein to inhibit starch-protein interaction is evident in the data shown in Table III. This effect occurred at an acidic pH (4.0) and at a nearly neutral pH (5.5). These pH values represent limits which might naturally occur in various food systems.

When flour-protein extract is combined with a solution of potato amylose (Superlose), complexing occurs immediately, forming a white coacervate which sediments on centrifugation. Combination of flour-protein extract with potato amylopectin (Ramalin G) does not show evidence of complexing; there is no visible formation of a coacervate.

Heat denaturation of wheat protein reduces its effect on the viscosity of a gelatinized wheat solution, as seen in Table IV. Viscosity of the starch-protein system was increased by simultaneously increasing starch and decreasing protein. However, viscosity was increased less in the system containing native wheat protein,

TABLE III. EFFECT OF HEAT ON ABILITY OF WHEAT PROTEIN TO INTERACT WITH STARCH

Protein Aliquot, ml.[a]	pH	Absorbance (650 nm.) of supernatant following centrifugation of wheat starch-wheat protein mixture
0	5.5	.51
3 (heat denatured)	5.5	.52
5 (heat denatured)	5.5	.53
3 (native)	5.5	.15
3 (heat denatured)	4.0	.53
3 (native	4.0	.17

[a]Volume of protein extract added to a system containing 10 g. gelatinized wheat-starch solution (5%) plus water to give added volume of 10 ml.

TABLE IV. THE EFFECT OF HEAT DENATURATION OF WHEAT PROTEIN ON VISCOSITY OF A GELATINIZED WHEAT STARCH–WHEAT PROTEIN SYSTEM

Ratio of Starch and Protein Components[a]		Viscosity, degrees MacMichael	
Starch solution	Protein extract	Unheated protein	Heated protein
5	15	5	5
10	10	7	12
15	5	12	18

[a]Volume ratios of a 2% gelatinized wheat-starch solution and an acetic acid (0.1M) extract of high-protein air-classified flour (1:5).

attributable to its interaction with starch. At least some of this capability of interaction with starch was lost upon heat denaturation of the protein.

DISCUSSION

A recent study of the interaction of starch and protein was reported by Takeuchi (5). The electrostatic attraction between negatively charged potato starch and positively charged alpha-casein was demonstrated by electrolytic conductance measurements. An actual equivalence point was observed at the intersection of two straight lines, which corresponded to the point at which the starch and protein charges would exactly neutralize each other.

This relates to the earlier work of Yoshino and Matsumoto, who conducted titrations of wheat proteins and flour (6). They used standard positive colloid and standard negative colloid in a titration procedure based on the attraction of oppositely charged particles; this procedure was developed by Terayama (7). The results of Yoshino and Matsumoto showed that wheat proteins are positively charged at pH 5 to 6 and behave like positively charged colloids.

The results of experiments reported here substantiate the effects of starch-protein interaction. Relating extracted flour protein to the total protein present in a flour of normal protein content (11.15%), as shown in Fig. 1, it is apparent that the amount of protein in flour is adequate for the complexing of the companion starch that would be present. This does not include that protein which is not extractable, which also can be expected to interact with starch. From the studies of Takeuchi (5) it is concluded that starch-protein interaction is a consequence of the attraction of oppositely charged colloids. At alkaline pH, both starch and wheat protein would bear negative charges; at acidic pH, wheat protein would bear a positive charge. The diminished interaction effects at alkaline pH in the system used in these studies can be explained by the presence of negative charge on both starch and protein.

The sensitivity to pH of rheological behavior of dough is well-documented. Bennett and Ewart made extensigraph measurements indicating decreasing extensibility and increasing resistance to extension with decreasing pH (8). This was confirmed in a study by Hlynka and Chanin (9) and by Tsen (10). In the work of Hlynka and Chanin, bread was baked from doughs of varying pH values (9). Optimal loaf volume occurred at pH 5.7 and diminished at pH 5.3 and pH 4.7. The smallest loaf volume occurred at pH 7.0.

Very early work conducted by Geddes demonstrated the loss of volume of bread baked from flour which had been systematically subjected to heat treatment at varying moisture levels (11,12). Concomitant with this change was a decreasing solubility of flour protein in various extractants. The changes in baking performance and protein solubility of heat-treated flour was attributed to heat denaturation of flour protein. Evidence from experiments reported here show that the effect of heat denaturation on wheat protein would strongly influence bread baking performance of flour.

The results obtained in experiments reported here correlate with the above-cited effects of pH change and heat treatment of flour. It is likely that starch-protein interaction in the dough stage is very important in the early stages of baking, making possible the formation of optimal dough structure prior to complete denaturation of protein during baking.

Further work is being conducted to more fully elucidate the mechanism of starch-protein interaction. It is hoped that the knowledge will relate to an improved understanding of flour functionality.

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