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EXTRACTABLE PROTEIN AND HYDRATION CHARACTERISTICS OF FLOURS AND DOUGHS IN DILUTE ACID[1]

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ABSTRACT

When extracted repeatedly with 0.01*N* acetic acid, freeze-dried flour-water doughs yielded more protein than the flour from which they were prepared. The additional extractable protein appeared to be derived largely from material which, in suspensions of the flour, settled rapidly and was highly hydrated.

The rate and extent of conversion to extractable protein in doughs mixed in a farinograph differed markedly among four flours. A mixograph converted more protein to an extractable form more rapidly than the farinograph. When sodium chloride (2%, flour basis) was added, both rate and extent of the changes were decreased. In doughs mixed in a nitrogen atmosphere, extractable protein initially increased more rapidly than in air, but in 20 minutes (farinograph) the increases in air were larger. In each case, the nature of the changes suggests that the conversion of protein to an extractable form is related to the characteristics of recording dough mixer curves.

Both 0.1*N* acetic acid and pH 3.1 aluminum lactate buffer (0.017*M* in aluminum) gave results similar to those with 0.1*N* acetic acid (one flour only).

The components of flour are modified in various ways when a dough is mixed. Examples of specific changes are the absorption of oxygen (2), the action of lipoxidase (8), and the loss of sulfhydryl groups (3,16,17). Lipids are bound to proteins (14), and, from rheological studies, a decrease in size of the structural unit in doughs has been suggested (6).

Evidence that such changes affect the characteristic pattern given by a dough in a recording mixer also has been reported. Thus, differences have been observed between farinograph curves from doughs mixed in air and in nitrogen (7,15). The addition of lipoxidase has been reported to change stability to mixing (10,15). Sulfhydryl-blocking reagents shorten mixing time to peak resistance and markedly

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reduce stability (11). Flours from which lipids have been extracted are slow to form a cohesive dough (4,12).

The nature of the changes thus far recognized, as listed above, indicates that protein constituents must be involved. Changes in *protein* properties (as distinct from *dough* properties) then ought to be observable as a result of mixing treatments, but this factor appears to have been given little consideration in protein characterization studies.

This paper reports observations which show that the extractability of flour proteins by dilute acetic acid is altered as a dough is mixed. The hydration of insoluble components, probably protein, also is changed. The extent and rate of changes in extractable protein were found to differ from flour to flour. Recognition of these alterations in protein properties may help to account for variations in the mixing characteristics of flours.

Materials and Methods

The flours are described in Table I. All were unbleached and

TABLE I

SOURCE AND ASH AND PROTEIN CONTENTS OF FLOURS

FLOUR	SOURCE	ASH (DRY BASIS)	PROTEIN (DRY BASIS)
Baart (White Spring)	Washington	0.62	13.0
Nebraska (HRW)	Nebraska	0.49	12.8
Commercial HRS	Montana	0.45	15.6
Lee (HRS)	North Dakota	0.39	17.5

straight-grade. All were milled on laboratory or pilot-plant mills, except the commercial hard red spring wheat flour.

Preparation of Doughs. When doughs were mixed in a farinograph, a 50-g. bowl was used. The standard procedure was followed (1). If salt was added, it was first dissolved in a portion of the water. For the preparation of doughs mixed in a nitrogen atmosphere, flour samples were deaerated in a desiccator by use of a vacuum pump. The vacuum was released with purified nitrogen and the process was repeated. Water was boiled to remove oxygen, and cooled under nitrogen. The farinograph bowl was covered with a tight-fitting lid and nitrogen was passed over the dough during mixing. The gas was first bubbled through water; this was necessary to avoid removal of water from the dough.

When doughs were mixed in the mixograph, 35 g. flour were used, with the absorption indicated by the farinograph mixing curve.

After mixing, doughs were immediately pressed into a thin sheet

on a block of solid carbon dioxide until frozen. They then were freeze-dried, and the dried material was ground in a small Wiley mill through an 80-mesh screen. The ground material was exposed in a thin layer to room air overnight or longer to permit it to reach a moisture content near equilibrium with the atmosphere, then held in screw-capped jars in the cold.

Extraction of Doughs. Acetic acid, 0.01*N* (150 ml.), in a 500-ml. round-bottom flask fitted with a ground glass closure with stopcock, was deaerated by subjection to reduced pressure (water aspirator, 5 minutes). The sample (5.0 g., except for dried doughs containing 2% salt of which 5.1 g. were taken) then was added without agitation so that most of it remained on the surface of the liquid. Most of the air again was pumped out of the flask (oil pump, about 1 minute). (This could not be done, because of uncontrollable foaming, unless the acid solution had been deaerated.) The flask then was shaken vigorously 50 times to suspend the sample; release of the vacuum then destroyed all foam. The suspension was transferred immediately to a 250-ml. glass-stoppered graduated cylinder with 100 ml. additional 0.01*N* acetic acid. The cylinder was stoppered, inverted gently 10 times, and allowed to stand 1 hour. In this time, a definite sediment layer had formed. The supernatant liquid was decanted into a tared 250-ml. centrifuge tube (retaining some supernatant in the cylinder as necessary to avoid loss of sediment), 0.01*N* acetic acid was added to the cylinder to restore the volume of suspension to 250 ml., and the cylinder was again inverted 10 times and allowed to stand 1 hour. This procedure was repeated until the sample had been extracted five times. (When sediment volume after a 1-hour settling period exceeded 50 ml., the volume of added acid was increased to give a suspension volume of 300 ml., in order to provide more thorough extraction.) After the final settling, the *sediment volume* was recorded.

The decanted liquid was centrifuged (30 minutes, relative centrifugal force 800 X gravity). The centrifuged extract was filtered through a loose mat of glass wool into a 1,000-ml. glass-stoppered graduated cylinder (again retaining liquid if necessary to avoid loss of residue). Successive supernatants from a sample were decanted into the same centrifuge tube and the precipitate from the previous centrifuging was resuspended by stirring.

The procedure was slightly different after the final settling. About 150 ml. supernatant were first decanted and centrifuged; then both the sediment and residual liquid were poured into the centrifuge tube, and the transfer was completed by washing the cylinder with about 70 ml. 0.01*N* acetic acid. After centrifuging, the extract was decanted

carefully, and the *residue weight* determined by weighing the tared centrifuge tube and contents. The firmness of the residue gels varied among samples, but in general the separation of residue and extract was satisfactory. (Gelatinous highly hydrated residues give high values.)

The combined filtered extracts (1,000 to 1,200 ml.) were mixed thoroughly and their total volume determined. Portions, usually 250 ml., were taken for Kjeldahl nitrogen determinations to permit the percent of *extractable nitrogen* to be calculated.

Precision and Completeness of Extraction. Although refinements could be made in this procedure, the precision of determinations was adequate to demonstrate changes in doughs with mixing. This may be judged from the data plotted in the figures, in which the individual points represent single determinations; the duplicate determinations were carried out on different days. An unsatisfactory step in the procedure was the formation of the first suspension of a sample. Flour samples were suspended uniformly, but the freeze-dried doughs showed progressively more tendency to form lumps as the dough mixing times increased.

The five portions of acid gave reasonably complete extraction of protein, even in the presence of salt. For example, from 5 g. commercial hard red spring wheat flour plus 100 mg. sodium chloride, four portions of 0.01*N* acetic acid extracted, successively, 65, 9, 2, and 1 mg. nitrogen. Corresponding values with a badly overmixed dough (mixed 10 minutes in a farinograph, Baart flour, no salt) were 70, 9, 4, and 3 mg. nitrogen.

Extracts of flours were nearly clear, but those of doughs were opalescent.

Results

Changes Observed with Mixing. The relationship of values obtained by the three measurements—sediment volume, residue weight, and extractable nitrogen—is shown in Fig. 1 for samples from the Lee flour. Sediment volume initially decreased rapidly with mixing; 5 minutes in the farinograph lowered the value to about one-fourth that of the flour. Prolonged additional mixing then changed the volume relatively little. Residue weight, in contrast, declined gradually throughout 20 minutes of mixing. The extractable nitrogen increased with mixing, the increase becoming less rapid beyond 10 minutes.

Mixing thus modified some of the unextractable flour protein to render it extractable from doughs, and this was accompanied by large decreases in the volume and weight of the hydrated insoluble fractions. The rapid decrease in sediment volume and the gradual

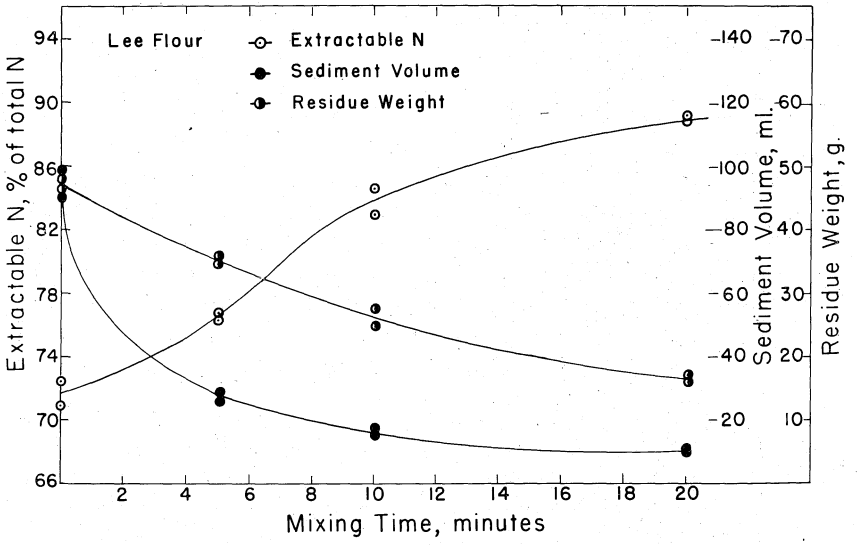

Fig. 1. Nitrogen extracted by 0.01*N* acetic acid, sediment volume, and residue weight changes in Lee flour doughs mixed in a farinograph.

change in residue weight suggested that most of the sediment-volume solids were quickly subdivided or otherwise changed so that they did not settle in 1 hour. They were still sedimented in the centrifuge, however, and so were included in the residue weight. The residue-weight solids appeared to be more slowly modified.

For comparison with the sediment-volume and residue-weight values, the distribution of protein was determined in another set of extractions. In these, the extractable nitrogen and the nitrogen in the sediment-volume fraction were determined, and the remainder calculated by difference. The results are given in Table II. Insofar

TABLE II

DISTRIBUTION OF NITROGEN IN FRACTIONS OF LEE FLOUR AND DOUGHS
(Expressed as percent of total sample nitrogen)

	SEDIMENT	PRECIPITATED BY CENTRIFUGING	EXTRACT
	%	%	%
Flour	23	6	71
Farinograph doughs			
5 minutes	8	16	76
10 minutes	7	9	84
20 minutes	6	5	89

as the *protein* components of the sediment-volume fraction are concerned, it is clear that mixing of a dough quickly modifies the major part so that they are precipitated only by centrifuging; with continued mixing, protein in the residue-weight fraction progressively becomes extractable.

Comparisons of Flours. The extraction procedure was applied to three additional flours and doughs prepared from them in the farinograph. Because of the rapidity of the changes observed with the Lee doughs, doughs mixed for only 2 as well as 5 minutes were included. The additional flours were chosen to provide marked differences in mixing characteristics; this is shown by the farinograph curves in Fig. 2. The Baart doughs developed very rapidly and had little

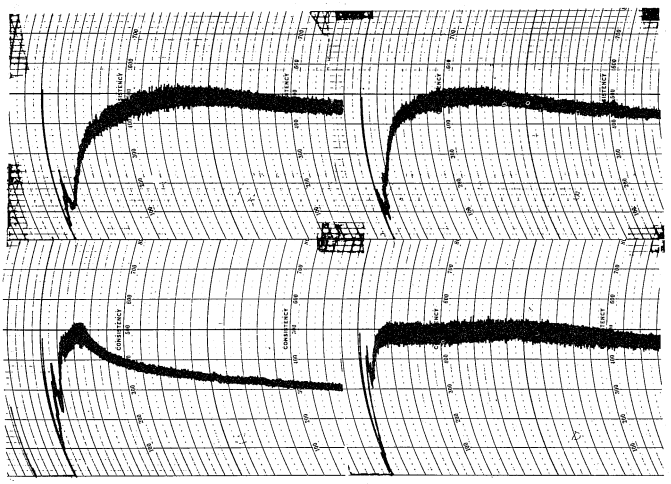

Fig. 2. Farinograph mixing curves for the four flours, A (upper left) Lee flour, B, (upper right) commercial hard red spring wheat flour, C, (lower left) Baart flour, D, (lower right) Nebraska flour.

stability; the Lee and commercial hard red spring doughs gave typical, although not unusually strong, curves; and the Nebraska hard red winter doughs showed marked stability to mixing.

The sediment volumes of the three additional flours showed the same trends with mixing as the Lee flour—a rapid initial decrease, with little change after the value fell below 20 ml. The decrease was less marked with the Baart doughs because the flour itself gave the low value of 40 ml., as compared to 80 for the Nebraska flour and 125 for the commercial spring wheat flour. The sediment volume thus differed markedly among the *flours,* but not among *doughs* from the different flours, particularly after 5 minutes or more of mixing

in the farinograph[3].

Residue weights for the three additional flours and their doughs are given in Table III. The changes with the commercial HRS samples

TABLE III

RESIDUE WEIGHTS FOR FLOURS AND FOR DOUGHS: COMPARISON OF FARINOGRAPH AND MIXOGRAPH DOUGHS
(Results are the average of duplicate determinations)

	LEE	COMMERCIAL HRS	NEBRASKA	BAART
	g	*g*	*g*	*g*
Flour	48	45	35	19
Farinograph doughs				
2 minutes	....	34	30	20
5 minutes	35	30	29	15
10 minutes	26	21	23	15
20 minutes	17	14	19	13
Mixograph doughs[a]				
Underdeveloped	36(1.5)	30(1)	29(2)	19(0.5)
Near curve peak	11(3)	13(3)	15(4)	10(1.25)
Overmixed	13(11)	13(11)	15(12)	8(8.25)

[a]Numbers in parentheses indicate mixing times in minutes.

are similar to those with the Lee samples; in both sets, the changes are more extensive and continue longer than in the Baart and Nebraska samples[4].

Of the three measurements made, however, changes in extractable nitrogen showed differences among the flours most clearly. The results are given in Fig. 3. Extractable nitrogen values for the commercial spring wheat flour doughs were quite similar to those for the Lee flour doughs (Fig.1); the 68% of flour nitrogen extractable increased to 89% in the 20-minute dough. The Nebraska flour contained about the same percent of extractable nitrogen as the commercial spring wheat flour, but this increased only to 80% in the 20-minute dough. There also was a marked delay, through the 2-minute dough, before an increase occurred. The Baart flour contained a larger percentage of extractable nitrogen (78%) than the other flours, but gave the smallest increase on mixing (to 84%). The 2-minute dough contained slightly less extractable nitrogen than the flour.

Effect of Type of Mixer. The mixing action of the farinograph is relatively gentle. To determine whether doughs mixed more vigorously

[3]A relationship between the sediment volume results and Zeleny sedimentation test values could be expected from the somewhat similar nature of the two procedures. The Zeleny test (1) applied to the four flours gave values as follows: Lee, 68; commercial spring wheat flour, 55; Nebraska, 41; and Baart, 24. The sediment-volume values reversed the order of the spring wheat flours, but the position of the Nebraska and Baart samples relative to the spring wheat flours is about the same by the two procedures. The Zeleny procedure applied to doughs gave no clear boundary; readings of 2 to 5 for doughs mixed 5 minutes in the farinograph were obtained, however.

[4]A reviewer has suggested that the residue weights indicate a more slowly hydrating protein system in the Lee and commercial HRS flours than in the Baart and Nebraska flours, and that this is consistent with the shapes of the farinograph curves also.

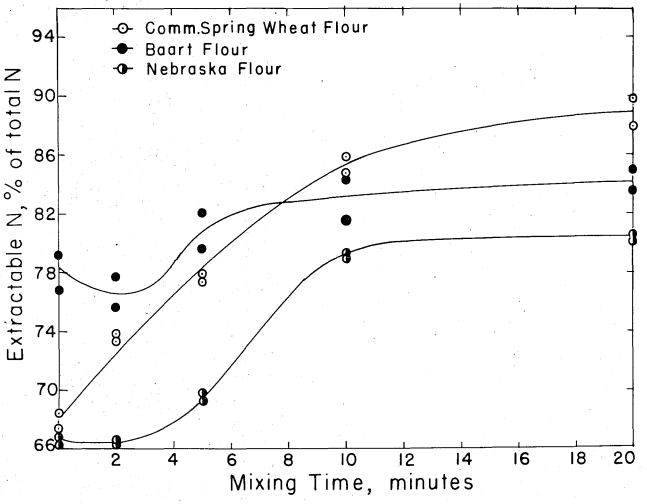

Fig. 3. Effect of mixing time in the farinograph on nitrogen extracted by 0.01*N* acetic acid from commercial spring wheat, Baart and Nebraska flour doughs.

would show changes that were similar to or more extensive than those found in the farinograph doughs, a mixograph was used. Three mixing times were selected for each flour to obtain doughs that were underdeveloped, developed to near maximum resistance, and broken down by overmixing.

As mixing time was increased, the sediment volumes in the mixograph doughs decreased so rapidly that only small differences were present between flours. The residue weights decreased rapidly and followed the sediment volume changes more closely in the mixograph doughs than in the farinograph doughs. (Residue weights are given in Table III.) The most striking difference is that the residue weight was at or near a minimum in mixograph doughs brought to the curve peak, while in the farinograph doughs residue weight decreased throughout 20 minutes of mixing.

The extractable nitrogen data are presented in Fig. 4, and the mixing curves in Fig. 5. Both show more rapid changes in mixograph as compared to farinograph doughs. The extractable nitrogen in the mixograph doughs also reached a higher maximum for each of the four flours. Extractable nitrogen from the extremely overmixed doughs was less than maximum (except for the Baart samples); the significance of the decreases may be questioned, however, because of the strong tendency of the overmixed doughs to form lumps when suspended and the consequent possibility of incomplete extraction.

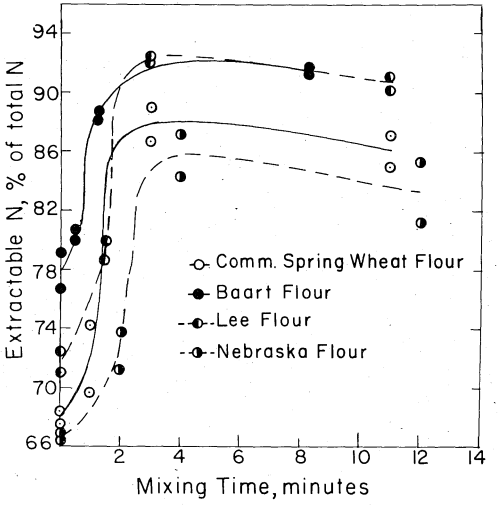

Fig. 4. Effect of mixing time in the mixograph on nitrogen extracted by 0.01*N* acetic acid from doughs of the four flours.

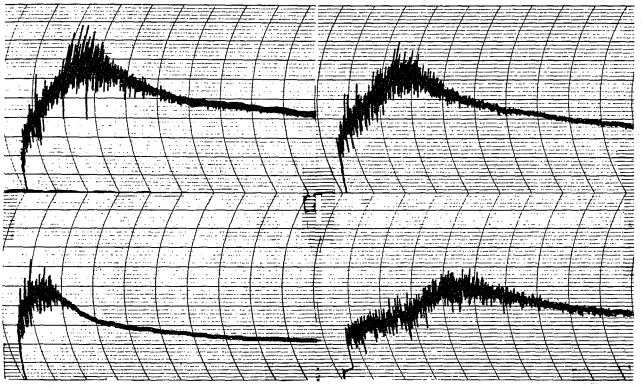

Fig. 5. Mixograph curves for the four flours. Identification as given for Fig. 2.

The rapid changes in hydration and extractability of protein in the mixograph doughs contrasted to the slower changes in the farinograph doughs emphasize that the changes are dependent on the mechanical action of the mixer and are not brought about only by wetting the flour.

Effect of Salt. It is known that the mixing development of doughs is less rapid in the presence of sodium chloride; in commercial practice, their development is sometimes speeded up by withholding salt in the early stages of mixing (5). It was expected then that changes of

the kinds described in preceding sections would occur less rapidly if salt was added to doughs.

Doughs containing 2% sodium chloride (flour basis) were prepared in the farinograph from the commercial spring wheat flour and subjected to the regular extraction procedures. The control extractions, with undoughed flour, were made with 100 mg. salt added to the first portion of 0.01*N* acetic acid in which the flour was suspended. The presence of salt decreased the total extraction of nitrogen only slightly; it delayed the hydration of the sedimented material so that its maximum volume was not reached until the last extraction was made but then led to a slightly higher volume. The results for extractable nitrogen are included in Fig. 6; the increase in extractable nitrogen

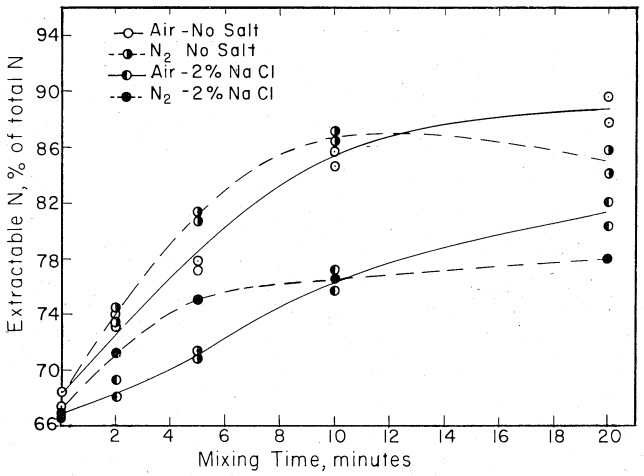

Fig. 6. Effects of 2% sodium chloride and nitrogen atmosphere on changes in extractable nitrogen in doughs. (Commercial spring wheat flour; doughs mixed in the farinograph.)

with mixing was slower in the presence of salt. Sediment volume and residue weight changes also were retarded. The results are consistent with the retarding action of salt on mixing development of doughs.

Doughs Mixed in Nitrogen. The possible involvement of sulfhydryl groups in the changes occurring during mixing and the effects of oxygen on mixing curves of some flours suggested extraction of doughs mixed in a nitrogen atmosphere. Results of determinations with the commercial spring wheat flour are shown in Fig. 6. The initial increase in extractability with mixing was somewhat more rapid in a nitrogen atmosphere than in air, both in the presence and absence of sodium chloride. Beyond 5 minutes, however, the change was slower in the

nitrogen atmosphere, and at 20 minutes the samples mixed in air contained more extractable nitrogen.

The farinograph curves of this flour were not markedly different when doughs were mixed in nitrogen rather than in air. In the absence of salt a small increase in absorption was required (65.8% in nitrogen vs. 65.0% in air), time to peak decreased (5.0 minutes in nitrogen to 7.0 minutes in air), but stability increased (9.5 minutes in nitrogen from 6.5 minutes in air). In the presence of 2% salt, no significant differences were present.

Sulfhydryl losses occur rapidly on mixing in air (17), and these are avoided on mixing in nitrogen. A more extensive occurrence of sulfhydryl-disulfide radical interchanges in the nitrogen atmosphere thus could be expected and might lead to the more rapid increase in extractable nitrogen.

Use of Other Extractants. The choice of 0.01*N* acetic acid had been made originally because, with repeated extraction, it removed a reasonably large proportion of protein and gave large sediment volumes with the spring wheat flours. Comparisons with 0.1*N* acetic acid and with the pH 3.1 aluminum lactate buffer (0.017*M* in aluminum) employed in electrophoretic studies of gluten proteins (9) are given in Table IV. The same trends are shown with the three extractants,

TABLE IV

COMPARISON OF THREE SOLVENTS FOR EXTRACTION OF FLOUR AND DOUGH SAMPLES (Commercial HRS wheat flour; doughs mixed in farinograph in nitrogen atmosphere)

ACETIC ACID, 0.01*N*			ACETIC ACID, 0.1*N*			ALUMINUM LACTATE		
	Dough			Dough			Dough	
Flour	5-minute	20-minute	Flour	5-minute	20-minute	Flour	5-minute	20-minute
Sediment volume, ml.								
125	13	14	64	10	11	22	8	7
Residue weight, g.								
44.5	23.5	19.5	30.5	15.0	11.5	13.0	9.5	8.5
Extracted nitrogen, %								
68.0	81.3	84.4	71.8	87.5	89.6	74.7	85.3	89.8

although at the higher acid concentration and especially in the presence of the buffer salts, the sediment volumes with the flour are much smaller. A larger fraction of total nitrogen is extracted with the 0.1*N* acetic acid and aluminum lactate buffers than with the 0.01*N* acetic acid, but the effect of dough mixing in increasing extractable nitrogen remains as marked as with the 0.01*N* acetic acid.

General Discussion

The observations reported above clearly show that changes in the extractability of flour proteins are brought about by mixing of doughs. The increase in extractable protein is at the expense of material which, when the original flour is suspended in dilute acetic acid, settles rapidly and appears to be highly hydrated. The latter material has not been shown to be entirely protein in nature, however.

The conversion of insoluble protein to an extractable form by dough mixing suggests that stability to mixing may depend upon the supply of convertible material in the flour, which would be indicated by sediment volume and residue weight values. There may be secondary, modifying factors, however, such as the *rate* at which the insoluble proteins can be converted and the dependence of this rate on the intensity of mechanical action. The latter possibility is suggested because the Nebraska flour was more stable, relative to the other flours, in the farinograph than in the mixograph.

In a recent paper, Meredith (13) has described the separation of an insoluble gel fraction from glutens by treatment with 0.01*N* formic acid to extract soluble proteins. The gel protein is present in flour and combines with acid-soluble flour proteins during dough mixing; both gel and acid-soluble proteins are recovered in gluten. He suggests that development and breakdown of a dough with mixing then reflects the formation, and later the breakdown, of structure within the protein combination.

That the gel protein described by Meredith and the insoluble, "convertible" protein referred to in this paper are basically the same seems likely, but the differences in experimental methods are too marked for certainty. Meredith has shown that oxidizing agents and lipids, as well as mixing, affect the proportion of gel protein found in gluten, but did not report differences among flours. In the present work, differences, especially in response to mixing, were readily shown. In this regard, acid-extraction of freeze-dried doughs rather than glutens presumably was advantageous by avoiding further modification and loss of proteins during the gluten-washing step.

In view of the changes which occur in a dough during mixing, both those cited earlier and those described in this paper, the nature of *flour* proteins may not be indicated adequately by characterization studies conducted with *gluten*. The use of flour as a starting material appears to be basically more sound for such purposes.

Acknowledgments

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Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. BAKER, J. C., and MIZE, M. D. Mixing doughs in vacuum and in the presence of various gases. Cereal Chem. **14:** 721-734 (1937).
3. BAKER, J. C., PARKER, H. K., and MIZE, M. D. The action of oxidizing agents on sulfhydryl compounds in dough. Cereal Chem. **21:** 97-107 (1944).
4. BLOKSMA, A. H. Influence of the extraction of lipids from flour on gluten development and breakdown. Chem. and Ind. **1959:** 253-254.
5. DALBY, G. The mixing of bread doughs. Baker's Digest **34** (4): 34,36,38-39 (1960).
6. DEMPSTER, C. J., and HLYNKA, I. Some effects of the mixing process on the physical properties of dough. Cereal Chem. **35:** 483-488 (1958).
7. FREILICH, J., and FREY, C. N. Dough oxidation and mixing studies. VII. The role of oxygen in dough mixing. Cereal Chem. **24:** 436-448 (1947).
8. IRVINE, G. N., and ANDERSON, J. A. Kinetic studies of the lipoxidase system of wheat. Cereal Chem. **30:** 247-255 (1953).
9. JONES, R. W., TAYLOR, N. W., and SENTI, F. R. Electrophoresis and fractionation of wheat gluten. Arch. Biochem. Biophys. **84:** 363-376 (1959).
10. KOCH, R. B. Mechanisms of fat oxidation. Baker's Digest **30**(2): 48-53, 68,71 (1956).
11. MECHAM, D. K. Effects of sulfhydryl-blocking reagents on the mixing characteristics of doughs. Cereal Chem. **36:** 134-145 (1959).
12. MECHAM, D. K. and MOHAMMAD, A. Extraction of lipids from wheat products. Cereal Chem. **32:** 405-415 (1955).
13. MEREDITH, P. A gel fraction of wheat gluten; mixing, oxidation, and lipid relationships. New Zealand J. Sci. **4:** 66-77 (1961).
14. OLCOTT, H. S., and MECHAM, D. K. Characterization of wheat gluten. I. Protein-lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction. Cereal Chem. **24:** 407-414 (1947).
15. SMITH, D. E., and ANDREWS, J. S. The uptake of oxygen by flour dough. Cereal Chem. **34:** 323-336 (1957).
16. SMITH, D. E., VAN BUREN, J. P., and ANDREWS, J. S. Some effects of oxygen and fat upon the physical and chemical properties of flour doughs. Cereal Chem. **34:** 337-349 (1957).
17. SOKOL, H. A., MECHAM, D. K., and PENCE, J. W. Sulfhydryl losses during mixing of doughs: Comparison of flours having various mixing characteristics. Cereal Chem. **37:** 739-748 (1960).