Changes in Flour Proteins during Dough Mixing[1]

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ABSTRACT

Acetic acid (0.05*N*) extracts of various flours and doughs were fractionated by gel filtration on a column of Bio-Gel P-150 into four major UV-absorbing components, I, II, III, and IV, representing primarily fractions of glutenins, gliadins, albumins, and nonproteins, respectively. When doughs were mixed in a farinograph for 3.0, 6.5, 15.5, and 31 min., the distribution of these components was changed; component I increased, but the other components did not change significantly with mixing. Mixing at a higher speed intensified the increase of component I. This change was also observed for wet gluten mixed for various periods. When the changes in the components' distribution were examined in relation to the mixing properties of flours, it was found that component I of weak flours increased faster than that of strong flours. Extracts of soft white winter wheat flours contained proportionally more of component I than those of hard red spring wheat flours.

Mixing properties are important parameters in the assessment of flour quality. Their importance has recently become more significant than ever before with the introduction of the new continuous breadmaking processes in which dough development is achieved primarily by high-speed mixing.

The mixing properties of dough largely depend on the viscoelastic properties of flour proteins. Mecham and co-workers (1,2,3) found that the amount of protein extracted from dough by dilute acetic acid increased with mixing. Rates of increase were different for flours of different mixing characteristics. The explanation for the increase in protein extractability was that dough mixing decreased the size of protein aggregates or particles in flour. Mullen and Smith (4,5) showed that the main difference between a short- and a long-mixing flour was that the former contained more of the beta gluten component and less of acid-insoluble proteins. Of the protein fractions, salt-soluble fractions (albumins and globulins) had little effect on mixing characteristics. Protein-starch residues (glutenins) had long mixing requirements, whereas the addition of water-solubles (gliadins) markedly shortened the mixing requirements. They postulated that alpha-glutenins in these protein-starch residues of the two flours might be different in their molecular sizes. Such differences could be responsible for mixing differences.

On the basis of the limited published information, it seems desirable to study changes in molecular size distribution of flour protein components, particularly glutenins and gliadins, with mixing, and to relate these changes to mixing characteristics of flour. Results of such a study are reported in this paper.

MATERIALS AND METHODS

Flours

The flours used in this study are described in Table I. All flours were unbleached and contained no improvers. Unless otherwise stated, a commercial straight-grade flour was used throughout the study. The other flours,

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TABLE I
DESCRIPTION OF FLOURS USED

FLOUR	ORIGIN	ASH	PROTEIN	STRENGTH CLASSIFICATION
		% (14% m.b.)	% (14% m.b.)	
(HRS) Commercial, No. 3 Manitoba Northern	Western Canada	0.45	13.6	Strong
(HRS) Minnesota II-54-29	Minnesota	0.46	16.6	Very strong
(HRS) Pembina	Manitoba	0.48	13.1	Very strong
(HRS) Prairie Pride	Manitoba	0.43	11.3	Weak
(HRS) Thatcher	Manitoba	0.46	14.6	Strong
(SWW) White winter, 2 C.E.	Ontario	0.33	7.2	Very weak
(SWW) Genesee	Ontario	0.48	11.0	Weak
(SWW) Richmond	Ontario	0.49	12.6	Weak
(SWW) Talbot	Ontario	0.48	11.4	Weak

selected to provide a range of mixing properties, were milled on a laboratory Buhler mill. All wheats were of the 1964-65 crop.

Gluten

Gluten was prepared by mixing 200 g. flour and 100 ml. 0.001*M* sodium chloride in a GRL mixer at 68 r.p.m. and 30°C. for 5 min., followed by washing with hand-kneading in a stream of 0.001*M* sodium chloride. The wet gluten was freeze-dried, ground in a micro Wiley mill (60-mesh), and stored at – 40°C.

Doughs

Doughs (50 g. flour, 14% m.b.) were mixed in a 50-g. farinograph mixer at 63 r.p.m. and 30°C. Absorptions were adjusted to give a dough consistency of 500 B.U. Figure 1 shows farinograms of flour-water doughs from these flours.

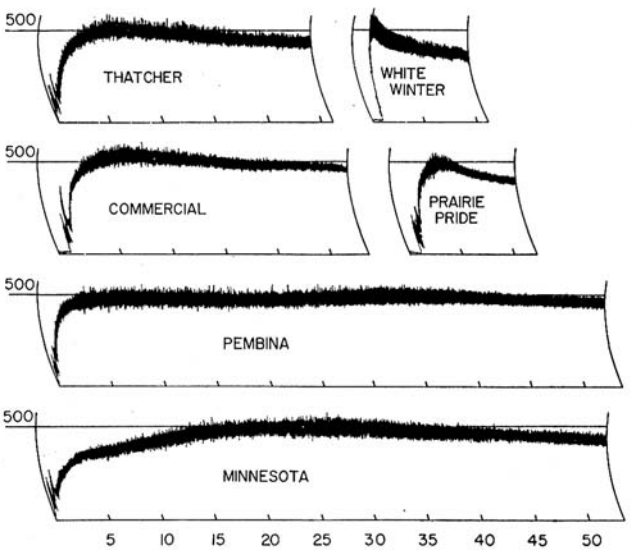


Fig. 1. Farinogram of flour-water doughs. Absorptions for the commercial, Minnesota II-54-29, Pembina, Prairie Pride (which is not a licensed variety in Canada), Thatcher, and two C.E. white winter wheat flours were 63.2, 67.8, 62.0, 64.6, 65.4, and 50.8%, respectively.

Freeze-dried gluten was rehydrated by mixing 50 g. gluten (14% m.b.) in a farinograph to an absorption at 78.26%; the technique used was that described by Bushuk (6).

Forty-gram samples of dough or wet gluten mixed for the required period were immediately frozen in liquid nitrogen, freeze-dried, ground, and stored at – 40°C. prior to analyses.

Extraction

A flask containing 2.5 g. flour or dough, or 0.4 g. dry or wet gluten (all weights on dry-matter basis), and a flask with 0.05*N* acetic acid were cooled in a refrigerator overnight. Cold 0.05*N* acetic acid (30 ml.) was added to the flask and the flask was shaken vigorously on a wrist-action shaker for 15 min. in a cold room (2°–3°C.). After this shaking, the suspension was centrifuged at 30,000 × *g* and 4°C. for 15 min. The supernatant is the acetic acid extract used for gel filtration.

Extracts of flours and glutens were clear, but those of doughs were slightly opalescent. The absorbance at 280 mμ of the diluted extract (1:20 dilution) was measured with a Beckman DU spectrophotometer. Total nitrogen content of the extract was determined by the Kjeldahl method.

Gel Filtration

The usefulness of gel filtration on Sephadex for the separation of wheat proteins has already been demonstrated (7–9). In this study, Bio-Gel P-150, a copolymer of acrylamide and methylene-bis-acrylamide (Control Number 3398, Bio-Rad Laboratories), was the gel filtration medium. A suspension was made by slowly pouring, with stirring, the dry Bio-Gel P-150 beads (50- to 150-mesh) into an excess of 0.002*N* sodium acetate buffer of pH 3.8, or aluminum lactate buffer ($\mu = 0.05$, pH 3.2). The suspension was evacuated and poured into the column (45 × 2.5 cm.). It was then allowed to pack by gravity to a bed height of 39.8 cm. The column was washed with about 500 ml. buffer before use. Throughout the run the column was kept at 30°C. by circulating water at this temperature around the column.

Unless otherwise stated, 3 ml. of the acetic acid extract was applied with a pipet to the top of the gel column. This was followed by 5 ml. of buffer. The column was then filled with buffer and connected to a Beckman Model 746 metering pump. The acetate buffer was pumped through the column at a rate of 10.5 ml./hr. The absorbance of the column effluent was monitored at 280 mμ and recorded by an automatic UV analyzer (Vanguard Model 1056–O.D.).

From time to time, the effluent fractions were pooled and most of the water was removed by evaporation in a digestion flask. Total nitrogen content of the effluent was determined by the Kjeldahl method, in addition to the routine check by measuring the absorbance at 280 mμ of the effluent for calculating recoveries. On the basis of these measurements, recoveries were 95.5% or better. The separation results are highly reproducible, and the positions of the elution peaks could be readily identified by elution volumes.

RESULTS AND DISCUSSION

Changes in the Extractability of Flour Proteins with Mixing

Table II shows that the protein content in the acetic acid extract and the percent of the extractable protein with respect to total protein of various flours and doughs increase with mixing. The rate of increase (during the initial mixing stage) is faster for the known weak flours (Prairie Pride and white winter) than for strong flours (commercial, Minnesota II-54-29, Pembina, and Thatcher). These results are in general agreement with the findings of Mecham and co-workers (1,2,3). Table II also indicates that dilute acetic acid extracts a higher percentage of the original protein from the weak flours than from the strong flours.

TABLE II

EFFECT OF DOUGH MIXING ON PROTEIN EXTRACTABILITY

Flour	Mixing Time	Protein Content of Extract	Extractable Protein	Flour	Mixing Time	Protein Content of Extract	Extractable Protein
	min.	*mg.*	*% total protein*		*min.*	*mg.*	*% total protein*
Commercial	0.0	245.4	62.1	Prairie Pride	0.0	240.6	73.6
	3.0	248.4	62.8		3.5	261.0	79.8
	6.5	278.4	70.4		5.5	271.2	82.9
	15.5	297.0	75.1		11.0	270.0	82.6
	31.0	319.8	80.9	Thatcher	0.0	266.4	63.0
Minnesota	0.0	302.4	62.6		3.5	270.6	64.0
II-54-29	3.5	301.8	62.5		7.5	291.6	68.9
	13.5	335.4	69.4		17.5	324.0	76.6
	27.0	387.6	80.2	White winter	0.0	145.8	69.7
	39.0	400.2	82.8		1.0	148.2	70.8
	70.0	396.6	82.1		3.5	156.6	74.8
Pembina	0.0	228.0	60.0		15.5	166.8	79.7
	3.5	233.4	61.4	Genesee	0.0	214.8	67.2
	10.0	262.2	69.0	Richmond	0.0	252.6	69.0
	35.0	286.2	75.3	Talbot	0.0	235.8	71.2
	55.0	295.2	77.6				

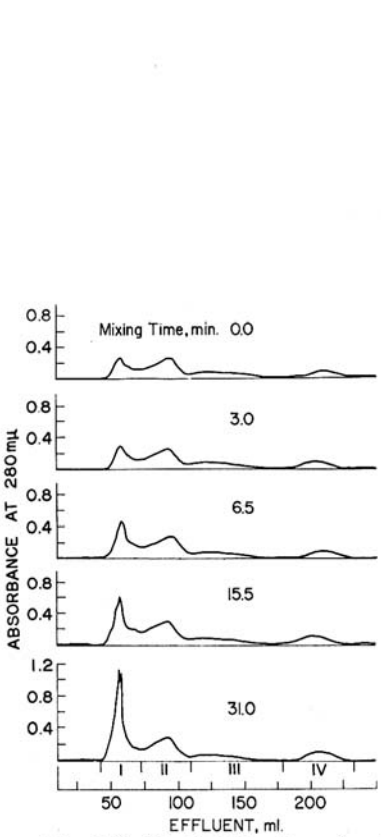
Gel Filtration of Acetic Acid Extracts from Doughs Mixed for Various Periods

Figure 2 shows that four major UV-absorbing components of the acetic acid extract of the commercial flour are separated by filtration on Bio-Gel P-150. The relative size and the positions of the four peaks are, in general, similar to those obtained by filtration on gels of Sephadex 100 or 200 by Wright, Brown, and Bell (8) and by Meredith and Wren (9), although these authors used different extracting and eluting agents. For ease of presentation the four major peaks are designated as components I, II, III, and IV.

To obtain information on the approximate molecular weight (MW) of the individual components, elution volumes under the same experimental conditions were estimated for thyroglobulin, aldolase, bovine serum albumin, cytochrome C, and tyrosine, of which MW's are known. The calibration indicates that component I, which is excluded from the gel, would represent flour proteins of MW over 150,000; this is most likely the glutenin fraction with its MW 1.5–2.0 million (10). Component II with an average MW about

100,000 probably represents the gliadin fraction (11). This designation is also supported by the results obtained from the gel filtration of a gluten sample (see Fig. 5). With gluten, the elution curve shows mainly components I and II (glutenin and gliadin fractions). The soluble components (III and IV) are apparently removed during the gluten-washing process. Component III consists of soluble proteins, mostly albumins, with their average MW of 15,000. Component IV appears to consist of soluble nonprotein fraction with MW under 1,000. The approximate MW's and the classification of these components (I–IV) in the present study, to a large extent, agree with those of Wright, Brown, and Bell (8) and of Meredith and Wren (9). Further studies are necessary for a more definite characterization of these components and more accurate determination of their MW's.

The most significant feature of Fig. 2 from the viewpoint of the present study is that when the doughs are mixed longer, component I increases, whereas the other components in the extract do not change significantly with mixing time.



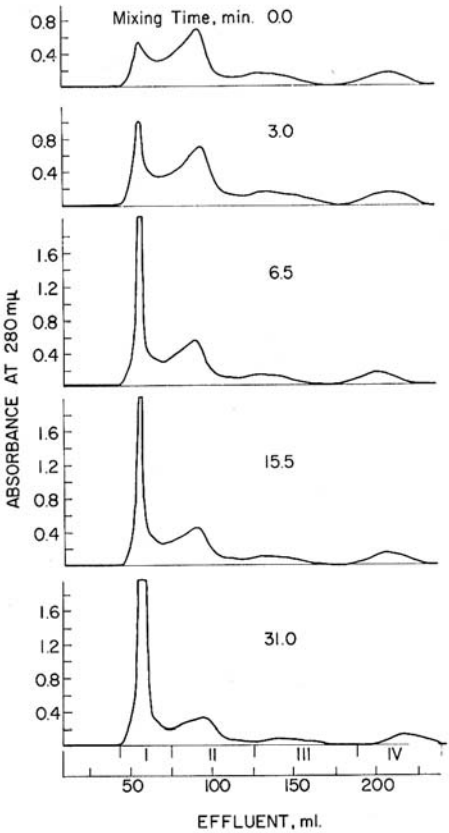


Fig. 2 (left). Gel filtration of acetic acid extracts from flour and flour-water doughs mixed for various periods.

Fig. 3 (right). Elution curves for protein components of flour and flour-water doughs extracted with acetic acid and chromatographed in aluminum lactate.

Analogous results were obtained in another experiment in which the same sample was used and in which the amount of extract was adjusted to contain a constant amount of protein (60 mg.), estimated by its absorbance and nitrogen content, instead of using a constant volume of extract (3 ml.) as for Fig. 2. In addition, aluminum lactate buffer ($\mu = 0.05$, pH 3.2) was used as the eluting agent instead of sodium acetate buffer. Results of this experiment, presented in Fig. 3, again show the marked increase in component I during mixing. Because total protein in the extract of this experiment is kept constant, there is a decrease in the relative size of the peaks for the other components, but this, of course, does not necessarily reflect a disappearance of these components during mixing.

Effect of High-Speed Mixing

Since mixing increases the amount of component I in the extract, it was of interest to determine if mixing at higher speed for a comparable time would have a similar effect. Results given in Fig. 4 show that mixing at a higher speed (94.5 instead of 63 r.p.m.) in the same mixer does indeed increase the amount of component I in the extract. At the same time the amount of protein extracted also increases, as shown in Table III.

TABLE III
EFFECT OF HIGH-SPEED MIXING[a] ON PROTEIN EXTRACTABILITY

FLOUR	PERIOD OF MIXING AT 94.5 R.P.M.	PROTEIN CONTENT OF EXTRACT	EXTRACTABLE PROTEIN
	min.	*mg.*	*% total protein*
Commercial	0.0	245.4	62.1
	3.0	303.0	76.6
	6.5	308.4	78.0
	15.5	317.4	80.3
	31.0	331.2	83.8

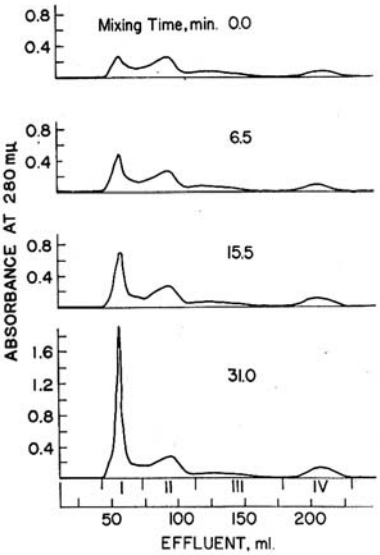
[a] 95 r.p.m.

Effect of Mixing on Wet Gluten

Since component I (the glutenin fraction) was affected most by mixing, a study was made of the effect of mixing of wet gluten instead of dough. In this experiment the volume of the acetic acid extract used for gel filtration was adjusted to contain 38.3 mg. of protein. The results, given in Fig. 5, show that mixing does produce some disaggregation of gluten; component I increases with mixing. Also analogous to the extraction results of flour and doughs presented before, the amounts of protein extracted by acetic acid for the wet glutens mixed for 0.0, 3.0, and 31.0 min. were found to be 36.9, 45.9, and 62.1% total gluten protein. The low extractability is largely due to the fact that freeze-dried and ground gluten samples do not disperse readily in acetic acid with shaking only.

Relation of Protein Components to Mixing Characteristics of Flour

Flours, such as those from white winter and Prairie Pride wheat, which produce doughs that develop and break down rapidly in the farinograph, are generally referred to as weak; whereas flours from wheats such as Pembina



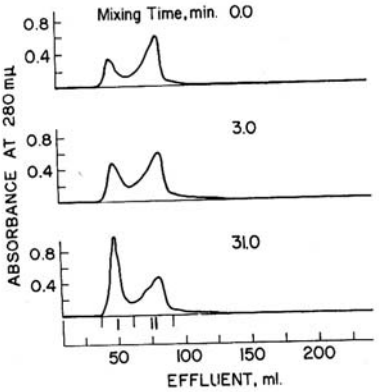
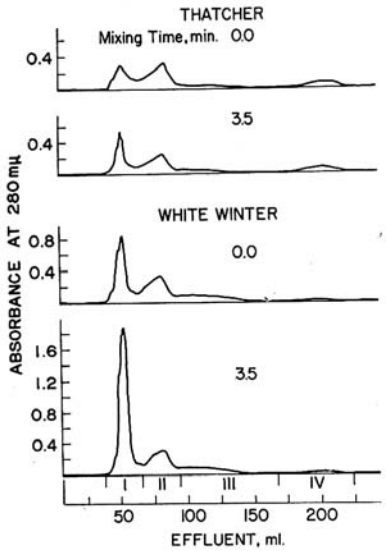


Fig. 4 (left). Gel filtration of acetic acid extracts from flour and flour-water doughs mixed at 94.5 r.p.m. for various periods.

Fig. 5 (right). Gel filtration of the acetic acid extracts from gluten and wet gluten mixed for various periods.



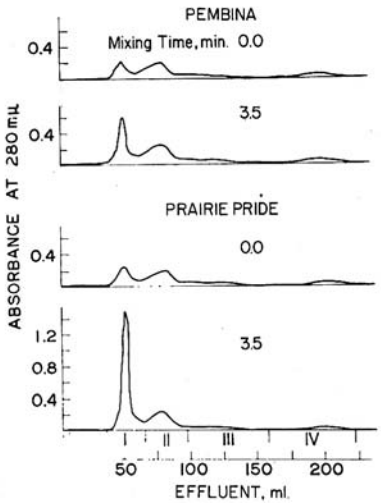


Fig. 6 (left). Elution curves for protein components of Thatcher and white winter wheat flours and their doughs extracted with acetic acid.

Fig. 7 (right). Elution curves for protein components of Pembina and Prairie Pride wheat flours and their doughs extracted with acetic acid.

and Minnesota II 54-29, whose doughs develop more slowly and tolerate longer mixing, are usually called strong (see Table I and Fig. 1). Experiments were therefore made to determine if these differences in mixing properties are reflected by a difference in the distribution of protein molecular sizes. All doughs used in this experiment were mixed for 3.5 min. For the white winter wheat flour, 8 ml. of the acetic acid extracts were used for gel filtration because of their low protein contents. For the other flours, the sample size was 4 ml. Results of this experiment are summarized in Figs. 6 and 7.

Figure 6 shows, first, that the extract from the white winter wheat flour contains a relatively higher proportion of component I than that of Thatcher flour, considering the total amount of protein placed on the column; and second, component I of white winter wheat flour increases much more during 3.5 min. of mixing than that of Thatcher wheat flour.

In view of the distinct difference in the amount of component I in the extracts from the white winter and Thatcher wheat flours, it seemed desirable to examine this component in the extracts of flours from pure varieties of soft winter wheat. The representative varieties selected are Genesee, Talbot, and Richmond. Results for these three varieties (not shown) were all similar to those for the white winter wheat flour shown in Fig. 6. It is therefore concluded that the difference shown in Fig. 6 probably is related to the difference in mixing characteristics between soft and hard wheat flours.

Figure 7 shows the comparative data of Pembina and Prairie Pride flours and their doughs. These flours are taken as examples of very strong and weak varieties of the same class of wheat (Hard Red Spring). There are no marked differences in the distribution of components in the extracts of these two flours. However, during mixing the amount of component I increases much faster in the Prairie Pride flour than in the Pembina flour. Results for Minnesota II 54-29 flour (not given), shown to be a very strong flour by the farinograph (see Fig. 1), provide further evidence that the rate of increase in the amount of component I with mixing time decreases as the strength (dough development time) of the flour increases.

GENERAL DISCUSSION

The presence of large aggregates which can be converted to extractable protein with mixing has been demonstrated by Mecham *et al.* (1, 2, 3). This study provides further information that the increase in the protein extractability during mixing arises mainly from the increase in component I (primarily glutenin fraction). The increase is not at the expense of other soluble components or gliadins, and therefore must result from the disaggregation of large protein aggregates present in the nondispersible fraction during mixing.

The nature of these aggregates is still obscure. Whether they are the protein bodies observed by Morton and his associates (12, 13), whether they are associated with the wedge or adhering proteins observed by Hess (14), or are just simple wheat protein clusters formed during the maturing and desiccation of wheat kernels is not known.

During mixing, these aggregates are hydrated and disaggregated to form more of component I. A number of factors could be responsible for this disaggregation: Mixing, through tearing and shearing, can break or separate the protein aggregates to form continuous protein films and a protein network (gluten matrix), thus making the protein more accessible to the acetic acid extraction. Once a continuous gluten matrix is developed in the dough, the breakdown of the gluten complex may become predominant through the scission of noncovalent bonds such as the hydrogen and hydrophobic bonds, and salt bridges. A third possibility for the mechanism of the formation of component I is the depolymerization of large protein aggregates by reduction of disulfide bonds. This reduction could be readily mediated by certain free sulfhydryl groups of water-soluble or water-insoluble flour proteins, causing the disulfide interchange in dough. All these possibilities, particularly those concerning the depolymerization through reduction and the scission of noncovalent bonds, are at present under study.

Different flours respond to extraction and mixing differently. Acetic acid extracts more glutenin fraction, relative to the other components, from the soft wheat flours than from the hard wheat flours. The difference in extractability or dispersibility with acetic acid indicates that the size of large protein aggregates of the soft wheat flours may be smaller than that of the hard wheat flours, or that the structure of large protein aggregates of the soft wheat flours may be more liable to dissociation (disaggregation) than that of the hard wheat flours. This finding supports the suggestion of Smith and Mullen that alpha glutenins of the short- and long-mixing flours might be different in their molecular size (4, 5). It also agrees with Pomeranz's observations that poor- and good-breadmaking flours differ in their protein dispersibilities in *3M* urea (15). With mixing, the rate of conversion of large protein aggregates to the glutenin fraction occurs more rapidly for weak flours than for strong flours (Figs. 6 and 7). This difference suggests again that the structural stability of large protein aggregates may vary with flours of different mixing properties.

Acknowledgment

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