WHEAT PROTEIN-STARCH INTERACTION. II. COMPARATIVE ABILITIES OF WHEAT AND SOY PROTEINS TO BIND STARCH[1]

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ABSTRACT

Differences of ability of wheat and soy protein isolates to bind starch are shown by tests on starch-protein mixtures. Also shown are differences of ability to bind dextrins. The ability of wheat protein to bind starch is greatly diminished by the action of a disulfide-splitting agent but not by a sulfhydryl blocking agent. Differences of binding action at various pH values are shown by a sedimentation test on starch-protein mixtures. Rheological behavior of protein-starch dough systems is demonstrated over a pH range by a titration procedure, using the farinograph.

A previous study of wheat protein-starch interaction, reported from this laboratory, showed the ability of wheat protein to bind to gelatinized wheat starch (1). This capability was impaired by heat denaturation of the protein and little binding occurred between gelatinized wheat starch and wheat protein at alkaline pH.

There was interest in testing the ability of wheat and soy protein isolates to bind various starches, including ungelatinized starches. The differences between wheat and nonwheat proteins, in this context, could help toward an understanding of the differences of functionality of wheat flour and nonwheat flours. Related to this is the usefulness of tests which might help in the research and development of high-protein food products, many of which are based upon composite flour blends. An example is the use of soy flour to increase protein content of bread without a depression of loaf volume.

The experimental data reported here include various starch-protein binding tests, involving gelatinized and ungelatinized starches and different forms of soy isolate. These tests include a sedimentation test similar to the procedures used to assess wheat flour quality (2). Also obtained were rheological measurements of protein-starch doughs at varying pH values, using the farinograph. These procedures have been found helpful in this laboratory for both research and control purposes. The primary objective, however, has been to collect data to shed further light on some of the unique properties of wheat proteins as compared to the properties of soy proteins.

MATERIALS AND METHODS

Wheat gluten used in these experiments was from a commercial source (General Mills) and contained 75% protein. The soy isolates were commercial products obtained from Ralston Purina and contained 95% protein. They are Edi-Pro A (isoelectric), Edi-Pro N (neutral), Supro 610 (modified), and Supro 7 (modified, soluble). Dextrins used were Maltrin 10 (D.E. 10) and Maltrin 050 (D.E. 5), obtained from Grain Processing Corporation. Commercial wheat and corn starches were employed.

[1]Presented in part at the 58th Annual Meeting, St. Louis, Nov. 1973. Mention of proprietary products does not imply their approval to the exclusion of other products.

Binding Tests

To test binding action of protein, a dispersion of protein was prepared by combining it with 0.1N acetic acid solution (1:5), stirring at 5-min. intervals for a period of 30 min., centrifuging, and then carefully neutralizing recovered supernatant with a minimum volume of concentrated NaOH solution. Ten milliliters of this protein dispersion was combined with 50 ml. gelatinized wheat starch solution, in one experiment, and centrifuged. A blue value of the supernatant was measured at 550 nm. on a Beckman spectrophotometer (3). Comparison with a control (water in place of protein dispersion) gave the percentage of supernatant starch bound by the protein.

The same procedure was followed when high amylose starch was used. The 2% dispersion of starch was boiled for 15 min.; high amylose starch does not fully gelatinize under these conditions but does release starch solubles.

Table I

Comparative Binding Action of Gluten and Soy Isolates to Gelantinized Wheat Starch Solubles (Starch Fraction Remaining after Centrifugation)

Protein	Per Cent Supernatant Starch Bound*
Gluten	46%
Soy isolate (isoelectric)	None
Soy isolate (neutral)	None
Soy isolate (modified)	None
Soy isolate (modified, soluble)	None

* (Supernatant starch measured following centrifugation of gelatinized starch-protein system)

Table II

Comparative Binding Action of Gluten and Soy Isolates to High Amylose Corn Starch Solubles (Starch Fraction Remaining after Centrifugation of Heated Dispersion of Corn Starch Containing 50% Amylose)

Protein	Per Cent Supernatant Starch Bound*
Gluten	69%
Soy isolate (isoelectric)	None
Soy isolate (neutral)	None
Soy isolate (modified)	None
Soy isolate (modified, soluble)	None

* (Supernatant starch measured following centrifugation of starch-protein system)

To test binding action of soluble protein to ungelatinized starch, 10 ml. aqueous dispersion of protein (1:10) was combined with 1 g. ungelatinized starch and centrifuged. The supernatant was measured for protein, using the biuret procedure (4). Comparison with the control (no starch) gave the percentage of protein bound by the starch. To obtain the dispersion of soluble protein, water and protein were combined (1:10), stirred periodically for 30 min., and then centrifuged. The supernatant was then used. Flour was extracted in this manner to obtain soluble wheat protein; neutralized acetic extract of flour was obtained by extracting with 0.1N acetic acid, centrifuging, neutralizing the recovered supernatant and centrifuging again, followed by recovery of the supernatant. A final dilution of 1:10 with respect to flour was obtained. In one experiment N-ethylmaleimide (NEMI) and dithiothreitol were added to the final dilution at a level of 4 and 5 μmoles per ml., respectively.

The procedure for testing binding of dextrins to protein consisted of mixing insoluble protein and dextrin solution, filtering, and measuring blue value at 550 nm. Comparison with a control (no protein) gave the amount of dextrin bound. In each case, 2 g. protein was combined with 50 ml. of a 1% solution of Maltrin 10, or 50 ml. of a 1% solution of Maltrin 050.

Sedimentation Volumes of Starch-Protein Dispersions

Ten grams of wheat starch plus 1 g. of soy isolate were weighed into an Erlenmeyer flask. One hundred milliliters of aqueous media was added and the contents stirred to obtain a uniform dispersion. The slurry was then transferred into a 100-ml. graduated cylinder and the time noted. The level of sediment was observed after 30 min. and recorded. The degree of clarity of the liquid above the sediment was also noted. At the conclusion of testing, the slurries were again stirred and pH measurement taken. Aqueous media included water, 0.1N HAc, and 0.05M borax.

Titrations

Titration of doughs in the farinograph consisted of adding 25 ml. of distilled water, 1N HCl or 1N NaOH over a period of 25 to 27 min. to a pre-formed doughlike mixture which had 5 min. mixing time for hydration and uniformity. These mixtures contained 200 g. of wheat starch, 20 g. of wheat gluten or soy isolate, and 105 to 135 ml. of distilled water. Gluten required 110 ml. of water, isoelectric soy protein (Edi-Pro A), 120 ml., neutral soy protein (Edi-Pro N), 105 ml., modified soy protein (Supro 7), 115 ml., and soluble soy protein (Supro 610), 135 ml. The water requirement was based upon visual performance in the bowl and not on objective reference value in Brabender units.

RESULTS

Results of binding between gelatinized starch and gluten and the four soy isolates are shown in Table I. Significantly, the binding action shown by gluten was absent in the soy isolates. Under the conditions of this test, no binding action was shown by gluten or soy isolate when the system was made acidic or alkaline.

For comparison, a suspension of high-amylose corn starch was prepared under the same conditions used to gelatinize wheat starch. The high-amylose starch (50% amylose) will not gelatinize under these conditions. However, some

starch will leach from the granules. A repeat of the procedure on gluten and soy isolates gave the same result as seen in Table II.

Results of binding between soluble proteins and ungelatinized starch are shown in Table III. Wheat starch granules did bind some of the protein of jack bean meal and none of the water solubles of flour. It did bind the soluble soy isolate protein and it did bind dilute acetic extract of flour that had been adjusted to neutrality.

The effect of NEMI and dithiothreitol on starch-binding action is shown in Table IV. The blocking agent, NEMI, exerted no effect; the sulfhydryl agent, dithiothreitol, almost completely destroyed the binding action.

Table III

Comparative Binding Action of Soluble Protein Extracts to Ungelatinized Starches

Protein	Per Cent Protein Bound*			
	Wheat Starch	Regular Corn Starch	Waxy Maize Corn Starch	High Amylose Corn Starch
Jack Bean Meal Solubles	8%	9.5%	6.5%	10.8%
Wheat Flour Water Solubles	0	0	0	0
Soy Isolate (modified, soluble)	23%	36%	8.4%	6.0%
Dilute Acetic Extract (neutralized)**	28.5%	30%	30.5%	32%

*(Supernatant protein measured following centrifugation of protein-starch system)
**(Represents protein remaining in supernatant following neutralization and centrifugation)

Table IV

Effect of N-Ethylmaleimide and Dithiothreolol on Binding Action of Dilute Acetic Extract (Neutralized) of Wheat Flour Water Solubles

Agent	Per Cent Protein Bound*			
	Wheat Starch	Regular Corn Starch	Waxy Maize Corn Starch	High Amylose Corn Starch
None, control	28.5%	30.0%	30.5%	32%
N-Ethylmaleimide	20.5%	25.0%	22.0%	26.5%
Dithiothreitol	0	8.0%	0	4.5%

*(Supernatant protein measured following centrifugation of protein-starch system)

Table V

Comparative Binding Action of Gluten and Soy Isolates (unmodified) to Dextrins

Protein	Per Cent Dextrin Bound*	
	Dextrin (D.E. 10)	Dextrin (D.E. 5)
Gluten	97.0%	80.5%
Soy Isolate (isoelectric)	37.6%	64.7%
Soy Isolate (neutral)	1.8%	73.0%

*(Dextrin measured following filtration of protein-dextrin system)

Table VI

Sedimentation Volumes of Wheat Starch-Protein Mixtures in Neutral, Acidic, and Basic Media

Protein	Volume, ml*		
	Water	Acetic Acid (0.1N)	Borax (0.05M)
Gluten	30 (pH 5.35)	30 (pH 3.45)	100 (pH 9.1)
Soy Isolate (isoelectric)	35 (pH 4.7)	20 (pH 3.5)	100 (pH 9.0)
Soy Isolate (neutral)	100 (pH 6.1)	21 (pH 3.7)	100 (pH 9.0)
Soy Isolate (modified)	100 (pH 6.5)	24 (pH 3.7)	100 (pH 9.1)
Soy Isolate (modified, soluble)	100 (pH 6.7)	25 (pH 3.75)	100 (pH 9.1)

*(After 30 minutes standing)

Filtration of a solution of dextrin, when combined with a suspension of the protein isolate, results in less dextrin in the filtrate, if binding occurs. As seen in Table V, the gluten had an affinity for the dextrins; the soy isolates had much less, particularly at the higher D.E. More of the lower D. E. dextrin was bound to both wheat and soy protein.

Experiments were conducted on sedimentation rates of starch granules in aqueous systems containing wheat and soy proteins. Conditions employed were similar to those described in the official AACC Methods for sedimentation tests on flour. The data are shown in Table VI.

As seen, only gluten and isoelectric soy isolate systems sedimented in water. The pH of the latter system was actually acidic (pH 4.7) because it assumed the

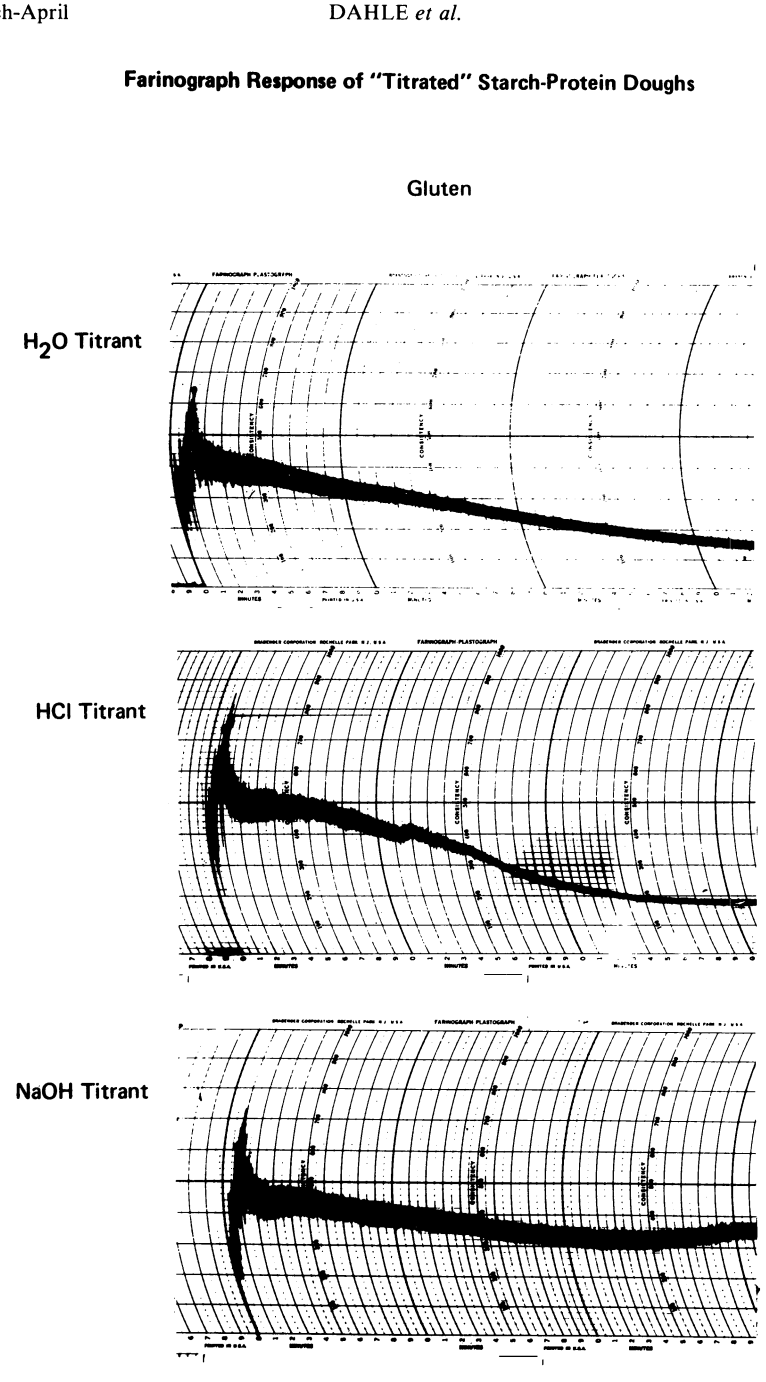
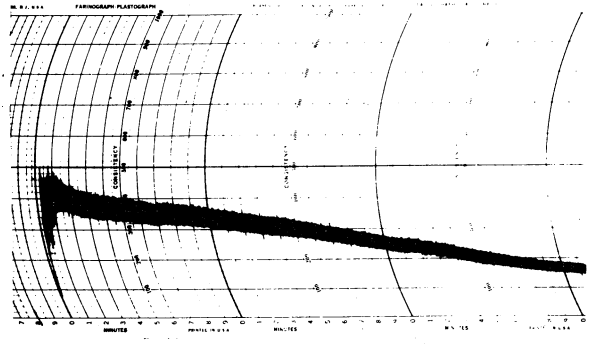


Fig. 1. The farinograph response of "titrated" starch-protein doughs. Titrant was added as mixing proceeded at the rate of 1 ml. per min. Concentration of HCl and NaOH was 1.0N.

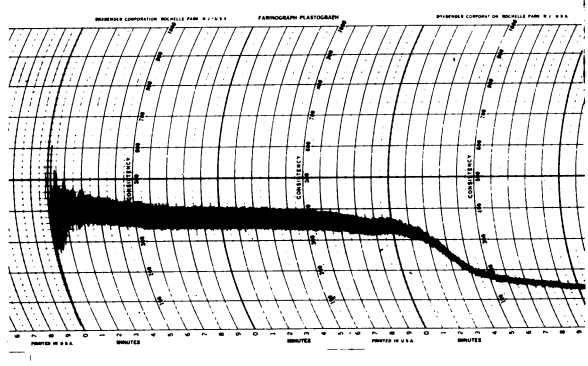
Farinograph Response of "Titrated" Starch-Protein Doughs

Soy Isolate
(isoelectric)

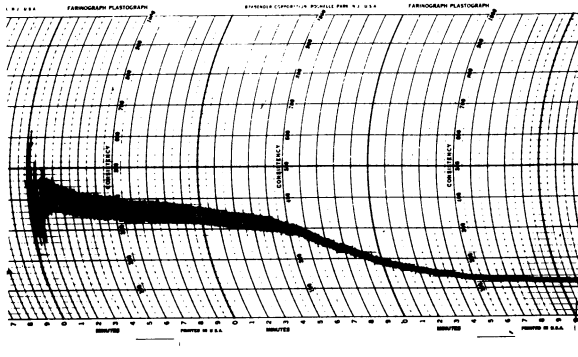
H_2O Titrant



HCl Titrant



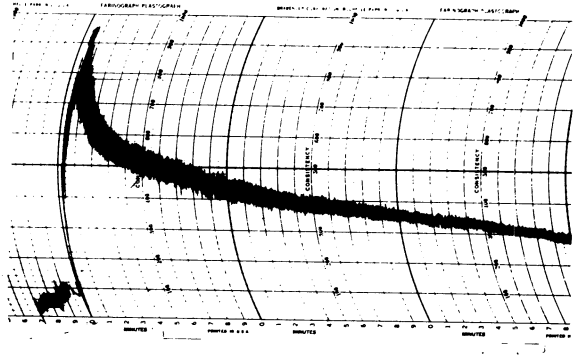
NaOH Titrant



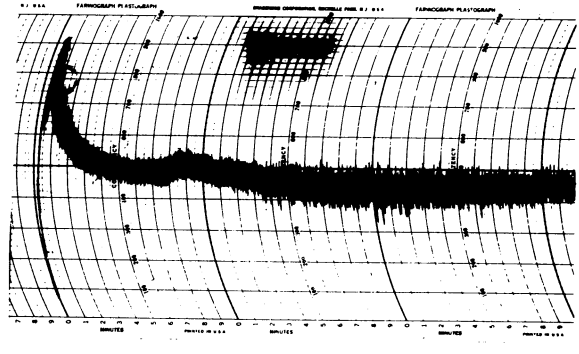
Farinograph Response of "Titrated" Starch-Protein Doughs

Soy Isolate (neutral)

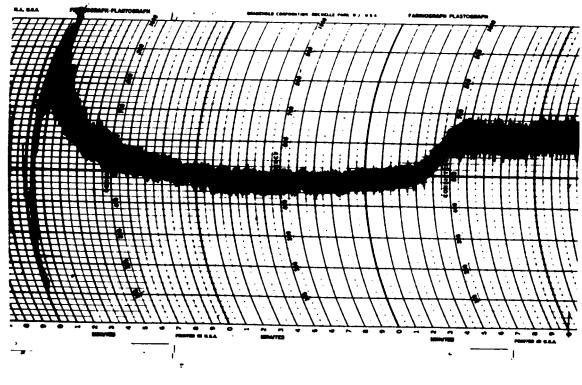
H_2O Titrant



HCl Titrant



NaOH Titrant



pH of the soy isolate. At acidic pH, all of the systems sedimented; at alkaline pH, none of the systems sedimented. Starch, in the absence of protein, sedimented in all three media.

As seen in Figs. 1 and 2, various farinograms were obtained. The result was curves that were characteristic of the particular protein isolate which showed behavior under acidic, neutral, and basic conditions. The wheat gluten-wheat starch system (Fig. 1) showed somewhat similar curves under neutral and acid conditions. There was evidence of dough breakdown observed in the mixing bowl toward the end of the "titration." By contrast, the curve under alkaline conditions showed a fairly even mixing curve.

When compared with unmodified soy isolates (Fig. 1) we find much greater mixing stability in the gluten system under alkaline conditions. Under acidic conditions, gluten had less stability than the isoelectric soy isolate. The isoelectric and neutral soy isolates had characteristically different curves under acidic and alkaline conditions.

Two chemically modified commercial soy isolates were tested in this system, as shown in Fig. 2. The one which is completely soluble offers practically no viscoelastic dough properties and very little mixing stability under neutral conditions. The other modified commercial soy isolate did not have the weakness of the curves shown by the soluble modified isolate under neutral conditions. However, it showed a characteristic difference under alkaline conditions, noted by an ascending curve of considerable amplitude.

DISCUSSION

The effects observed in the experiments on gelatinized starch confirmed earlier results (1) on experiments where dilute acetic acid extracts of flour were neutralized and combined with gelatinized wheat starch, resulting in decreased amounts of measurable supernatant starch following centrifugation of the system. The results of experiments reported here showed this type of binding action to be much less in soy. Under conditions of above, soy was unable to bind significant amounts of soluble starch, either from regular or high-amylose starches.

Dilute acetic extract of wheat gluten and soluble soy isolate was bound to ungelatinized starches, in varying amounts. Soluble wheat protein did not bind starch; this is expected from the conditions of its separation from flour. The binding to starch of jack bean protein is expected from the ability of one of its proteins, concanavalin A, to bind carbohydrate (5).

The unmodified soy isolates bound considerable amounts of the dextrin of D. E. 5, but only little of the dextrin of D. E. 10. Gluten bound considerable amounts of both dextrins.

The sulfhydryl groups and the disulfide links of wheat flour proteins have an important role in rheological behavior of wheat flour (6,7). The effect of *N*-ethylmaleimide (NEMI), sulfhydryl blocking agent, is well documented (8). The farinograph curve is severely weakened upon the addition of 1 μmole per g. flour or less. Similarly, the disulfide cleaving action of a thiol accompanies a severe weakening of the farinograph curve (9). It is interesting to observe that NEMI did not disrupt starch-binding action of wheat protein, whereas the thiol, dithiothreitol, did destroy binding action. Under conditions of the test it is likely

Farinograph Response of "Titrated" Starch-Protein Doughs

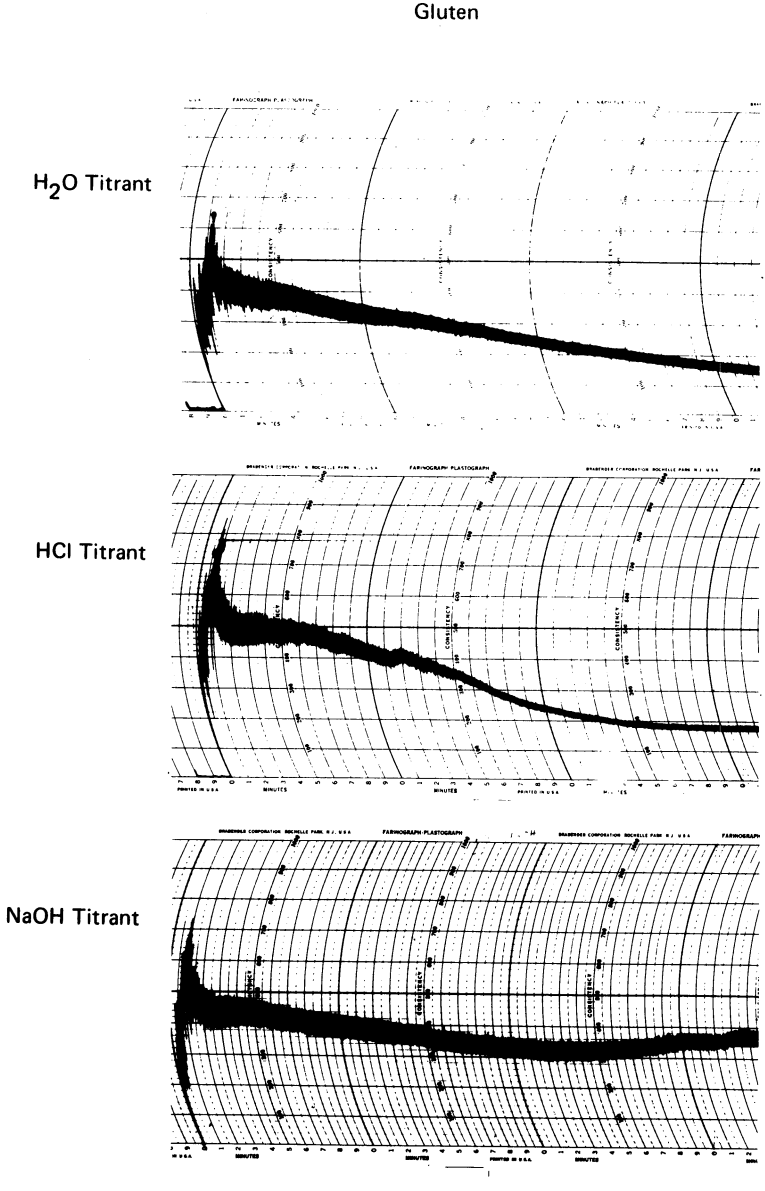
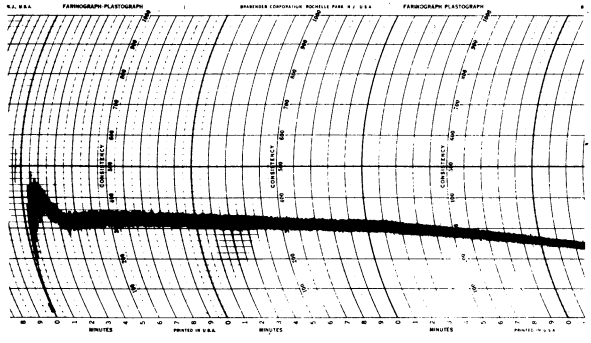


Fig. 2. The farinograph response of "titrated" starch-protein doughs. Titrant was added as mixing proceeded at the rate of 1 ml. per min. Concentration of HCl and NaOH was 1.0N.

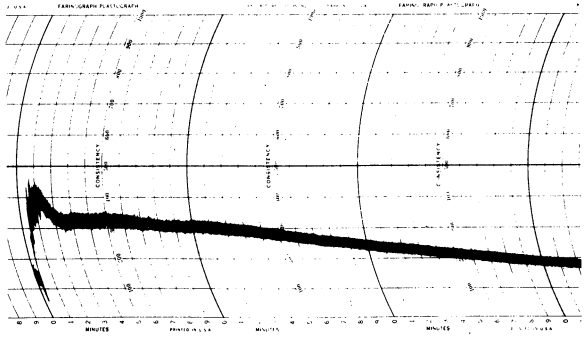
Farinograph Response of "Titrated" Starch-Protein Doughs

Soy Isolate
(modified)

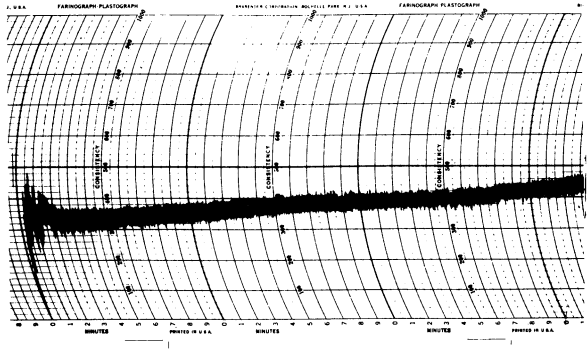
H_2O Titrant



HCl Titrant



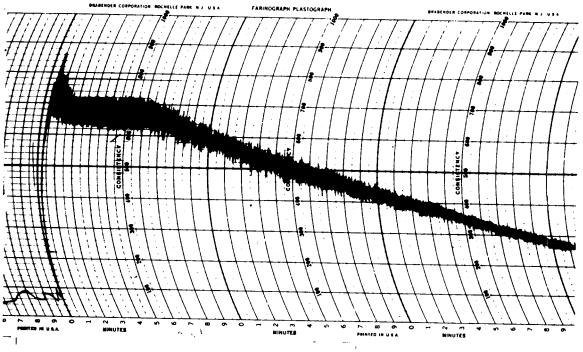
NaOH Titrant



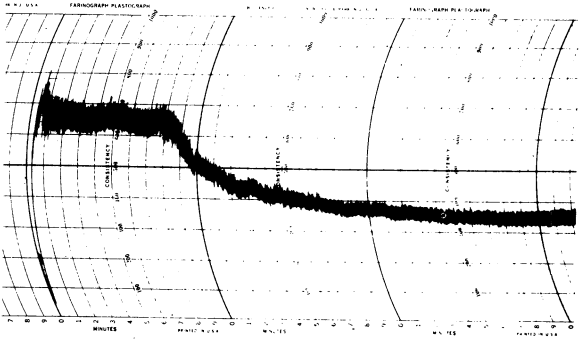
Farinograph Response of "Titrated" Starch-Protein Doughs

Soy Isolate
(modified, soluble)

H_2O Titrant



HCl Titrant



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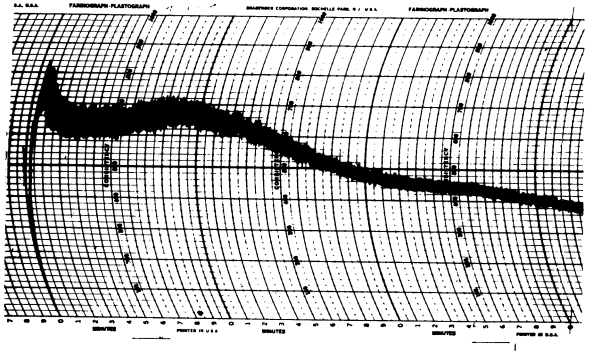

that the integrity of the protein structure was more severely affected by disulfide cleavage than by sulfhydryl blocking.

The conditions employed in the sedimentation experiment indicated the sensitivity of protein-starch interaction to pH. The results suggested that when protein is positively charged (acidic pH) it would bind to starch and the resulting mass would sediment; when protein is negatively charged (alkaline pH) the binding capability is lost and there is no sedimentation. Gluten appeared to have a capacity for binding at pH near neutrality that soy protein did not possess. This could be explained by the negative charge on soy protein at neutral pH. A study by Yoshino and Matsumoto (10) showed that wheat proteins are positively charged at pH 5 to 6. Takeuchi made a study of the interaction between protein and starch which showed it to be an attraction between positively charged protein colloid and negatively charged starch colloid (11).

It is evident, upon examination of the farinograph responses, that rheological behavior of protein-starch systems varies with the nature of the protein. To what extent protein-starch binding contributes to the mixing action is not easily ascertained. The effects of pH on protein behavior by itself (such as disulfide cleavage at alkaline pH) would be superimposed upon any changes in protein-starch interaction induced by pH change. Apart from theoretical interpretation, the characteristic curves developed with specific protein isolates can be useful for assessing uniformity of ingredient quality and help predict behavior in product systems.

Murthy and Dahle showed rheological behavior of dough systems formed from starch, gliadin, and glutenin components, which revealed the importance of both gliadin and glutenin to give the rheology similar to a flour dough, and the sensitivity of gliadin to disulfide cleaving and sulfhydryl blocking agents (12). Smith and Mullen reported experiments showing the mixing behavior of wheat flour doughs at different pH conditions (13). The increased mixing stability observed by them at alkaline pH was also observed in the farinogram responses of gluten-starch doughs reported here.

Some work by Sandstedt, reported several years ago, implies some importance to protein-starch interaction in bread systems (14). When nonwheat starches were substituted for wheat starch in gluten-starch systems, some of these were observed to function poorly. Specifically, wheat and potato starches made bread of adequate volume and texture when combined with gluten; rice and corn starches made bread of inadequate volume and texture.

The phenomena involved in flour-based food systems are complex. Yet a more complete understanding of it is necessary and relevant to progress in innovative or newly engineered food systems, many of which involve nonwheat proteins. It is hoped that some of the results reported here will stimulate further interest and research.

Literature Cited

1. DAHLE, L. K. Wheat protein-starch interaction. I. Some starch-binding effects of wheat-flour proteins. Cereal Chem. 48: 706 (1971).
2. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC. Method 56-60, Sedimentation Test for Flour.
3. McCREADY, R. M., and HASSID, W. Z. The separation and quantitative estimation of amylose and amylopectin in potato starch. J. Amer. Chem. Soc. 65: 1154 (1943).

4. JOHNSON, R. M., and CRANEY, C. E. Rapid biuret method for protein content in grains. Cereal Chem. 48: 276 (1971).
5. AGRAWAL, B. B. L., and GOLDSTEIN, I. J. Specific binding of concanavalin A to cross-linked dextrin gels. Biochem. J. 96: 23c (1965).
6. SULLIVAN, B., HOWE, M., and SCHMALZ, F. D. On the presence of glutathione in wheat germ. Cereal Chem. 13: 665 (1936).
7. SULLIVAN, B., DAHLE, L. K., and LARSON, E. The oxidation of wheat flour. I. Measurement of sulfhydryl groups. Cereal Chem. 38: 272 (1961).
8. MECHAM, D. K. Wheat and flour proteins. Baker's Dig. 47: 24 (1973).
9. DAHLE, L. K., and HINZ, R. S. The weakening action of thioctic acid in unyeasted and yeasted doughs. Cereal Chem. 43: 682 (1966).
10. YOSHINO, D., and MATSUMOTO, H. Colloid titration of wheat proteins, dough, and flour. Cereal Chem. 43: 187 (1966).
11. TERAYAMA, H. Method of colloid titration (a new titration between ions). J. Polymer Sci. 8: 243 (1952).
12. MURTHY, P. R., and DAHLE, L. K. Studies on a simplified dough system composed of gliadin, glutenin, and starch. Cereal Chem. 46: 463 (1969).
13. SMITH, D. E., and MULLEN, J. D. Note on the effect of pH and oxygen on the mixing properties and protein solubility of flour doughs. Cereal Chem. 47: 650 (1970).
14. SANDSTEDT, R. M. The function of starch in the baking of bread. Baker's Dig. 36 (June 1961).

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