

Development and "Undevelopment" of Wheat Dough by Mixing: Physicochemical Studies¹

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ABSTRACT

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The changes in flour proteins during dough development and undevelopment by mixing were investigated using three flours selected to represent very strong, strong, and medium dough mixing strengths. As judged by the loaf volume and other loaf properties, the negative effect of undevelopment on bread quality was more pronounced for stronger flours. When undeveloped doughs were redeveloped by mixing at higher speeds, the strongest flour was the only one that increased in loaf volume in relation to that of the optimally developed dough while maintaining overall organoleptic quality of the loaf. Undevelopment produced a decrease in the amount of acetic acid-extractable protein. Subsequent remixing at higher speed reversed this extractability. The amounts of soluble glutenin and insoluble residue protein, obtained by the modified Osborne procedure, were related to the energy (work) input during dough mixing. Glutenin

increased, and residue protein decreased with increasing energy. Undevelopment slowed the change of protein solubility with work input. This effect was more evident for the two stronger flours. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns showed that the unreduced glutenin fraction of underdeveloped doughs (premixing and undermixing stages) contained a number of low molecular weight subunits that entered the gel on electrophoresis. These subunits were not present in the patterns of the analogous fraction from developed doughs. Presumably, these units associate during development to form aggregates extractable with the solvent used but too large to enter the gel. Undevelopment caused an increase in the intensity of some of the bands in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of reduced glutenins.

From studies designed to investigate flour protein solubility during dough mixing, Mechem et al (1962, 1963, 1965) concluded that mixing results in a gradual increase in acetic acid-soluble protein. They also concluded that less protein was extracted from doughs of strong flours than from doughs of weak flours. These findings were later confirmed by Tsen (1967). Tanaka and Bushuk (1973a, 1973b) found that extended mixing in the farinograph, beyond maximum consistency, resulted in the conversion of insoluble glutenin (residue protein) into the soluble type.

The term "undevelopment" in this article describes physicochemical and functional changes that occur when an optimally developed dough, produced by high-speed mixing, is mixed for a further period of time at a slower speed. "Undevelopment" by mixing is synonymous to "unmixing," described previously by Tipples and Kilborn (1975, 1977, 1979). "Undevelopment" is offered as an alternative because it is analogous to the common usage of "development" to describe the desirable changes in dough produced by mixing.

During undevelopment, dough changes in character and assumes the appearance of a dough that is undermixed (underdeveloped). If an undeveloped dough is remixed at higher speed, a second development is obtained. The development-undevelopment-development cycle can be repeated several times (Tipples and Kilborn 1977). A somewhat similar phenomenon is the redevelopment of overmixed doughs by remixing after a period of relaxation.

Bread baked from an undeveloped dough has lower loaf volume and inferior external appearance and crumb structure compared to bread baked from the same dough that is properly developed.

Because of the central role of gluten in rheological properties of dough and in bread quality, one might speculate that undevelopment results from unknown physical changes in this constituent. The purpose of the present study was to obtain information on the nature of the gross changes that occur in the gluten properties during undevelopment. Such information is fundamental to understanding development.

MATERIALS AND METHODS

The three wheat cultivars Glenlea, Neepawa, and Fredrick were selected on the basis of their known dough-mixing properties. The first two cultivars are hard red spring wheats. Fredrick is a soft white winter wheat.

Glenlea and Neepawa samples were milled into straight-grade flours on a Buhler experimental mill after an overnight tempering to 15.5% moisture. The Fredrick sample was tempered to 14.5% moisture and milled on the pilot mill at the Canadian Grain Commission Grain Research Laboratory. Flour extraction was 68.1, 72.7, and 70.0% for Glenlea, Neepawa, and Fredrick, respectively. Glenlea flour was of medium protein content (12.6%, N × 5.7, 14% mb) and had very strong dough-mixing characteristics as assessed with the farinograph. Neepawa flour had a higher protein content (14.1%) and strong mixing characteristics. Because Fredrick was considered too weak to use as a bread flour, the third experimental flour was prepared by blending equal amounts of Fredrick and Neepawa (Fr/Np). The blended flour had medium protein content (11.8%) and medium dough strength. Glenlea, Neepawa, and Fr/Np flours had a mixing tolerance index of 25, 40, and 50 Brabender units, and a dough stability of 11.5, 7.5, and 6.0 min, respectively.

Preparation of Doughs and Baking

Dough was mixed on a variable speed GRL-200 mixer (Voisey and Kilborn 1974) with the pin speed at 165 ± 2 rpm and a temperature of 35 ± 0.2°C. The GRL-200 mixer measures the power and energy used by the motor and records the energy input

TABLE I
Mixing Treatments of Dough Samples Studied

Sample	Mixing Treatment
1	Initial blending of ingredients (premix) 1 min at 37 rpm
2	Optimal development at 165 rpm (peak)
3	First undevelopment stage (peak + 1 min at 37 rpm) ^a
4	Second undevelopment stage (peak + 8 min at 37 rpm) ^a
5	Third undevelopment stage (peak + 16 min at 37 rpm) ^a
6	Second development (remix) ^b
7	Undermixing (20 min at 37 rpm)
8	Overmixing (1.8 × peak work input at 165 rpm)

^aThe mixing times for undevelopment, which were the same for all doughs, were chosen arbitrarily.

^bOptimal development + 8 min undevelopment at 37 rpm + mix to peak at 165 rpm.

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TABLE II
Mixing Times and Energy Inputs for the Dough Samples Studied

Sample ^a	Mixing Time (min)			Energy Input (whr/kg)		
	Glenlea	Neepawa	Fr/Np ^b	Glenlea	Neepawa	Fr/Np
1	1.0	1.0	1.0	0.1	0.2	0.1
2	18.4	3.3	3.0	14.3	3.0	2.9
3	19.4	4.3	4.0	14.5	3.2	3.1
4	26.4	11.3	11.0	14.6	3.8	4.0
5	34.0	19.3	19.0	14.8	4.5	5.1
6	31.0	13.3	12.4	18.1	6.6	6.5
7	20.0	20.0	20.0	0.6	2.3	2.1
8	25.4	5.7	5.0	26.0	5.5	5.2

^a As in Table I.

^b Blend of equal amounts of Fredrick and Neepawa.

into the dough during mixing in watt hours per kilogram of dough (whr/kg). The result is a "mixing" curve.

Bread was baked according to the GRL-Chorleywood method (Kilborn and Tipples 1981b) using the same formula for all doughs. The resulting bread was scored subjectively (Kilborn and Tipples 1981a). In this scoring, the external appearance is graded 0.5–10, with 10 being best; letters may be added to denote apparent under- or overoxidation (eg, green = g, old = o). For crumb structure, the numerical value relates to the thickness of the cell walls; the thinner the wall, the higher the score. Letters may be added to denote overall gas cell size (eg, coarse = c, open = o). The numerical values used for crumb color scores indicate visual brightness; the higher the number, the brighter the crumb. Letters may be added to indicate other features such as grey (g), dull (d), and yellow (y). The same formula was used for doughs, for baking, and for analyses.

The experimental variables constituting the eight mixing treatments selected for this study are summarized in Tables I and II. After mixing, the doughs were divided into pieces of about 10 g and quickly frozen by immersion in liquid nitrogen. The frozen doughs were freeze-dried and ground on a Cyclone sample mill (Udy Analyzer Co.) to pass through an 80-mesh screen. Ground samples were kept in desiccators at 4°C and removed as required for analyses.

Extraction and Fractionation of Proteins

Samples of ground freeze-dried dough were extracted with 0.05M acetic acid by the method of Tanaka and Bushuk (1973a). The protein contents ($N \times 5.7$) of the supernatants were determined by the micro-Kjeldahl procedure (method 46-13, AACC 1976).

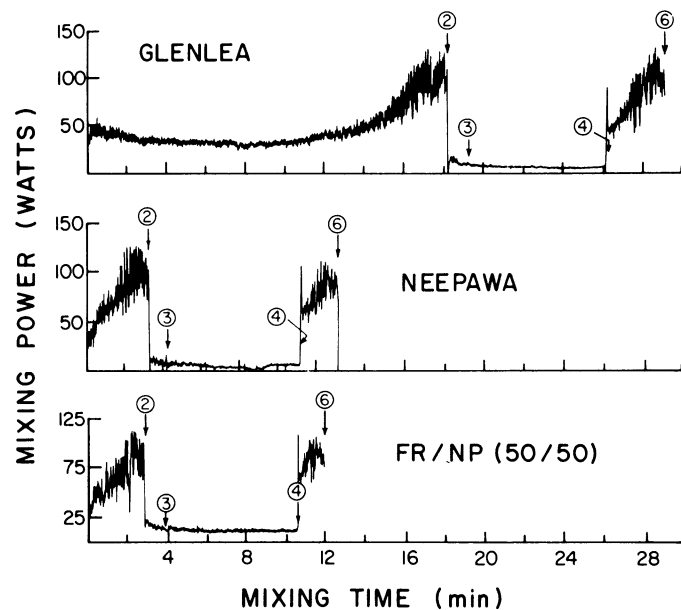


Fig. 1. Dough mixing curves. For identity of mixing treatments 2–6, see Table I.

The proteins of the ground freeze-dried dough were also extracted and fractionated by the modified Osborne procedure of Chen and Bushuk (1970). The freeze-dried protein fractions were stored in desiccators at 4°C and used as required for analyses.

SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed at pH 8.4 according to the procedure of Khan and Bushuk (1979a, 1979b). When required, the protein samples were reduced with 1% mercaptoethanol (v/v) for 1 hr at 50°C or overnight at room temperature.

RESULTS AND DISCUSSION

Dough-Mixing Results

The mixing treatments used to prepare the experimental dough samples are given in Table I. All doughs were mixed in a normal atmosphere. Because of the different mixing strengths of the three flours, equivalent doughs, on the basis of development, required markedly different mixing times and energy inputs (Table II). The first peak in the mixing curve (dough sample 2, Fig. 1) was taken as the point of optimum dough development. For the undevelopment phase, the same mixing times and speeds were used for the three flours.

The net energy used to mix the eight doughs from each flour ranged from 0.1 to 26.0 whr/kg for Glenlea, from 0.2 to 6.6 whr/kg for Neepawa, and from 0.1 to 6.5 whr/kg for Fr/Np. Glenlea required 14.3 whr/kg (18.4 min of mixing) to reach the first peak, whereas Neepawa and Fr/Np required only 3.0 whr/kg (3.3 min of mixing) and 2.9 whr/kg (3.0 min of mixing), respectively (Tables I and II).

All but two of the dough samples for each flour were obtained from replicate mixes represented by the mixing curves in Fig. 1. Dough samples 1 (initial blending) and 5 (third undevelopment stage) are not indicated. Doughs 7 (undermixed, not shown) and 8 (overmixed, Fig. 2) were produced by separate mixes.

Mixing curves (Figs. 1 and 2) show the amount of power used by the mixer to maintain constant preset mixing speed. At a constant mixing speed, a change in the curve height is directly related to a change in dough consistency. The power required to reach peak consistency by remixing at 165 rpm after undevelopment by mixing for 8 min at 37 rpm, was considerably lower than the power required to initially reach the first development peak.

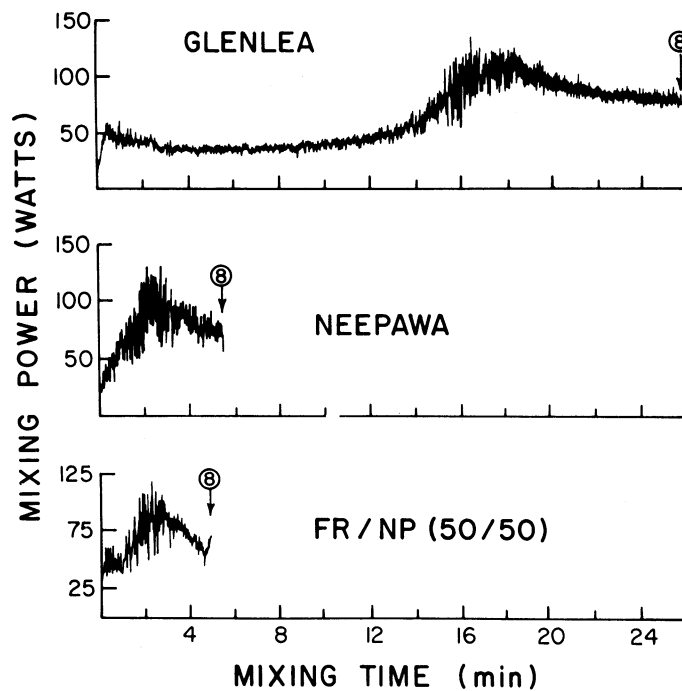


Fig. 2. Mixing curves for the overmixed doughs (sample 8 in Table I).

Overmixed doughs were prepared by applying 1.8 (arbitrary value) times the net energy used to mix the doughs to their first development peak (Table II and Fig. 2). Attention is drawn to the extremely high energy requirements of the very strong Glenlea flour, in comparison with those of the strong and medium flours (ie, Neepawa and Fr/Np).

Effect of Mixing Treatments on Bread Quality

The external and internal appearance of the breads produced from Neepawa flour subjected to various dough-mixing treatments is presented in Fig. 3 (Glenlea and Fr/Np results not shown). Table III gives the baking results for all three flours used in this study. Underdevelopment of all doughs produced bread of less than optimum quality. For Glenlea, 1 min of underdevelopment caused a marked deterioration in the bread quality. Loaf volume decreased by 20% compared with bread baked from optimally developed dough. For the same mixing treatment, the decrease in loaf volume was 4% for Neepawa and 2% for Fr/Np. Loaf volume decreased further with continued underdevelopment. The decrease after 16 min of underdevelopment was 62% for Glenlea, 38% for Neepawa, and 34% for Fr/Np. The negative effects of underdevelopment were also evident in the external appearance of the loaves, and in crumb structure and color (Table III and Fig. 3). Longer underdevelopment resulted in decreased bread quality as indicated by both objective and subjective quality parameters. The negative effects of underdevelopment were more pronounced for stronger flours, in agreement with the earlier results of Tipples and Kilborn (1975).

As expected, the bread from the two underdeveloped doughs of all three flours (samples 1 and 7) was extremely poor (the loaves are identified as 1 min and 20 min at 37 rpm in Fig. 3).

The loaf volume of the bread from the remixed Glenlea dough was slightly higher than that obtained for first peak development. The differences in loaf volumes of the remixed and the initially developed doughs were positive for Glenlea and negative for Neepawa and Fr/Np.

Overmixing caused a decrease in loaf volume and a slight deterioration in loaf quality of bread from all three flours (Table III).

Effect of Work Input on Protein Solubility

Extraction of Protein with 0.05M Acetic Acid. For the two stronger flours, Glenlea and Neepawa, the amount of protein extracted with acetic acid increased with initial blending (Fig. 4) and continued to increase with dough development. For the weakest flour, Fr/Np, the proportion of acetic acid-soluble protein increased slightly with blending but decreased with subsequent mixing to optimum development.

For all three flours, the amount of extractable protein decreased with mixing at slow speed after optimum development (ie, during underdevelopment). This suggests that underdevelopment promotes the formation of aggregates that are acetic acid-insoluble.

Subsequent redevelopment produced a reversal in the solubility

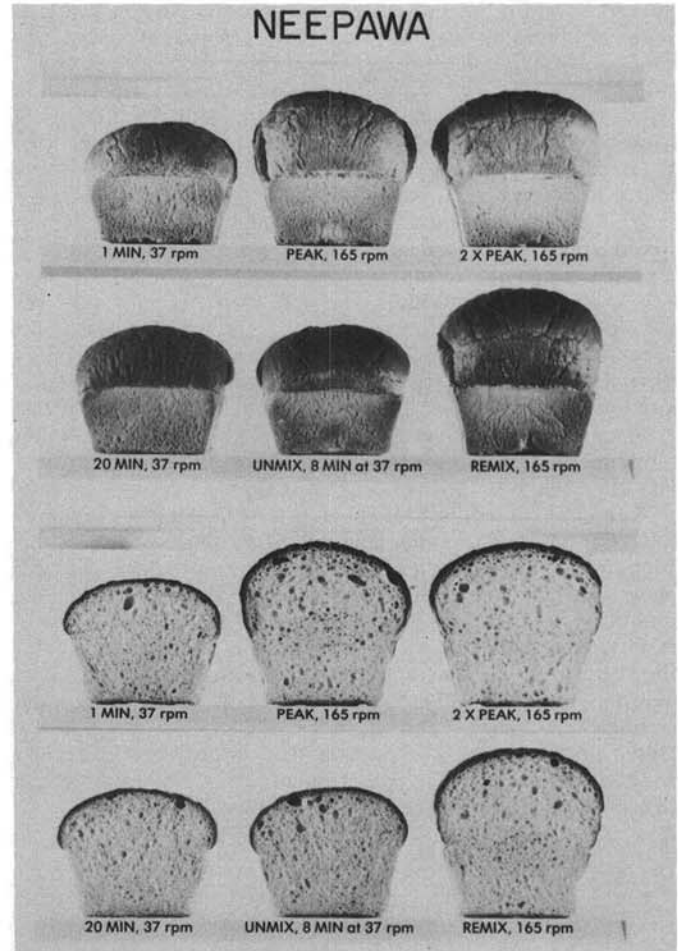


Fig. 3. External and internal appearance of pup loaves obtained from Neepawa doughs subjected to various mixing treatments. Mixing treatments: top (left to right), 1, 2, and 8; bottom (left to right) 7, 4, and 6. Table I contains details.

TABLE III
Effect of Mixing Treatments on Bread Properties for Glenlea, Neepawa, and Fredrick/Neepawa Flours

Flour	Mixing Treatments ^a							
	1	2	3	4	5	6	7	8
Glenlea								
Loaf volume, cc	515	990	795	405	375	1,045	380	870
External appearance	3.0,vo ^b	8.0	7.5	1.0,vo	0.5,vo	8.0	0.5,vo	8.0
Crumb structure	3.0,c,vo	6.5,o	5.8,o	1.0,c,vo	0.5,c	6.8,o	1.0,c,vo	6.0,o
Crumb color	1.5,g-y	11.0	8.0	2.0,g	15,g-y	10.2	0.5,g-y	9.0
Neepawa								
Loaf volume, cc	650	1,045	1,005	695	650	1,025	675	990
External appearance	5.0,old	9.0	8.5	5.2,old	5.5,old	8.8	5.0,old	8.0,g
Crumb structure	5.0,vo	6.0,vo	6.2,o	5.0,vo	4.0,c,o	6.0,o	4.8,vo	6.0,vo
Crumb color	4.0,g-y	8.0	8.0	5.0,g-y	4.0,g-y	8.5	4.5,g-y	8.0
Fredrick/Neepawa								
Loaf volume, cc	660	935	915	680	615	880	645	895
External appearance	5.5,old	8.2	8.0	6.2,old	5.5,old	8.0	5.0,old	7.8,vg
Crumb structure	4.5,c-o	6.0,o	6.0,o	5.2,c-o	4.0,c-o	6.0,o	3.8,c-vo	6.0,o
Crumb color	4.5,d-y	8.2	8.0	5.5,d-y	5.0,d-y	8.0	4.0,d-y	8.2

^a As in Table I.

^b Key for sensory evaluations: c = coarse; d = dull; g = grey; g-y = grey-yellow; o = open; vg = very green; vo = very old; vo = very open; y = yellow.

resulting in an increase in the amount of extractable protein (eg, compare values for samples 6 and 5 in Fig. 4). Overmixing resulted in higher protein extractability as compared to undevelopment but lower extractability as compared to redevelopment. The highest protein solubility for Glenlea and Neepawa doughs was obtained from those doughs that were remixed. The percentage of protein extracted was 79.2% from Glenlea and 80% from Neepawa. The weakest flour, Fr/Np, showed maximum protein solubility for the dough produced by the gentle mixing treatment used to blend the ingredients (sample 7).

The observed influence of undevelopment on protein extractability by acetic acid are consistent with the findings of Arakawa et al (1976, 1977), who reported that the tendency of gluten protein to aggregate, especially the glutenin, was directly related to the mixing strength of the flour.

Osborne Protein Fractionation. The most prominent effect of mixing treatment was on the amount of glutenin (results not shown) and insoluble residue protein. Optimally developed doughs from the two stronger flours contained more glutenin than the corresponding flour. The increase was greater for Glenlea than for Neepawa. For the weaker Fr/Np flour, the amount of the glutenin remained relatively constant for all mixing treatments. Compared with optimally developed doughs, undeveloped doughs of the two stronger flours had reduced amounts of glutenin, and the amount continued to decrease as the length of undevelopment increased. This effect was not observed for Fr/Np flour.

The amount of residue protein was inversely related to the energy input during dough mixing (Fig. 5). Also, the solubility of this fraction was related to the mixing strength of the flour. Figure 5 shows that at equivalent mixing treatments, decreases in the amount of residue protein were higher for the Glenlea flour; in other words, solubility was greater for the stronger flour. This observation is in general agreement with published results (Huebner and Wall 1976; Mecham et al 1962, 1963; Mullen and Smith 1965; Tanaka and Bushuk 1972, 1973a; Tsen 1967). Moreover, the present work showed that the difference in protein solubility is related to the energy input during mixing, which, in turn, is related to mixing strength of the flour.

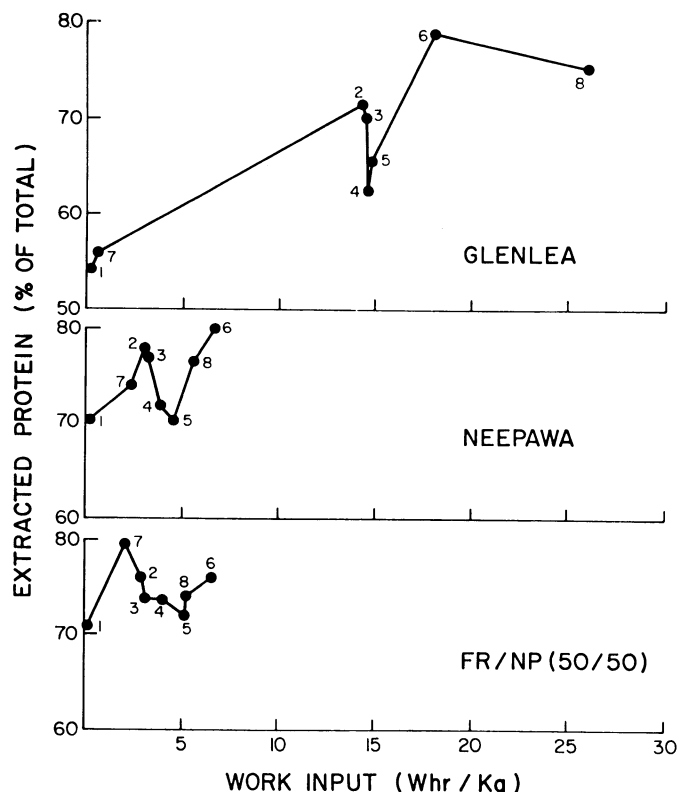


Fig. 4. Relationship between proportion of protein extracted with 0.05M acetic acid and work (energy) input during dough mixing. Table I identifies mixing treatments 1-8.

SDS-PAGE of Proteins During Dough Mixing and Unmixing

Dough development and undevelopment produced only minor qualitative and quantitative changes in the SDS-PAGE patterns of unreduced and reduced albumins, globulins, and gliadins (not shown). SDS-PAGE patterns of unreduced glutenin from undeveloped dough showed the presence of six components of 58,000, 51,000, 46,000, 26,000, 18,000, and 16,000 mol wt. The first three stained faintly and were not visible in the photograph (Fig. 6). These components were not present in the patterns of analogous fractions from any other dough samples. Presumably, these components remained at the origin in the form of high molecular weight aggregates unable to enter the gel. The presence of these components in the fraction was confirmed by the SDS-PAGE patterns of reduced glutenins. Pattern C of reduced glutenin showed some qualitative (a resolved component of 14,300 mol wt) and quantitative (bands of higher intensity) changes in relation to the other patterns; no explanation is available for this behavior. These results support the Khan and Bushuk (1979a, 1979b) model of functional glutenin, which is based on the aggregation of certain glutenin subunits during dough development and undevelopment.

Results for the other two flours used in this study (Neepawa and Fr/Np) were analogous to those for Glenlea shown in Fig. 6 and are, therefore, not reported.

As in the case of the solubility results, the effect of undevelopment on SDS-PAGE patterns (increase in intensity of some bands) depended on the mixing strength of the flour. The effect was greatest for the strongest flour (Glenlea). The observed relationship between the magnitude of the undevelopment effect and flour strength parallels the observed deleterious effect of

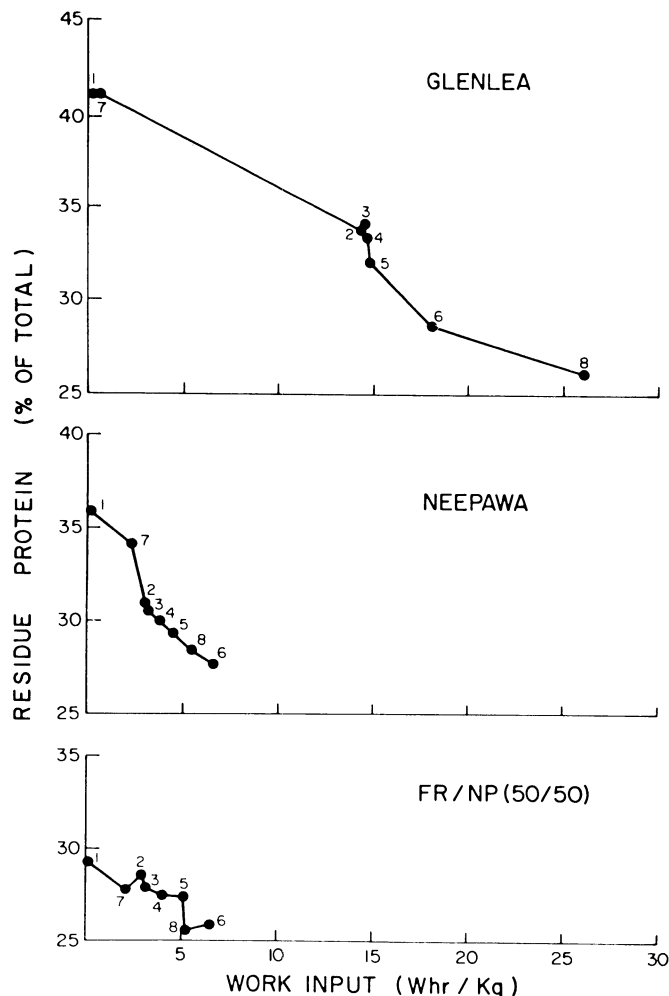


Fig. 5. Relationship between proportion of residue protein (by Osborne fractionation) and work input during dough mixing. Table I identifies mixing treatments 1-8.

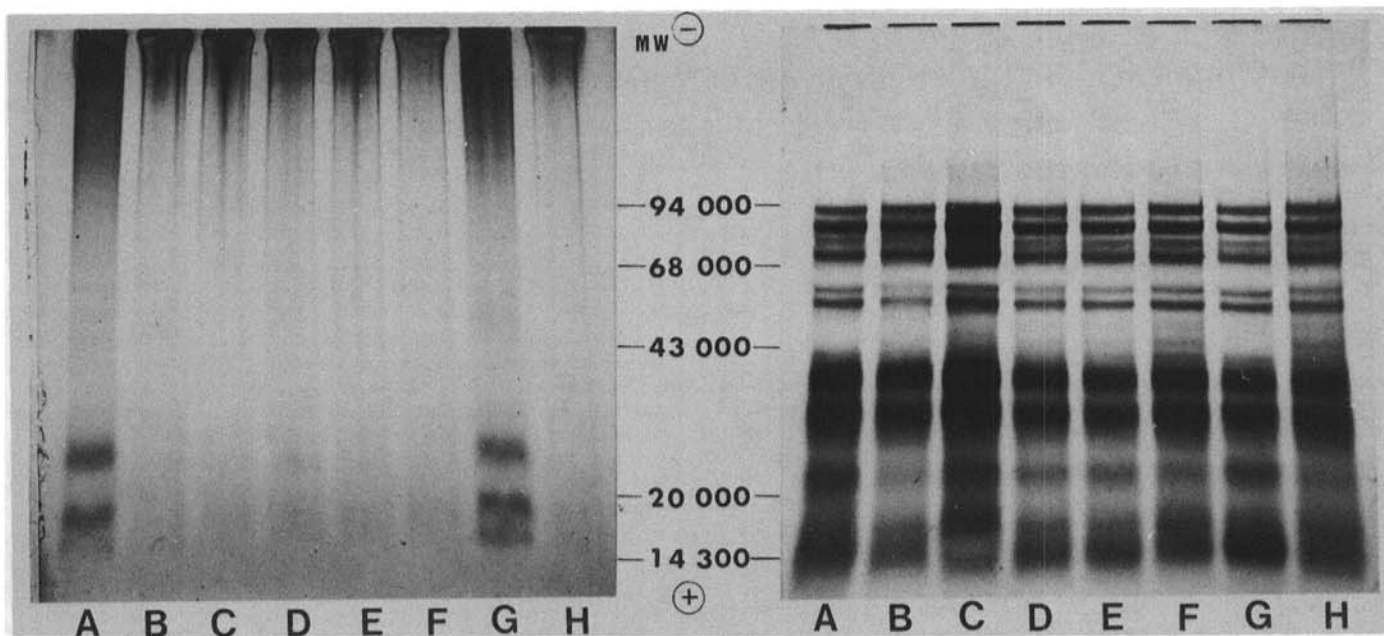


Fig. 6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of acetic acid-soluble proteins (glutenins) isolated from Glenlea doughs subjected to various mixing treatments. **Left**, unreduced proteins, **right**, reduced proteins. Patterns A-H represent mixing treatments 1-8 of Table I, respectively.

undevelopment on the bread-making quality. The detrimental effect was greatest for the strongest flours.

Protein solubility and SDS-PAGE results suggest that dough undevelopment results in the aggregation (insolubilization) of gluten proteins, particularly the glutenins. Presumably, this change favors the conversion of the membranous structure of developed gluten to one comprised of fibrils and globules (Paredes-Lopez and Bushuk 1982). Recent studies have emphasized the concept that an important characteristic of functional glutenin is its tendency to aggregate by secondary forces (Ewart 1979, Huebner and Wall 1980, Kasarda et al 1976, Khan and Bushuk 1979a, 1979b). The tendency of glutenins to aggregate during the dough stage in breadmaking may be a key factor in the nature of bread-making quality of wheat proteins.

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