PHYSICOCHEMICAL CHANGES IN FLOUR PROTEINS DURING DOUGH MIXING

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ABSTRACT

The changes in flour proteins during mixing under a variety of conditions were examined by various physical and chemical techniques using three flours of widely different mixing strength. Extended mixing in the presence of relatively high concentrations of N-ethylmaleimide and potassium iodate was used to produce accentuated breakdown. Exhaustive extraction of the proteins from freeze-dried doughs with 0.05N acetic acid indicated that under mixing conditions where doughs showed a marked decrease in consistency, there was a marked increase in the amount of protein that was soluble in this solvent. This was attributed to the depolymerization of the high molecular weight glutenin. Results of the solubility fractionation and the gel filtration experiments were consistent with the depolymerization hypothesis. Concomitantly with the decrease in the amount of the insoluble protein, increases were observed in the amounts of the alcohol-soluble and the acetic acid-soluble proteins of the dough samples. These solubility changes during dough mixing produced only a minor change in the SDS-PAGE patterns of the reduced protein fractions. The minor change was in the alcohol-soluble protein of doughs that suffered extensive mixing breakdown. This fraction contained several high molecular weight components that were detected only in the patterns of the reduced acetic acid-soluble and the insoluble residue protein fractions of doughs that did not show extensive breakdown. The effects of mixing on the phy-

sical properties of the gluten proteins under conditions of extensive breakdown were analogous to effects of disulfide reducing agent cysteine. On the basis of this evidence, it is concluded that the depolymerization involves reduction of disulfide bonds. Chemical analyses did not show any change in the number of disulfide bonds. Also there was no change in the number of free amino groups that would result from cleavage of peptide bonds nor was there any change in the binding of lipid by the gluten. It is therefore suggested that the depolymerization occurs through disulfide interchange reactions involving low molecular weight sulfhydryl peptides and proteins. Accordingly, the mixing strength of flour would depend directly on its content of these low molecular weight peptides as well as on the intrinsic structure of the high molecular weight gluten proteins.

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INTRODUCTION

Dough mixing is the most critical step in the breadmaking process. In conventional breadmaking its effect persists through the many subsequent steps to the point where it has a distinct influence on the quality of the bread that is obtained. In continuous breadmaking processes mixing plays a more prominent role in that much of the dough development is achieved by the mechanical action of mixing.

For any one breadmaking process, using a particular flour and formula, there is an optimum mixing time with each type of mixer. Too little or too much mixing will give inferior results. Accordingly, it is obvious that the function of dough mixing is much more complex than just the physical blending of ingredients. As will be seen from the detailed review of the literature that follows, there are many changes in the dough components that go together to produce the overall effect of mixing. Of the dough components that seem to occupy the key position in these changes are the flour proteins. Accordingly, this component has been studied most extensively. In brief, the published work on the changes in the flour proteins during dough-mixing can be summarized as follows:

1. The amount of protein that can be dissolved from freeze-dried doughs by water, dilute acetic acid solution, aqueous urea, or other appropriate solvents increases markedly with mixing time. The increase is greater at higher mixing speeds. The change in solubility in resting doughs is negligible; mixing is therefore prerequisite to the increased solubility.

2. The rate of protein solubilization by any one type of mixing action depends on the type of flour. Protein content is an important factor, but other, as yet undefined, qualitative factors play a significant role.

3. Solubilization is faster when the mixing is carried out in air instead of in nitrogen, or in the presence of oxidizing dough improvers. The rate at which the oxidant reacts in dough appears to be important but mixing is required to produce these effects. Sulfhydryl-blocking agents such as N-ethylmaleimide also produce a marked increase in the amount of extractable protein. Sulfhydryl groups are obviously involved in the solubilization process but the mechanism of this involvement is unknown.

These observations are generally consistent with the hypothesis that dough-mixing decreases the size of the particle or aggregate of the matrix proteins in doughs and thereby increases their solubility. This was also adopted as the working hypothesis for the study on which this thesis is based.

There are a number of possible mechanisms by which the protein particle or aggregate size could decrease. It could involve physical breakdown of flour particles, dissociation of physical or non-covalent bonds such as hydrogen bonds, hydrophobic interactions and salt linkages, and/or depolymerization of the protein macromolecules as a result of cleavage of covalent bonds. The primary objective of this study was to determine which of these mechanisms gives rise to the increased extractability or solubility of flour proteins during dough mixing.

The experimental approach adopted for this study is given below. Three flours were selected to give a wide range of mixing characteristics. The doughs were subjected to various mixing conditions to produce normal and accentuated breakdown, for example:

1. Variable mixing time.

2. Mixing in nitrogen and air.

3. Mixing in the presence of cysteine (a reducing agent).

4. Mixing in the presence of two concentrations of potassium iodate in air and in nitrogen.

5. Mixing in the presence of two concentrations of N-ethylmaleimide in air and in nitrogen.

The doughs were freeze-dried after mixing and then their proteins were characterized by extractability, solubility fractionation, gel-filtration chromatography, electrophoresis and other ancillary techniques. The results obtained are described in this thesis.

LITERATURE REVIEW

Chemical and Physical Bonds in Dough Structure

In recent years it has become clear that, to explain chemical and physical changes that occur in dough during mixing, it is essential to know the structure of the components of the dough and the nature of the interactions among them (Lasztity 1962; Vakar et al. 1965; Hampel 1968; Wall and Beckwith 1969). In the present investigation attention is focussed on the changes that occur in the flour proteins.

For the purpose of this review the linkages that determine the structure and behavior of the flour proteins in dough will be divided into two general types: (1) covalent bonds such as carbon-carbon, peptide and disulfide bonds, and (2) non-covalent bonds, namely ionic, hydrogen and hydrophobic bonds. The literature dealing with each type of linkage in dough will be reviewed in separate sections.

Covalent Bonds

Peptide Bond. A protein chain is composed of amino acid residues linked together linearly by peptide bonds (-CO-NH-). These bonds are formed when the α-carboxyl group of one amino acid is linked to the α-amino group of another amino acid with the elimination of water. Many of the specific properties of polypeptides arise directly from the structure of the peptide bond. This feature applies to all proteins in general. The peptide bond has a very high bond energy (69.9 kcal. per mole), but it can be readily hydrolyzed by the action of a proteolytic

enzyme. Normal bread wheat flours are low in proteolytic activity (Pyler 1969; McDonald 1969). However, the possibility still exists that this enzyme might be involved in dough breakdown that occurs during mixing. It has been suggested that the main difference between strong and weak mixing flours is in their proteolytic activities (Bushuk et al. 1971). The proteolytic action hypothesis would be consistent with the Jørgensen hypothesis (Jørgensen 1945) for the mechanism of dough maturing by oxidizing agents. Further work is required to clarify this point.

Carbon-Carbon Bonds. This bond is part of the structure of the protein backbone chain along with the peptide bond. Its bond energy (83.1 kcal. per mole) is higher than that of the peptide bond. There are no reports in the literature on the scission of carbon-carbon bonds in any of the flour components in the transformation of wheat to bread. A number of workers have suggested that such a scission could occur during milling of wheat or during high speed mixing of doughs with the formation of free radicals (Redman et al. 1966; Dronzek and Bushuk 1968). Free radicals have been detected in freshly ground flour (Redman et al. 1966; Wasik 1971); however, the nature of these radicals has not been identified and it could well be that they result from scission of bonds other than carbon-carbon.

Disulfide Bond. Of all the covalent bonds in flour proteins, the disulfide bond is considered to be most important in the changes that

occur in flour proteins during dough mixing (Dimler 1963, 1965; Wall 1964, 1967; Sullivan 1965; Holme 1966; Pomeranz 1968). Such bonds in proteins have an energy of 49 kcal. per mole which is somewhat lower than the energy of the two types of covalent bond mentioned above.

About 1.4% of the amino acids in flour proteins (Woychik et al. 1961; Wall 1964; Tkachuk 1966; Ewart 1967A, 1967B; Krull and Wall 1969) are either cystine or cysteine. The disulfide bonds of cystine can link together portions of the same or different polypeptide chains and thereby play an important functional role in the rheological properties of dough (Bloksma 1972). A protein network with a high concentration of disulfide cross-linkages is usually insoluble, has little viscous flow and is highly elastic (Bushuk 1961). This behavior is typical of glutenin, one of two major protein components of wheat gluten.

Chemical agents that break S-S bonds have long been known to bring about a rapid breakdown of dough. This effect of disulfide reducing agents was first described by Freilich and Frey (1944) in their article on comparative study of the effects of oxidizing and reducing agents. Later, Merritt and Bailey (1945) reported that the addition of bisulfite (an S-S reducing agent) resulted in an increased extensibility and a decreased resistance to extension of doughs tested with the extensigraph. Hlynka (1949) studied the effect of bisulfite using both the extensigraph and the farinograph and interpreted his rheological results in terms of the cleavage of the cross-linked network structure of dough. Udy (1953) also suggested that the effect of bisulfite on dough was through the

reduction of protein S-S crosslinks. Matsumoto et al. (1960) confirmed this cleavage by showing that there was a concomitant increase in SH content in flour proteins treated with bisulfite.

Disulfide interchange may occur in doughs during mixing (Goldstein 1957), with the sulfhydryl (SH) groups contributed by low molecular weight SH compounds present in the flour (Frater and Hird 1963; Stewart and Mauritzen 1966; Mauritzen 1967; Hird et al. 1968; Kuninori and Sullivan 1968; McDermot et al. 1969). Apparently, low molecular weight S-S compounds can also interchange with the S-S bonds of the gluten protein to produce a rheological effect similar to that of reducing agents (Sullivan and Dahle 1966; Mauritzen 1967; Jones and Carnegie 1969). The occurrence of S-S interchange reactions between added S-S compounds and flour proteins in dough during mixing was demonstrated for the first time by McDermott and Pace (1961). They added thiolated gelatin to dough, isolated gluten from it, and found hydroxyproline in its hydrolysate. This amino acid is present in gelatin, but not in gluten. Similar results have been obtained with 2-mercaptoethanol, 2-mercaptoethylamine hydrochloride and reduced glutathione (Tao and Pomeranz 1967; Kuninori and Sullivan 1968; McDermott et al. 1969), radioactively labeled amino acids (Mauritzen and Stewart 1963), and other sulfhydryl compounds (Lee and Lai 1967). Interchange reactions appear to be governed largely by the reactivity of low molecular weight SH compounds which can diffuse freely in the dough (Kuninori and Sullivan 1968). In spite of the extensive studies on the different aspects of disulfide interchange reactions

in dough, as yet no one has succeeded in obtaining direct evidence that these reactions occur among flour proteins during dough mixing.

Interchange reactions that break intermolecular S-S bonds of glutenin molecules can relax stress on maximally extended chains and thereby they are extremely important in the rheological properties of dough (Hird 1966; Frazier and Muller 1967; Ewart 1968). It has been suggested that, in the absence of SH groups, stresses imposed by mixing may cause a mechanical breakdown of S-S and other bonds with formation of free radicals (Axford and Elton 1960; Redman et al. 1966). Presumably the free radicals from breakage of S-S bonds would be converted to SH groups by abstraction of hydrogen, and these would be capable of further breakdown of gluten molecules by subsequent reaction with their S-S groups (Ewart 1968).

Removal of SH groups from dough proteins by reaction with oxidizing agents such as iodate or bromate, or with blocking agents such as N-ethylmaleimide (NEMI), generally toughens the dough. Presumably in such doughs there are fewer SH groups that can initiate the interchange of S-S cross-linkages (Sullivan 1954; Goldstein 1957; Hird 1966). Although the formation of interpolypeptide S-S bonds was observed in reoxidation of reduced gluten in concentrated solutions, there is no unequivocal analytical evidence that this reaction occurs in doughs when oxidizing maturing agents are added (Beckwith and Wall 1966). This is not surprising since the expected increase in S-S from the optimal oxidation of SH would be within the experimental error of the S-S determination.

The possibility that protein disulfide reductase might be active in the hydrolytic cleavage of S-S groups during mixing was suggested by two groups of Soviet workers (Agatova and Proskuriakov 1962; Averkieva and Vakar 1969). This enzyme was detected in flours from sprouted wheat; however, normal flours had no activity. Accordingly, it can be concluded that enzymic cleavage of S-S bonds is probably not involved in the changes in dough proteins during mixing.

Non-covalent Bonds

Ionic Bonds. Ionic bonds (also called salt linkages) result from electrostatic attractions of ionized groups. This type of linkage, although non-specific, can give rise to an interaction energy as high as 110 kcal. per mole. Accordingly, a small number of these bonds could make a major contribution to the stability of a concentrated protein system such as dough.

Flour proteins contain a number of amino acids that have ionizable groups. Among these are the carboxyl groups of aspartic and glutamic acids, the ϵ-amino group of lysine, the imidazole group of histidine, and the guanidino group of arginine (Butler 1970).

At the pH of dough both positively and negatively charged groups can exist in the flour proteins. Accordingly, the existence of ionic attractions in dough can be postulated *a priori*.

Direct evidence of the presence of salt linkages in dough has been obtained by studying the effects of various electrolytes and of pH. Watanabe *et al*. (1964) used McIlvaine's buffer (pH 2 to 12) as mixing

water in a farinograph, and observed marked weakening after peak time and a decrease of development time as the pH was decreased from 12 to 2. However, the maximum consistency remained essentially the same over the pH range investigated. On the other hand, Bayfield and Young (1964) found no change in dough strength, an increase in development time, and a marked decrease in consistency over a similar pH range. Tanaka et al. (1967) reported that the consistency of dough increased with decreasing pH, obtained by additions of acetic acid in the absence of salt, whereas it tended to decrease with decreasing pH in the presence of salt (sodium chloride). A study by Bennett and Ewart (1962) with the Simon Research extensometer showed that the extensibility and resistance decreased markedly when the pH of the dough was lowered from 6 to 2 in the presence of 2.5% sodium chloride. Between pH 4.2 and 7.3 the resistance of dough to extension in the extensigraph decreased with increasing pH (Tanaka et al. 1967, 1968; Tsen 1966).

Hlynka (1962) showed that 1% salt decreased farinograph consistency of dough by 70 B.U., and 2% salt decreased it by 90 B.U. This effect was attributed to the effect of salt on the hydration capacity of gluten proteins (Bushuk and Hlynka 1964).

The effect of salt on the extensigram was studied by Fisher et al. (1949) and compared with its effect on the farinogram. They found that addition of salt produced an increase of both extensibility and resistance to extension. Some of the results obtained with the extensigraph appear to be inconsistent with farinograph results. However the studies

mentioned above provide ample evidence that salt linkages are involved in the structure of dough. It is not yet possible to establish an overall mechanism that will explain the effects of salt measured under different conditions with various rheological techniques.

Some divalent ions, for example calcium, appear to have quite a different effect on dough from that of monovalent ions. Fullington (1967, 1969) suggested that divalent ions can form bridges between flour proteins and phospholipids. These complexes could have a significant effect on the rheological properties of dough through their effect on the solubility of gluten proteins.

Hydrogen Bonds. Hydrogen bonds result from the affinity of electropositive hydrogens of hydroxyl, amide or carboxyl groups for electronegative atoms such as oxygen, sulfur or nitrogen. These bonds are highly nonspecific and relatively weak (2.7 kcal. per mole). However, two protein molecules can form a large number of hydrogen bonds to give a total interaction of relatively high energy. The insolubility of gluten proteins in water is attributed mostly to the presence of a large number of hydrogen bonds (Cunningham et al. 1955; Holme and Briggs 1959; Beckwith et al. 1963).

Most of the hydrogen bonds in proteins involve the peptide group. In addition to the peptide group, wheat flour proteins contain a large number of side chain amino acid residues that can readily form hydrogen bonds (Wall and Beckwith 1969). Among these, the amide functions of glutamine and asparagine appear to be the most important. Glutamine

forms over a third of the amino acids in flour proteins (Tkachuk 1966). Evidence of the involvement of the amide groups in the interaction of dough proteins was published by Holme and Briggs (1959). These workers showed that, when the amide groups of gliadin were removed by acid hydrolysis, the tendency of gliadin to interact with itself was decreased considerably. Krull et al. (1965) and Krull and Wall (1966) demonstrated the presence of hydrogen bonds in dough by their studies of the effects on dough of water-insoluble and-soluble synthetic polypeptides containing side-chain amide groups. Barney et al. (1965) showed that acetylation of the free amino groups of gluten destroyed its cohesiveness. This effect was explained on the basis of the removal of the amino groups from participation in the hydrogen bonded structure of gluten. The important functional role of hydrogen bonds in dough was originally determined from the effects on dough properties of various chemicals such as urea and guanidine hydrochloride that are known to disrupt these bonds in proteins.

Another indirect experimental approach on the functional role of hydrogen bonds in dough has been through the use of heavy water (D_2O). Kretovich and Vakar (1964) found that, when gluten was solvated with D_2O, its extensibility was considerably lower than that of the same gluten hydrated with ordinary water. Analogous results were obtained by Tkachuk and Hlynka (1968) who showed that dry gluten mixed with D_2O showed greater stability in the farinograph than gluten mixed with H_2O. In addition, they found that flour doughs prepared with D_2O instead of H_2O appeared considerably stronger and tougher. The results of both groups of workers

were explained on the basis of the higher energy of the deuterium bond as compared with the hydrogen bond. Presumably the hydrogens which are involved in the hydrogen bonds in dough readily exchange with deuterium when the gluten (or flour) is mixed with D_2O.

Doguchi and Hlynka (1967) reported that the addition of urea or guanidine to the solution used to hydrate the gluten produced a lowering of the gluten consistency values obtained on the farinograph. Similar results were obtained for flour doughs by Jankiewicz and Pomeranz (1965). Although it is generally presumed that the effects of chemicals such as urea and guanidine result from their ability to disrupt hydrogen bonds, there is also a possibility that they may be capable of disrupting hydrophobic (see later) bonds (Levy and Magoulas 1962; Whitney and Tanford 1962). Their strong solubilizing effect suggests that they may be capable of breaking both types of bonds.

Hydrophobic Bonds. This is the physical bond that results from the Van der Waals interactions of nonpolar side groups of proteins in the presence of water. Kauzmann (1959) proposed the term "hydrophobic bond" to account for the forces responsible for the tendency of nonpolar residues to adhere to one another to avoid contact with an aqueous medium. The involvement of hydrophobic bonds in dough structure was inferred from the effects of organic solvents (Ponte et al. 1967) and hydrocarbons (Elton and Fisher 1968; Pomeranz et al. 1970) on physical properties of doughs.

If the amino acid composition of a protein is known, its hydropho-

bicity can be calculated by the method of Bigelow (1967). Hydrophobicity of proteins range from 440 to 2000 cal per amino acid residue. Hydrophobicities of glutenin and gliadin obtained by this calculation are 1016 and 1109 respectively (Wehrli and Pomeranz 1969). Thus, theoretically the hydrophobicity of both gluten proteins is high enough to make intra- and intermolecular hydrophobic bonds feasible.

Hydrophobic bonds can contribute to both plasticity and elasticity of dough. Although the energy of individual hydrophobic interactions is low, the cooperative effect of a large number of these interactions can make a substantial contribution to the overall structure of a protein system. Functionally, because these bond energies are low, it is possible to have rapid interchange of interactions at room temperature. Furthermore, when a dough is subjected to stress as in mixing, the tension could be readily relieved by a rapid interchange of the relatively mobile hydrophobic bonds. The tendency of hydrophobic groups to interact with each other rather than with water could contribute substantially to the elasticity of dough.

Mixing in Breadmaking

The elementary function of mixing is the uniform blending of all the dough ingredients. Water is one of the major ingredients; accordingly, its distribution in dough is an important phase of mixing. Hydration of all the flour components is a prerequisite for proper dough formation (Greer and Stewart 1959; Larsen 1964; Bushuk 1966, Bushuk *et al*. 1968).

Incorporation of air, particularly oxygen, is also important. The entrapped gas bubbles form the nuclei of gas cells produced by fermentation and the oxygen contributes to the improver reaction (Baker and Mize 1937; Freilich and Frey 1939; Dempster et al. 1954; Smith and Andrews 1957).

In addition to blending of ingredients, mixing must be optimal to achieve the proper development of physical properties for subsequent handling and gas retention (Bushuk et al. 1968). The term dough development is generally used to describe this optimization of physical properties of dough by mixing (Bushuk et al. 1968).

If the mixing is taken beyond the optimum the physical properties of the dough change quite drastically. The dough surface takes on a sheen and becomes sticky, and is difficult to handle. This overmixing, which usually produces a deleterious effect on the ultimate quality of the bread, is called dough breakdown.

In summary, the incorporation and blending of the dough ingredients and dough development are the two main functions of mixing. Superposed on these direct effects, mixing time allows for proper hydration of the flour particles. In most standard dough mixers uniform distribution of dough ingredients can be achieved in two to three minutes. Hydration and development take considerably longer. Gracza et al. (1965) reported that, in the farinograph, ten minutes are required to produce complete hydration of a standard bread flour. Muller and Hlynka (1964) indicated that, under usual mixing conditions, the rate of hydration of flour is rapid and

precedes development of gluten. In mechanical development baking processes the rate of hydration appears to be a very relevant phase in dough making since the residual time of the dough in the developer is extremely short.

The importance of mixing in the incorporation of oxygen was recognized by Baker and Mize (1937). Dempster and Hlynka (1958) showed, by means of an extensigraph, that mixing had two distinct effects: the direct effect on dough structure per se as indicated by a decrease in extensibility, and the indirect effect due to the incorporated oxygen as indicated by an increase in resistance to extension. A more detailed study of the effects of various gases on the mixing tolerance of dough was carried out by Meredith and Bushuk (1962). They also observed a decrease in extensigraph extensibility of doughs mixed in oxygen. In addition to changes of physical properties, these workers studied the changes in the amount of the so-called "gel" protein in the gluten during mixing. Doughs mixed in nitrogen, in air, and in oxygen were quite dissimilar on the basis of the extensigraph test, but they contained essentially the same amount of "gel" in the gluten. It was concluded that changes in physical properties of doughs can result from very subtle changes in gluten proteins that are difficult to detect analytically.

It appears now that dough development involves the combined effects of mixing and oxidation. This is supported by the fact that large amounts of oxidants are usually required for proper dough development in all the mechanical breadmaking processes used currently (Baker 1960; Chamberlain

et al. 1962; Snyder 1963; Blanchard 1965). In spite of the large number of studies on mechanical and chemical development of dough, it has not been possible to separate distinctly the changes produced by the physical action of mixing from those resulting indirectly from chemical reactions that occur during mixing.

Since the main emphasis of the present study is on the changes in the proteins, the literature dealing with previous studies of effects of mixing on this component will be reviewed in some detail. This part of the review will be divided into four subsections: 1) effects on protein solubility, 2) role of sulfhydryl and disulfide groups, 3) interaction with lipids, and 4) interaction with carbohydrates. Finally, a short subsection will summarize the possible mechanisms of the changes in proteins that occur during dough mixing.

Effect on Protein Solubility

Mecham and coworkers (Mecham et al. 1962, 1963, 1965; Mecham 1964) found that the amount of protein extracted from dough by dilute acetic acid increased with extended mixing. Rates of increase were different for flours of different mixing characteristics. To explain this increase in extractability it was postulated that mixing decreased the size of protein aggregates in the flour particles. The same workers also showed that extractability increased much faster and to a higher maximum if sulfhydryl-blocking reagents were added to dough (Mecham 1959, 1968). This was later confirmed by Tsen (1969).

Mullen and Smith (1965, 1968) and Smith and Mullen (1965) showed that the main difference between a short- and a long-mixing flour was that the former contained less acetic acid-insoluble protein. The salt-soluble fractions (albumins and globulins) have little effect on mixing characteristics. The protein-starch residue, after extraction with acetic acid, increased the mixing requirements to optimum in the farinograph, whereas the water-soluble gliadins markedly shortened the mixing requirements.

Tsen (1967, 1969) reported that the amount of flour protein that was extractable with 0.01N acetic acid and the distribution of protein components in the extract (as determined by molecular sieve chromatography) were significantly altered by dough mixing. Mixing at higher speeds intensified the increase of "glutenin" (first chromatographic peak) component. Weak flours showed this change more rapidly than strong flours. The changes became intensified when dough was treated with either oxidizing or reducing agents. The oxidizing agent produced an increase in the glutenin peak whereas the reducing agents caused an increase in both the glutenin and gliadin peaks. Mixing did not seem to enhance the effect of reducing agents as markedly as the effect of oxidizing agents.

Pomeranz (1965) showed that the amount of protein extracted by 3M urea was higher for weak flours than for strong flours. The extractability of protein with this solvent also increased with mixing time (Mamaril and Pomeranz 1966).

Meredith and Wren (1966, 1969) developed a highly effective aqueous

solvent system for solubilizing wheat flour proteins. This solvent comprised 3M urea, 0.1M acetic acid, and 0.01M cetyltrimethyl ammonium bromide (AUC). They and others (Bushuk and Wrigley 1971) have shown that this solvent can extract over 90% of the flour proteins. In the original work using this dissociating solvent Meredith and Wren (1966, 1969) showed that dough mixing increased the extractability of flour proteins from 95% to almost 100%. Using molecular sieve chromatography, they showed that it was the high molecular weight (glutenin) fractions that became more soluble during mixing. There were essentially no changes in protein fractions other than glutenin.

Role of Sulfhydryl and Disulfide Groups

Disulfide bonds are very important in determining the rheological properties of dough. By reacting with sulfhydryl groups, disulfide groups can interchange and provide the mobility (Goldstein 1957; Mecham 1959; Frater et al. 1960; Mauritzen 1963; Sullivan and Dahle 1966) required to relieve the stress imposed by mixing.

During dough mixing only 1 to 2% of all gluten disulfide bonds can be broken by exchange with free SH groups (Mauritzen 1967). As pointed out by Kuninori and Sullivan (1968) and Bloksma (1972), it appears that only a few of the S-S bonds in gluten proteins are crucial to rheological properties of dough. Tsen and Bushuk (1968) showed that total and reactive SH groups, and the ratio of reactive to total SH groups, increased with decreasing mixing strength. On the other hand, total S-S content decreased slightly, whereas the number of reactive S-S groups increased

with decreasing mixing strength. Thus, it appears that mixing strength of flour is inversely related to reactive SH and S-S contents of its proteins.

The effects of oxidizing and SH-blocking agents on the structure (rheological properties) of dough may be explained by the removal of rheologically active SH groups from participation in the interchange of S-S bonds (Bloksma 1968). On the other hand, reducing agents have a more pronounced effect arising from the decrease in the number of S-S bonds and an increase in SH groups.

Interaction with Lipids

Wheat flour lipids play an important role in determining the rheological properties of dough by interacting strongly with the gluten proteins (McCaig and McCalla 1941; Daniels 1963; Daniels et al. 1966). They may also be involved indirectly by reacting with protein SH groups (Balls and Hale 1940; Sullivan 1940; Balls et al. 1942).

The literature on studies of lipid-protein interactions in dough is extremely voluminous. Accordingly, only those references that are considered to be pertinent to the present study will be reviewed.

The strong interaction of flour lipids and proteins during dough formation has been demonstrated by the rapid decrease in the amount of lipid that can be extracted by common lipid solvents. Olcott and Mecham (1947) and Davies et al. (1969) showed that there was a rapid drop in lipid extractability as a consequence of adding water to flour even when mechanical work was excluded. Mixing produced a further decrease in lipid

extractability.

Daniels and co-workers (1966) found that lipid binding increased also with the rate of dough development. In addition, they showed that lipid distribution was markedly affected by the atmosphere used in the dough mixing chamber as well as by the rate and final level of work input (Daniels et al. 1969, 1970).

Most cereal chemists are of the opinion now that flour lipids are essential for the development of a proper bread dough. Indeed, Hoseney et al. (1970) have suggested that phospholipids form an integral part of gluten by forming a highly specific type of secondary linkage between gliadin and glutenin. Solubility data obtained by these workers suggested that phospholipids interact with gliadin through hydrogen bonds and with glutenin through hydrophobic bonds.

There is some evidence that the binding of phospholipids to protein is through ionic or salt linkages. It is well known that extractability of lipid from dough can be increased by the addition of sodium chloride (Mecham and Weinstein 1952). An ionic bond could be formed between the trimethyl ammonium group of lecithin and the negatively charged groups on the protein molecules (Glass 1960, 1962).

Interaction between phospholipids and wheat proteins may take the form of mixed chelation through a divalent metal ion (Fullington 1967, 1969). This interaction is very specific for certain proteins from wheat flour depending on the lipid-metal combination used (Fullington 1969).

In addition to extractability studies, x-ray diffraction studies

have been of considerable value in providing information on lipid-protein interaction in dough and gluten. Savary et al. (1957), on the basis of their x-ray work, suggested that gluten existed as elongated protein fibers held together by phospholipid molecules arranged as bimolecular leaflets between the fibers.

On the basis of similar x-ray diffraction studies, Grosskreutz (1960, 1961) proposed a somewhat different gluten structure. He visualized gluten as a sheet 100-10,000 Å thick and consisting principally of protein platelets about 70 Å thick, held together by a bimolecular layer of phospholipid. The two-dimensional mobility of gluten occurred along the lipid layers.

Several groups of workers have suggested that lipids might be involved in the oxidative improver reactions in dough through their oxidation products (Bushuk and Hlynka 1961; Glass 1962; Tsen and Hlynka 1962, 1963). Glass (1962) postulated that lipids could function as a vital intermediate between oxidative agents and the flour protein. Bushuk and Hlynka (1961) suggested that, in bromated doughs mixed in air, lipid hydroperoxides can effectively compete with the bromate ion for the SH groups. Subsequently, Tsen and Hlynka (1962) confirmed the formation of lipid peroxides in dough during mixing and the oxidation of SH by these peroxides. Dahle and Sullivan (1963) also showed that lipids were oxidized in dough during mixing, but they found only a slight reaction of protein SH groups with the oxidized lipid.

Interaction with Carbohydrates

In recent years considerable evidence has been accumulated which indicates that the carbohydrates of flour interact strongly with its proteins during dough mixing, and thereby have a definite role in the breadmaking process (Kulp and Bechtel 1963; Cawley 1964; Medcalf 1968; Dahle 1971; D'Appolonia and Gilles 1971). For many years starch was considered to be a relatively inert filler in dough (Medcalf and Gilles 1968). However, there is now ample evidence which indicates wheat starch interacts quite specifically in a dough system (Stamberg 1939; Harris 1942; Sandstedt 1961).

Hoseney et al. (1971) showed that, of all the known starches, only barley starch could adequately replace wheat starch in a reconstituted flour. Accordingly, it can be concluded that wheat starch is quite essential for the breadmaking quality of bread flours.

Udy (1957) studied the interaction of gluten proteins with the water soluble pentosans of flour. His results showed that this interaction was through weak, secondary bonds. The degree of interaction was found to depend on the molecular size of the pentosans. Neukom et al. (1967) observed that the flour pentosans can greatly modify the solubility properties of gluten proteins. It is, therefore, essential to eliminate this component from purified preparations of flour proteins in order to determine the true solubility properties of the proteins.

Certain glycolipids, because of their dualistic hydrophobic and hydrophilic character, can have a substantial effect on the rheological

behavior of dough and gluten (Pomeranz 1971). These observations have led to the development of a variety of fatty acid esters of sucrose which are being marketed as bread dough conditioning agents.

Summary

When flour and water are blended together to form a dough the first important physical change is the hydration of the flour components. This then enables the components to become mobile and interact with each other to varying degrees of specificity. With subsequent mixing, the rheological properties of the dough are optimized for the baking process. During this development of optimal properties flour proteins undergo a variety of physicochemical changes. Some of these changes, such as increased solubility and loss of SH, have been studied quite extensively. The mechanism of the latter change appears to be straightforward. However, the mechanism(s) of the interactions and reactions that lead to increased protein solubility (and concomitant changes in rheological properties of dough) are not understood completely. Their complete understanding has immense technological implications in the conversion of wheat into bread.

MATERIALS

Three wheat varieties of widely different dough mixing characteristics but of similar protein contents were selected for this study.

Red River 68. This variety is a hard red spring wheat developed in the U.S. by a private wheat breeding company, World Seeds, Inc. Its parentage, as far as it can be established, is Tezanos Pintos Precoz x Sonora 64A. Red River 68 is a semi-dwarf variety with a strong white stem and red kernels. The peculiar endosperm proteins of this variety are reflected by its overly strong dough mixing characteristics for most standard baking processes.

Manitou. This is a Canadian hard red spring wheat variety developed by the Canadian Department of Agriculture for the rust area of Western Canada. Its parentage is (Thatcher^{7} - Frontana x Thatcher^{6} - Kenya Farmer) x Thatcher^{6} - P.I. 170925. It is essentially the same as the Thatcher variety but contains two extra genes for stem rust resistance, Sr 6 from P.I. 170925 (a red Egyptian wheat) and Sr 7 from Kenya Farmer, and a gene for leaf rust resistance from Frontana. This variety was licensed in Canada in 1965, and in 1971 formed over 40% of the wheat acreage of western Canada. Compared with Thatcher, Manitou has better rust resistance, gives higher yields, is earlier maturing, is usually slightly higher in protein content, and is therefore better in overall breadmaking quality. It is considered to be of medium mixing strength.

Talbot. This variety is a soft white winter wheat grown in the winter wheat areas of Ontario. Its parentage is (Trumbull - Hope - Hussar) x F. (of Dawsons G.C.^{2} - Ridit Cornell 595). It was licensed in Canada in 1963 because of its superiority in lodging resistance compared with the then leading soft white winter variety, Genesee. This variety was selected for the present study because it has very weak mixing characteristics compared with the two hard red spring wheat varieties.

Some pertinent technological properties of the wheat and flour of the three varieties are given in Table 1. The flour was milled on an experimental Buhler mill using an overnight tempering to 16.5% moisture and the same milling procedure for all three wheats.

Table 1. Pertinent Technological Data on Flours From the Three Wheat Varieties.

	Red River 68	Manitou	Talbot
Moisture, %	13.6	13.6	14.0
Protein, %	10.9	10.8	11.3
Ash, %	0.57	0.45	0.46
Starch Damage, %	33	35	5
Gassing Power, mm.	350	350	265
Diastatic Activity, mg.	214	244	111
Amylogram Viscosity, B.U.	960	810	395
Falling Number, sec.	490	390	300
Loaf Volume, cc.	600	580	680

METHODS

Proteolytic Activity Determinations of Flour

Proteolytic activities of the flours were determined by a modified Ayre-Anderson method (Chua 1969) using hemoglobin as the substrate and the Lowry method (Lowry et al. 1951) to estimate the amount of tyrosine released during the incubation. The activity was expressed as μ moles of tyrosine formed per minute per g. of flour.

Preparation of Doughs

Doughs were mixed in the farinograph mixer using the 50-g. bowl and the normal mixing speed (63 r.p.m.). The constant flour procedure was used, and the water absorption was varied to give a peak consistency of 500 B.U. The absorptions were 63.8, 63.4 and 53.0% for Manitou, Red River 68 and Talbot respectively. Doughs containing various additives were mixed in air and nitrogen for various times. The experimental variables that were examined are listed in Table 2.

A special cover for the mixer bowl, with vents to permit circulation of gas and addition of the liquids, was fabricated. For doughs mixed under nitrogen the farinograph bowl was flushed with nitrogen gas before the flour was introduced, and the flow was continued for 5 minutes with the mixer running prior to the addition of the liquids. The liquids were deaerated by bubbling nitrogen through them for 5 min. Air was excluded from the bowl throughout the mixing by a continuous positive pressure of nitrogen.

Table 2. Experimental Conditions under Which Doughs Were Mixed.

Atmosphere	Air		Nitrogen	
Mixing time	5 min.	15 min.	5 min.	15 min.
Controls	0*	0	0	0
+NEMI	0.4	0.4	0.4	0.4
(μeq./g. flour)	2.0	2.0	2.0	2.0
+Iodate	0.4	0.4	0.4	0.4
(μeq./g. flour)	2.0	2.0	2.0	2.0

*No additives besides flour and water.

After an appropriate mixing time the dough was quickly frozen by immersion in liquid nitrogen. The frozen dough was freeze-dried, pulverized by hand, and ground on a coffee grinder. The ground samples were kept in a laboratory refrigerator (4°C) and removed as required for analyses.

Exhaustive Extraction of Protein with 0.05 N Acetic Acid

One gram of freeze-dried ground dough or flour was dispersed in 17 ml. of 0.05 N acetic acid and extracted for various periods in a cold-room (4°C) in a Potter and Elvejhem homogenizer. Extraction times used were 5, 10, 30, 60, and 120 min. The suspensions were centrifuged and the supernatants were freeze-dried. The protein contents of the dried solids were determined by the macro Kjeldahl method.

Extraction and Fractionation of Proteins

The proteins of the ground dough and flour samples were extracted and fractionated by a modified Osborne procedure as described by Chen and Bushuk (1970). The procedure shown schematically in Fig. 1 is as follows.

Flours (10g. d.b.) were first extracted with 70 ml. of 0.5 M. sodium chloride solution by mild stirring with a magnetic stirrer in a centrifuge bottle for 1 hr. Each suspension was centrifuged for 30 min. at 1800 xg and the supernatant was decanted. This was followed by a second extraction with 50 ml. of 0.5 M. sodium chloride solution for 1 hr. The residue was washed with 50 ml. of distilled water for 10 min. to remove residual salt. The three supernatants were combined, dialyzed against cold distilled

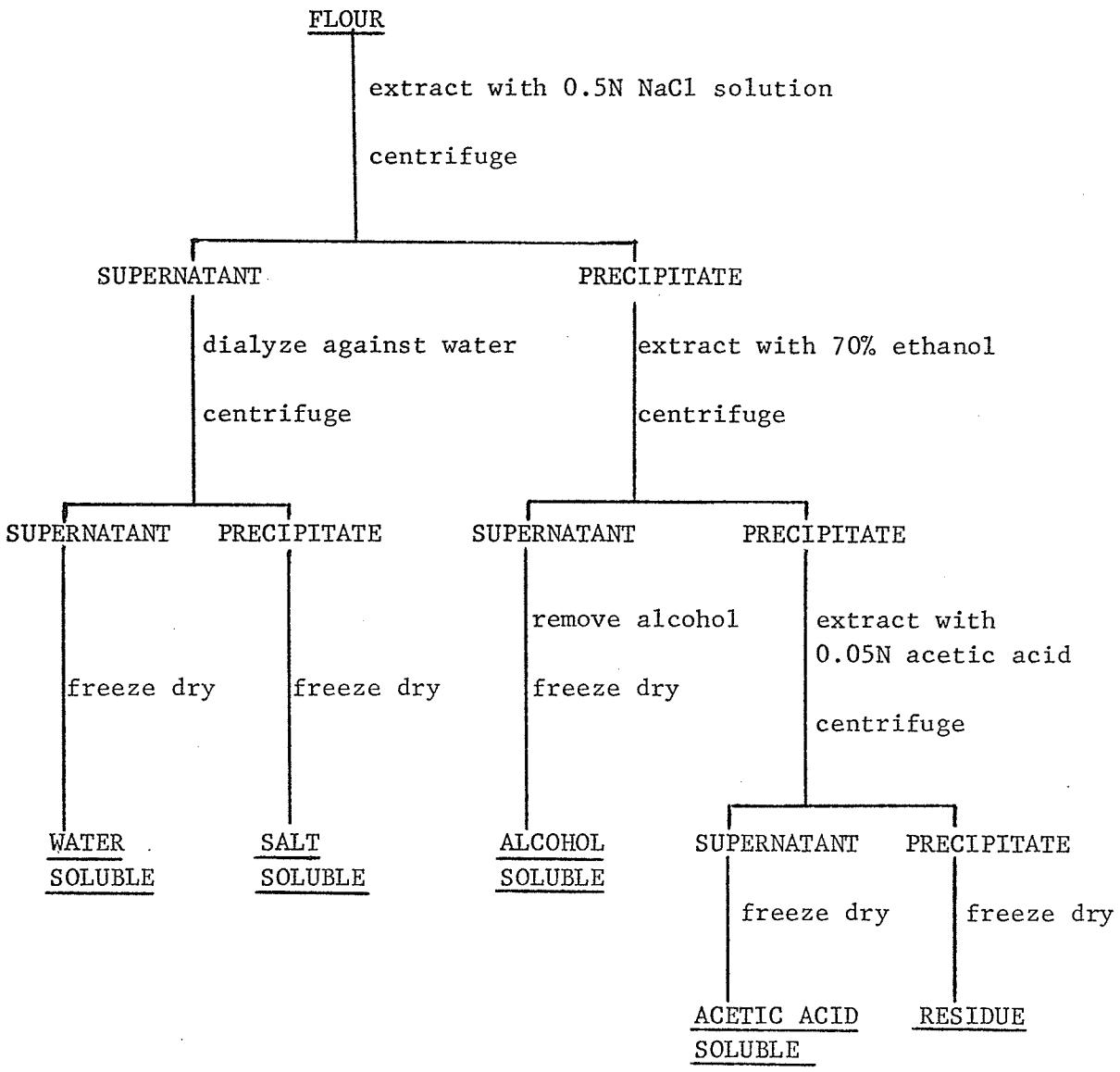

Fig. 1. Summary of Protein Fractionation Procedure.

water for 48 hr., and centrifuged to separate the precipitated salt-soluble proteins. Water-soluble proteins remained in the supernatant. The residue remaining after extraction of the flour with salt solution was suspended consecutively in 70 ml. and then 50 ml. portions of 70% ethanol solution. After each suspension, the liquids were separated by centrifugation and combined. The resulting residue was further extracted twice with 0.05 M. acetic acid solution, again using 70 ml. and then 50 ml. portions. Ethanol was removed from the combined ethanol supernatants in a rotary evaporator. The four soluble fractions and the final residue were freeze-dried, weighed, analyzed for protein content by a modified automated Kjeldhal procedure (Mitcheson and Stowell 1970), and stored in a refrigerator. The five protein fractions obtained by this method are: (1) albumins (water-soluble proteins), (2) globulins (salt-soluble proteins), (3) gliadins (alcohol-soluble proteins), (4) glutenins (acetic-acid soluble proteins), and (5) insoluble residue. All extractions were made in a cold room (4°C) to minimize effects of enzyme or thermal denaturation. The fractions were subsequently examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Gel Filtration

The proteins of freeze-dried ground doughs and flours (1 g.) were extracted in a Potter and Elvejhem homogenizer for 15 min. with 20 ml. of the AUC solvent of Meredith and Wren (1966). AUC is a strongly dissociating solvent containing 0.1 M. acetic acid, 3 M. urea, and 0.01 M. cetyl trimethylammonium bromide in distilled water. The mixture was

centrifuged for 25 min. at 20,000 x g. The supernatant was used for gel filtration chromatography after additional centrifugation for 30 min. at 100,000 x g. to further clarify the solution.

Preparation of the chromatographic columns and their use for fractionating AUC extracts of flour or freeze-dried doughs was as described by Hwang (1972). This procedure uses Sephadex G-150 as the support material and an upward flow of the eluting solvent. The upward flow is preferred since it increases the number of times that the column can be used without repacking.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was used according to the procedure of Orth and Bushuk (1972B). The use of SDS-PAGE for separation and determination of molecular weight of proteins was originally described by Weber and Osborn (1969). This technique was first applied to wheat glutenin by Bietz and Wall (1972). This method allows determination of the number and molecular weights (MW) of protein units in a complex mixture. When applied to reduced proteins, it can be used to determine the number and size of polypeptide subunits that are joined by S-S bonds to form large protein molecules. This aspect is particularly relevant to the structure of glutenin.

SDS complexes of reduced glutenin were prepared for electrophoresis as follows. The protein (10 mg.) was shaken overnight at 40°C in 1 ml. of protein solvent containing 1% (w/v) SDS and 1% (v/v) β-mercaptoethanol. This allowed for reduction and complexing with SDS. Sucrose (10% w/v) was added to the SDS-protein solution to increase the density, and bromophenol

blue (20 µl. of 0.3% solution per 1 ml. of protein solution) was included as the electrophoretic front marker. Proteins of known molecular weight, required for calibration, were complexed with SDS as above at a concentration of 1 mg./ml.

Where a comparison between reduced and intact proteins was required, a separate electrophoresis experiment was carried out. For this experiment, the SH groups of the intact protein were reacted with N-ethylmaleimide. To obtain a complete reaction 10 mg. of protein was allowed to react with 10 mg. of N-ethylmaleimide in 1 ml. of solution overnight at 40°C.

Myoglobin (10 µl. of 1 mg./ml. solution) was included as a reference protein in each SDS-PAGE experiment and was placed always in the first left slot on the gel. For this electrophoresis, the residue proteins were extracted from 2-g. samples with 20 ml. of AUC by stirring overnight in a cold room (4°C). The suspension was centrifuged at 20,000 x g. for 25 min. and the supernatant was freeze-dried. The freeze-dried protein was reduced and complexed with SDS as described above.

Determination of Sulfhydryl and Disulfide Contents

Sulfhydryl and disulfide contents of the samples were determined by amperometric titration as developed by Sokol et al. (1959) and modified by Tsen and Anderson (1963). According to this method, SH content is measured by titration with silver nitrate. S-S groups are first reduced to sulfhydryl groups by pretreatment with sodium sulfite, and then the total SH is determined by titration. Disulfide content is calculated

from the difference in SH content after and before reduction.

Determination of Amino Groups

The content of amino groups in the proteins that could be solubilized in 0.17 N (1%) acetic acid was determined for all dough and flour samples. The soluble proteins were first extracted with the acetic acid solution, using a flour to solution ratio of 1 to 10, by magnetic stirring for two hours in a cold room. The suspension was centrifuged at 20,000 x g. for one hour and the supernatant was used for analysis of amino groups. This extraction procedure solubilized about 70% of the total protein for all three varieties used in this study. For the analysis, an aliquot (0.025 ml.) of the clear supernatant was mixed with 1 ml. of 2% ninhydrin reagent in a test tube. The mixture was heated at 100^{o}C for 30 min. to develop the characteristic purple color, and the optical density of the solution at 570 nm. was determined on a Zeiss spectrophotometer against a water blank. The amount of amino nitrogen, expressed in micro equivalents per g. protein, in the aliquot was read off a standard curve which was constructed using a series of solutions, each containing a mixture of known amounts of the 18 common amino acids.

Extraction of Free Lipid

Free lipids were extracted from 3 g. of ground dough or flour with 50 ml. of petroleum ether in a Soxhlet extraction apparatus for 8 hrs. The amount of free lipids was weighed after the solvent was evaporated.

RESULTS AND DISCUSSION

The main purpose of this study was to examine the changes in the proteins of wheat flour that occur during dough mixing. Accordingly most of the experiments were designed with this purpose in mind. A number of ancillary experiments involving other flour components were carried out because of their possible involvement in the changes in the proteins. The results of the investigation will be presented and discussed in a series of sections that follow.

Farinograph Mixing Curves

A farinograph mixer was used to mix the doughs from the three flours to various degrees of development (or breakdown) using 5- and 15-min. mixing times and the normal mixing speed of 63 r.p.m. (slower blade). Doughs were mixed in air and in nitrogen with additions of potassium iodate and of N-ethylmaleimide (NEMI) at two concentrations, 0.4 and 2.0 μeq. per g. of flour. These chemicals were used because of their ability to accentuate mixing breakdown, especially when they are used at concentrations higher than those used for optimal improver effect (Meredith and Bushuk 1962). In all, a total of 60 doughs were mixed and freeze-dried for subsequent analyses.

Figures 2, 3 and 4 show the farinograms for Red River 68, Manitou and Talbot flours respectively. Only the 15-min. curves are shown. Farinograms for the doughs containing 0.4 μeq./g. of iodate and NEMI are not shown. The effects of the two chemicals at this lower concentration on the

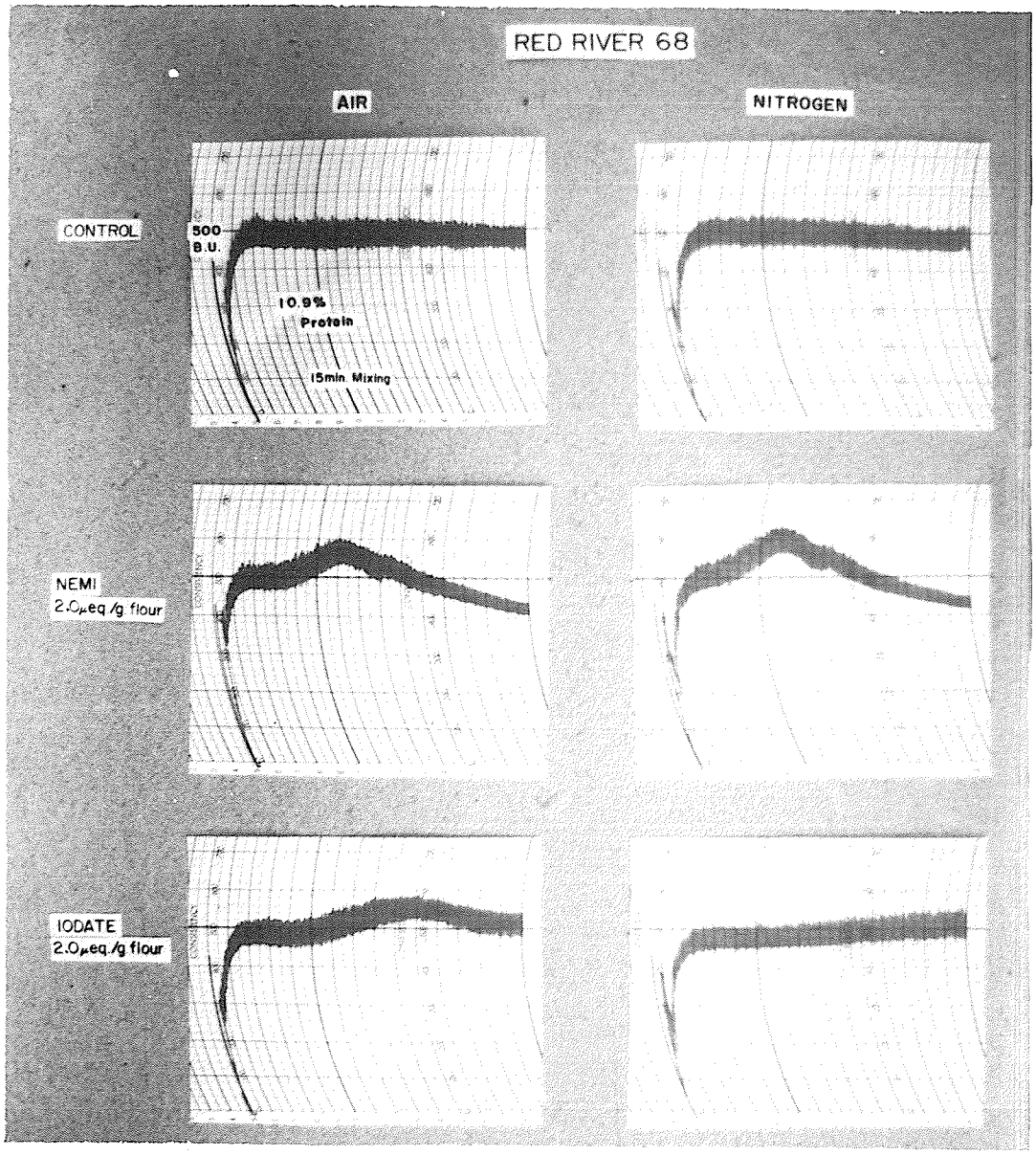

Fig. 2 Farinograms for Red River 68.

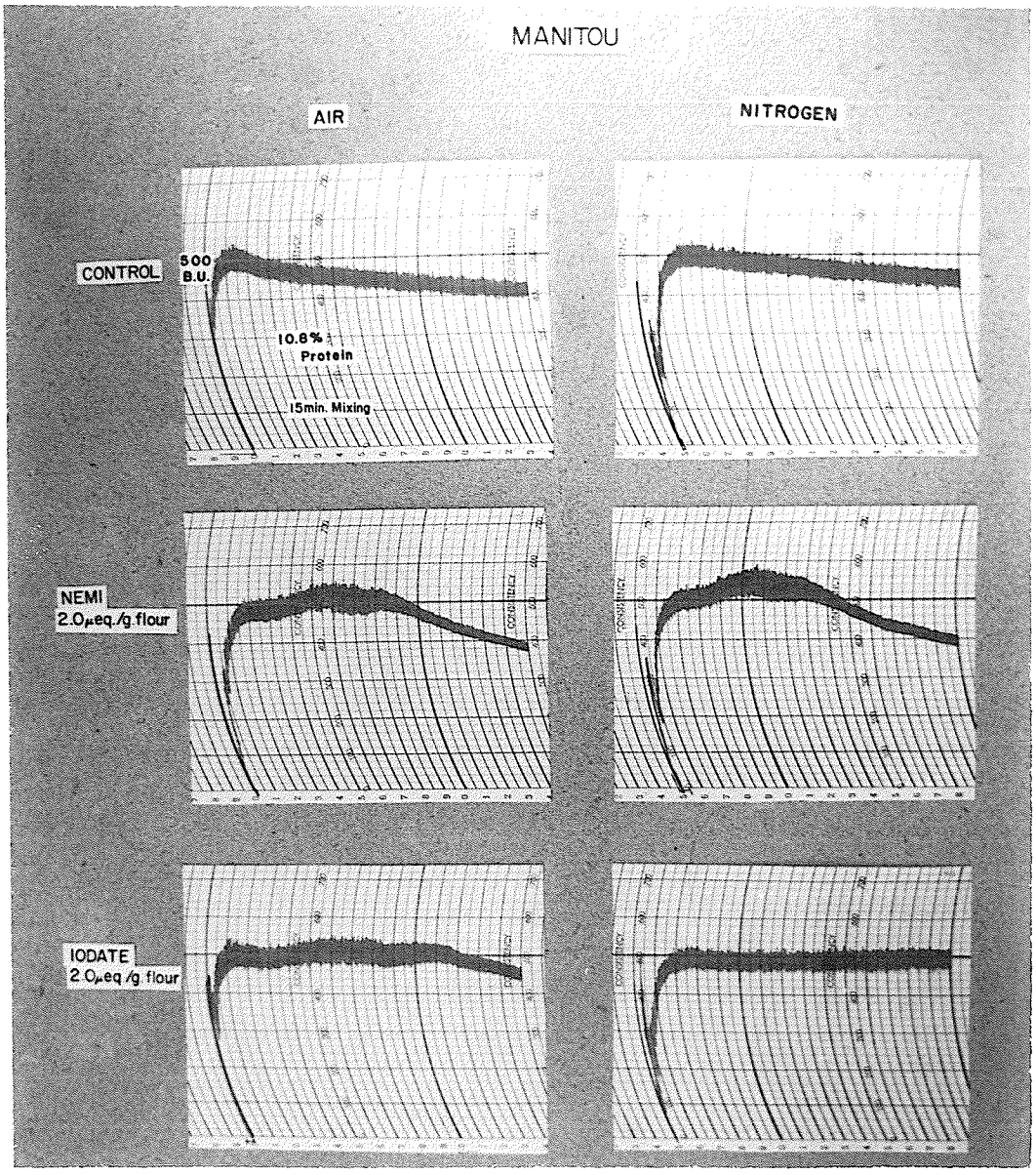

Fig. 3 Farinograms for Manitou.

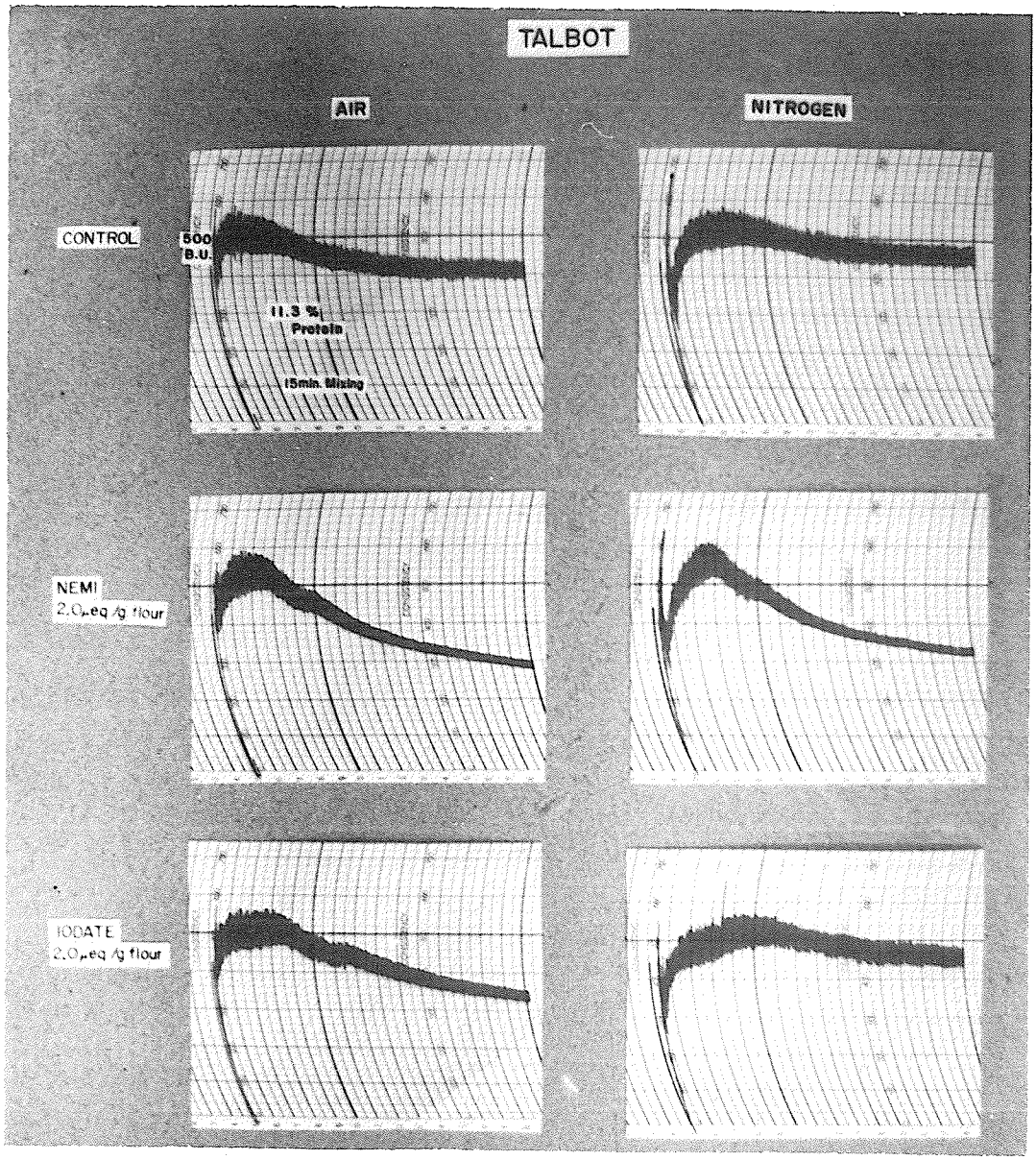

Fig. 4 Farinograms for Talbot.

farinograms were considerably less than those at the higher concentration.

The farinograms for the control doughs (mixed in air; no additives) showed that the three varieties selected covered a relatively wide range of mixing properties from very strong through medium strong to weak. When the same doughs were mixed in nitrogen, all farinograms showed an increase in strength. The effect of oxygen on mixing properties, which has been extensively studied (see literature review), appeared to be more prominent in the weak dough than in the strong dough.

The effects of iodate and NEMI on farinograph mixing properties were the same as obtained by others (Meredith and Bushuk 1962). In doughs mixed in air, both chemicals increased the rate of breakdown after the optimum. Doughs containing iodate showed the additional competitive effect of oxygen which was not apparent at the higher level of NEMI. In the stronger doughs (see Figs. 2 and 3) iodate and NEMI slowed down the attainment of maximum consistency. The iodate-containing doughs mixed in nitrogen did not show any breakdown, even after 15 min. of mixing. Analogous doughs from a flour of higher protein content (milled from a wheat variety similar to Manitou) showed a breakdown after about 10 minutes of mixing (Meredith and Bushuk 1962). Accordingly, it appears that, for a specific variety of wheat, the rate of dough breakdown during mixing is dependent on the protein content of the flour. Doughs from flours of lower protein content developed slower and therefore suffered less breakdown beyond optimum development after a specific total mixing time.

The mixing results presented above indicate that the varieties and

mixing conditions selected for this investigation cover, and extend beyond the range of mixing properties normally encountered in baking technology.

Proteolytic Activity of the Flours

Proteolytic enzymes degrade protein molecules into smaller fragments. Flours with high proteolytic activity produce doughs that break down readily during mixing. Accordingly, some cereal technologists have suggested that significant differences in mixing strength could result from very small differences in proteolytic activity (Bushuk et al. 1971).

The proteolytic activities of the three flours used in this study are given in Table 3. Although the activities were low and the absolute differences among the three varieties small, the ranking of varieties according to proteolytic activity followed the order according to mixing strength, with the weakest dough being from the flour having the highest activity. The results for the three varieties used in this study fit the hypothesis that mixing strength is inversely related to proteolytic activity.

Formation of Free Amino Groups During Mixing

If proteolytic enzymes are active during mixing of doughs, then it should be possible to detect this activity by the determination of amino groups that would be formed. Accordingly, the content of amino groups was determined for the doughs used in this study (Table 4). Talbot flours and doughs had the highest, Manitou doughs medium, and Red River 68 doughs the lowest amino group contents. These results are consistent with the order

Table 3. Proteolytic Activity* of the Flours.

Flour	Activity x 10^3
Red River 68	1.14
Manitou	1.25
Talbot	1.36

*Activity --- μeq. tyrosine/min./g. flour.

Table 4. Contents of Amino Group of Flours and Doughs Mixed under Various Conditions.

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
Flour	2.3				2.8				3.4			
Control	2.3*	2.5	2.5	2.4	3.0	3.0	2.8	2.8	3.4	3.7	3.7	3.6
NEMI												
0.4 µeq./g. flour	2.6	2.4	2.7	2.6	2.7	3.0	3.0	2.9	3.2	3.4	3.4	3.4
2.0 µeq./g. flour	2.6	2.5	2.6	2.5	2.7	2.8	3.2	3.0	3.3	3.3	3.5	3.3
Iodate												
0.4 µeq./g. flour	2.7	2.6	2.6	2.7	3.1	2.8	2.7	3.1	3.5	3.3	3.9	3.7
2.0 µeq./g. flour	2.5	2.6	2.8	2.7	3.1	3.0	3.2	3.2	3.5	3.2	3.8	3.8

* µeq./mg. protein x 10^2

of proteolytic activities for the three varieties.

However, for doughs of any one variety, the amino group content remained constant during mixing up to 15 minutes. Mixing in nitrogen instead of air or in the presence of iodate or NEMI also had no effect on the amino group content. Presumably the level of activity in the flour is not sufficient to produce a change in the amino group content that could be detected by the analytical procedure used.

Exhaustive Extraction of Protein with 0.05N Acetic Acid

This experiment was included to investigate the possibility that mixing breakdown results from depolymerization of protein molecules rather than disaggregation of protein complexes. It was postulated that depolymerization would lead to increased maximum extractability whereas disaggregation would not, since, in exhaustive extraction, all the protein aggregates would be eventually destroyed. In this experiment, dry ground dough samples were dispersed in 0.05N acetic acid solution in a Potter and Elvejhem homogenizer at high speed. Extraction was continued until no more protein could be solubilized even by the high shear forces of the homogenizer used. The specific doughs selected for this exhaustive extraction were those that showed greatest differences in mixing properties.

Representative results are presented in Figure 5, and complete data in Tables 1 to 3 in the Appendix. Figure 5 shows the amount of extractable protein (as a percentage of total) as a function of extraction time. The top part of the figure gives the curves for the strongest variety, the

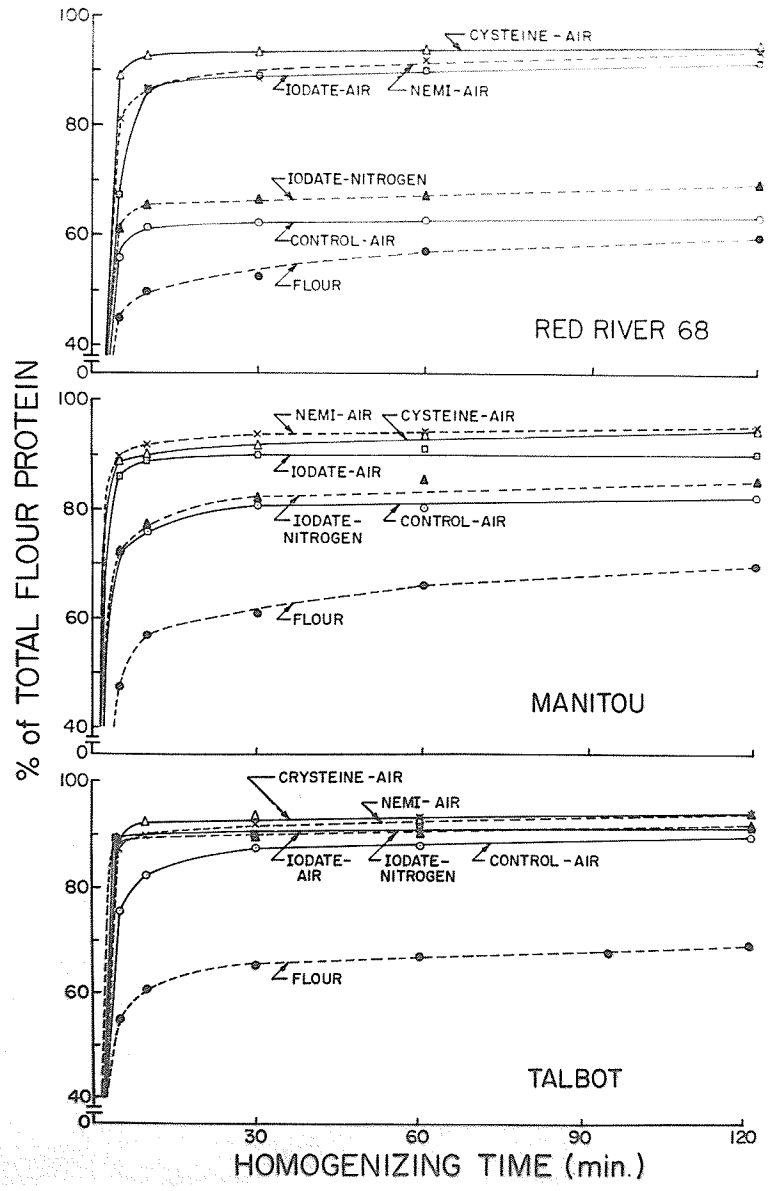

Fig. 5 Exhaustive extraction of the protein with 0.05N acetic acid from the flours and various doughs mixed for 15 minutes.

middle for the next strongest, and the bottom for the weakest.

Results shown are for doughs containing 2.0 μeq. of iodate or NEMI. In addition to the regular doughs, for this experiment only, doughs containing 2.0 μeq. of cysteine hydrochloride were prepared for comparison purposes. Results in Fig. 5 are for doughs mixed for 15 minutes.

Results for the control doughs will be discussed first. The final extractability for the strongest variety was 61%. This value was only 3 percentage units above the value for the flour. This small increase in extractability results from 15 minutes of mixing. Analogous data for the two weaker varieties in order of decreasing strength were 66 and 69% for total extractability and 15 and 20 percentage units for the increase during 15 minutes of mixing. Increases in extractability of the flour proteins by dough mixing were observed by others (Mecham et al. 1962; Tsen 1967). However the differences among flours of different strength were not emphasized previously.

The results for the control doughs suggest that the main direct effect of mixing is the disaggregation of flour particles and protein aggregates as postulated by Tsen (1967). If the curves for the flours and the 15-minute doughs mixed in air are compared, it is seen that the ultimate protein extractability is higher for the doughs than for the flours. Also the extractability increases faster with extraction time for the control doughs than for the flours. Additions of chemicals such as iodate, NEMI or cysteine produced additional effects. These effects appear to be dependent on the degree of mixing. These are discussed below.

When the doughs were mixed in the presence of cysteine, iodate or NEMI, there was a marked increase in extractability. Under these conditions extractabilities of over 90% were obtained for some of the doughs (see Fig. 5).

The magnitude of the effect of added chemicals depends on the mixing strength of the flour (wheat variety). In doughs of the weakest flour, the additional effect of additive was small. It was greatest in doughs of the strongest flour and intermediate in the Manitou doughs which were also of intermediate strength. In the doughs of the two stronger varieties, the effects of NEMI and iodate-air were analogous to that of cysteine. The effect of NEMI on protein extractability was the same in doughs mixed in air and in nitrogen. On the other hand, iodate without atmospheric oxygen, increased the extractability only slightly above the values for the control doughs. These results are consistent with those of Meredith and Bushuk (1962), who showed that the effects of iodate and oxygen on the physical dough properties appear to be synergistic.

On the basis of the results presented here, it is postulated that the additional increase in extractability caused by iodate and air and by NEMI is due to depolymerization of protein molecules by the combined action of mixing and the reagents. The similarity of the effects of these chemicals to the effect of cysteine suggests that disulfide bonds are probably involved in this depolymerization.

Effect of Dough Mixing on Solubility of Flour Proteins in Various Solvents

The proteins of the flour and freeze-dried ground dough samples were extracted and fractionated on the basis of solubility by the modified Osborne technique. This fractionation gives five fractions: (1) water-solubles, (2) salt solution solubles, (3) ethanol solution solubles, (4) acetic acid solution solubles, and (5) insoluble residue protein. It was felt that results of this fractionation would provide some evidence as to the mechanism of dough breakdown during mixing. Specifically, the working hypothesis was that, if the solubility fractionation is essentially complete, then disaggregation during dough mixing should not affect the subsequent fractionation results. On the other hand, depolymerization during mixing probably should lead to major changes in some of the fractions.

The data for the solubility fractionation are given in Tables 4 to 9 in the Appendix. The amounts of water-soluble protein and a salt-soluble protein were not affected by dough mixing under the conditions used in this study. Accordingly these data will not be considered any further.

The changes in the amount of alcohol-soluble protein with mixing in the control doughs and those treated with 2.0 μeq. of iodate or NEMI are shown in Fig. 6. The results for the lower concentration of iodate or NEMI were intermediate between the analogous values for the controls and the doughs that were treated with 2.0 μeq. of each reagent. These were not included in Fig. 6.

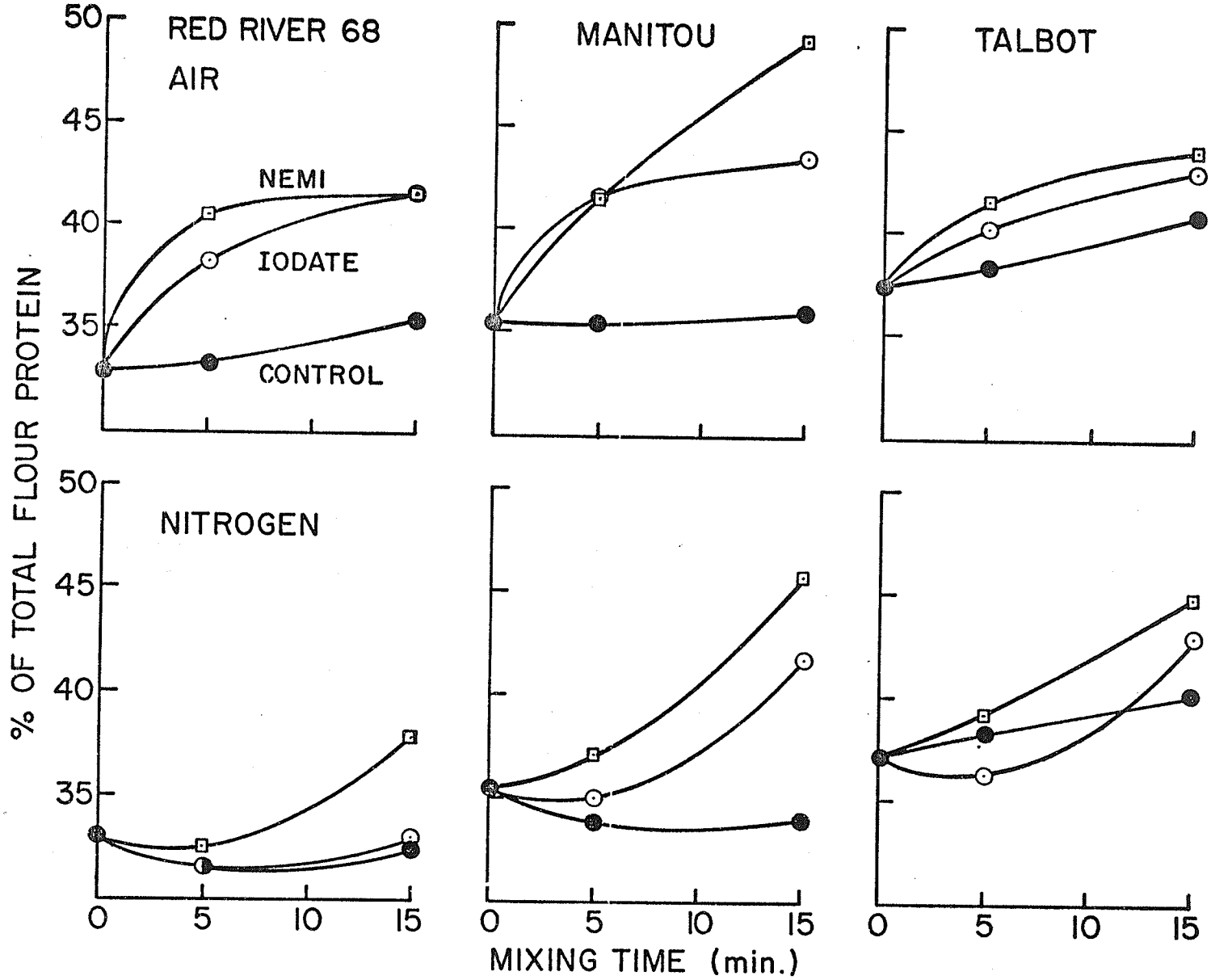

Fig. 6 Changes in the amount of alcohol-soluble protein during mixing of various doughs from flours of the three wheat varieties.

In the controls mixed in air, there was a gradual slight increase in the amount of alcohol-soluble protein with mixing. Additions of iodate or NEMI produced a large increase in this amount. Most of this increase occurred during the first five minutes of mixing. Additional 10 minutes of mixing produced very little further increase, except in the case of the Manitou dough containing NEMI. This dough showed a continuous, almost linear, increase in the amount of this protein with mixing.

The behaviour of the doughs mixed in nitrogen was quite different. In some cases, there was a slight decrease in the amount of alcohol-soluble protein during the first 5-minutes of mixing. Again, the control doughs showed little change. Addition of iodate or NEMI increased the amount of this fraction. The increase was almost negligible after 5 minutes of mixing but became quite large after 15 minutes of mixing. That is, the effects of the two chemicals and mixing appear to be synergistic. On basis of the amount of alcohol-soluble protein, the analogous 5-minute doughs mixed in air and nitrogen appear to be quite different. These differences became negligible after 15 minutes of mixing except for the iodate dough which contained significantly more alcohol-soluble protein when mixed in air than when mixed in nitrogen.

Comparison of the results for the three wheat varieties, shows that the magnitude of the effects of iodate and NEMI depends on variety. The two stronger varieties were affected much more than the weak one. These different susceptibilities might well be related to the subtle differences

in physicochemical properties that are important to functional qualities, e.g., breadmaking.

The changes in the amount of acetic acid-soluble protein with mixing for selected doughs are shown in Fig. 7. It is seen that, in most cases, there was an increase with mixing. Oxygen, iodate and NEMI each produced an additional increase. For the weakest variety, Talbot, the amount of this fraction was maximum after 5 min. mixing in the doughs containing iodate and NEMI. Subsequently there was a slight decrease as mixing was extended to 15 min.

There are several other interesting features in the results of Fig. 7. The effect of oxygen with iodate is synergistic, but not so with NEMI. Also, the effect of iodate in doughs mixed in air and in nitrogen increases in the ascending order: Manitou, Red River 68, Talbot. This result may be due to differences in accessibility of the component(s) that react with iodate. This factor does not appear to be important in the reaction with NEMI.

The changes in the insoluble residue protein with mixing for selected doughs are shown in Fig. 8. In all cases, the amount of residue protein decreased with increasing mixing time. Although the decrease for the control doughs mixed in nitrogen was extremely small, the decreasing trend was still quite obvious. Oxygen, iodate and NEMI each produced additional decreases in the amount of residue protein. The effects of the higher concentrations (2.0 μeq./g.) of iodate and of NEMI were extremely drastic. Again the synergistic effect of oxygen with iodate was particularly evident,

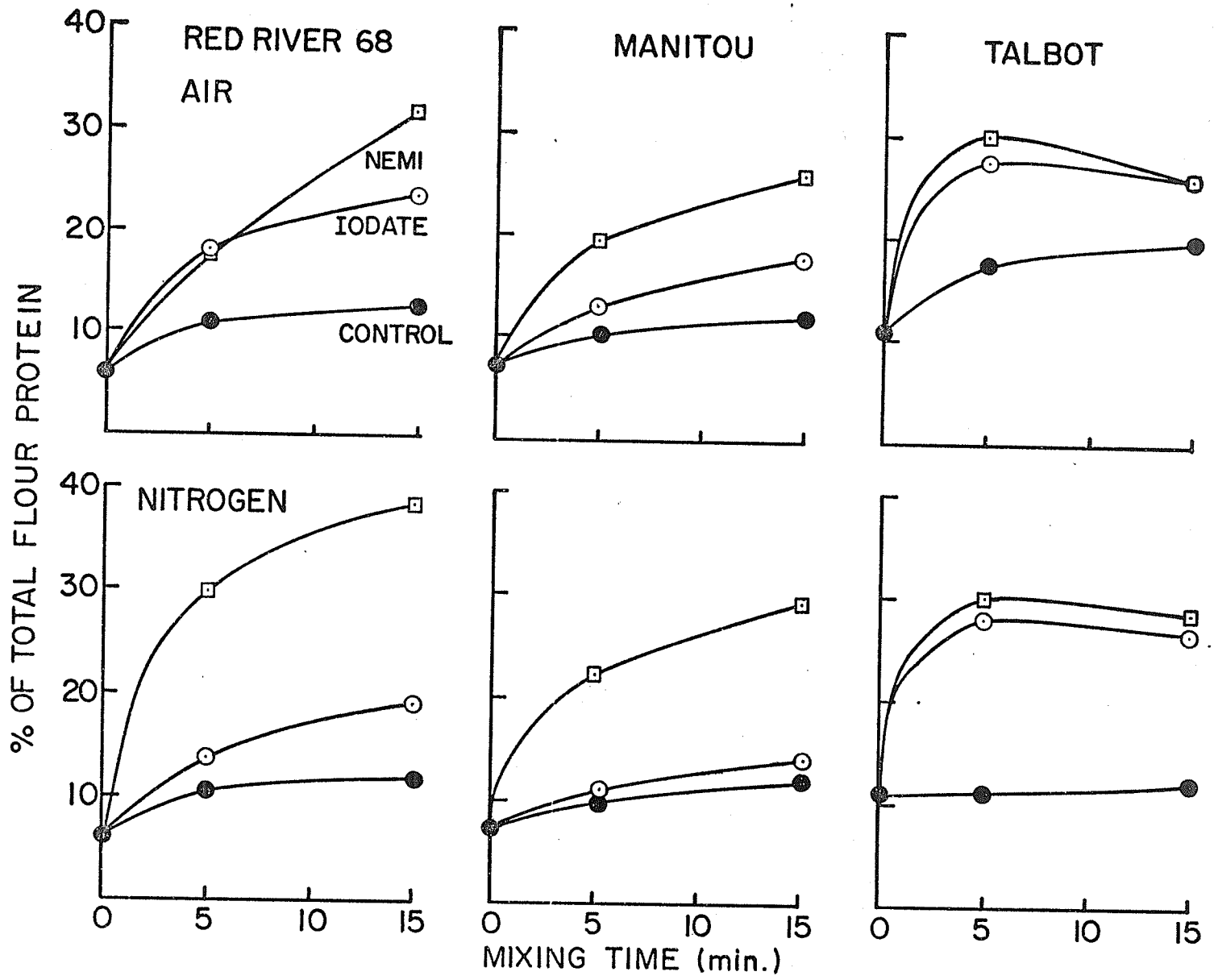

Fig. 7 Changes in the amount of acetic acid-soluble protein during mixing of various doughs from flours of the three wheat varieties.

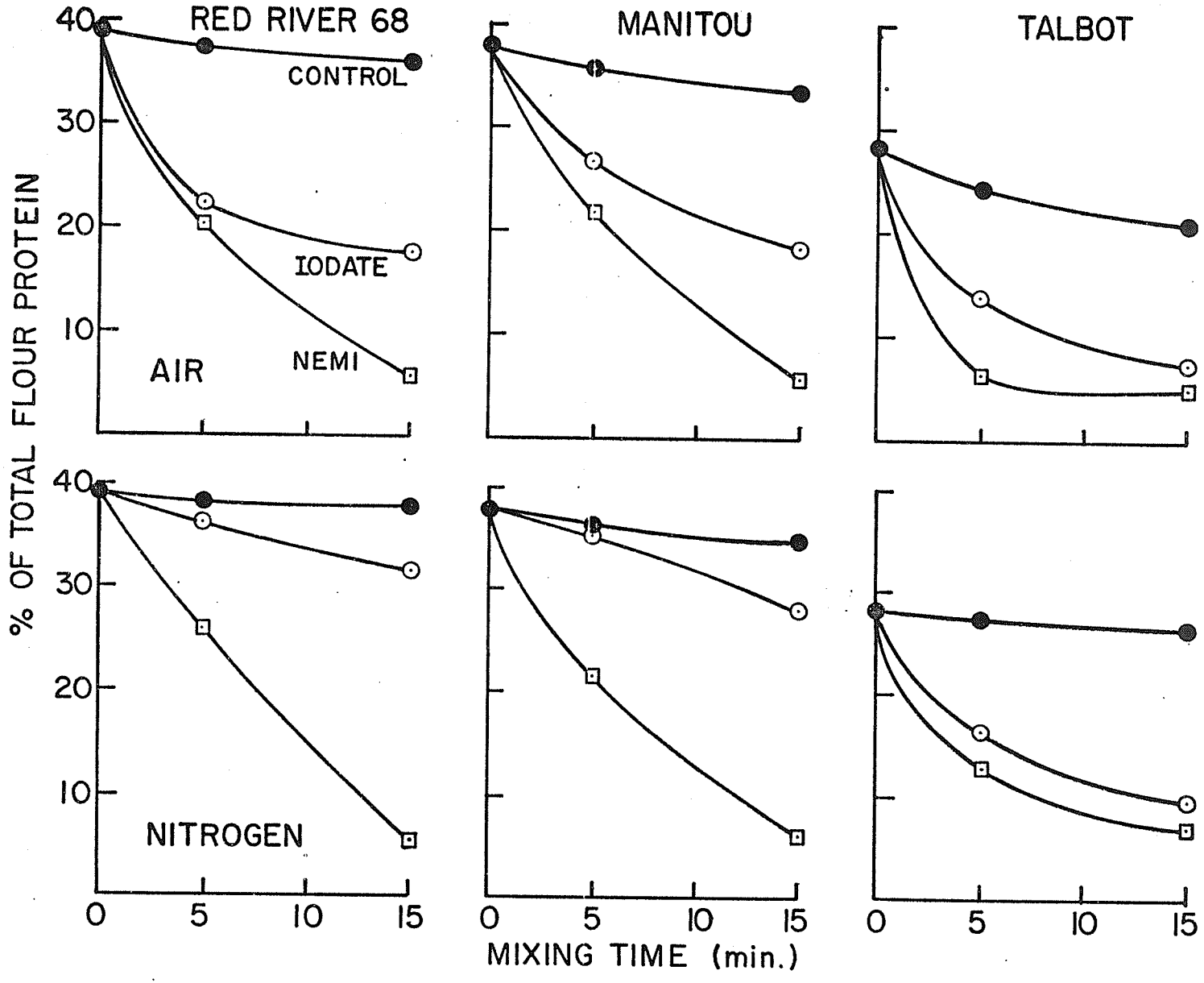

Fig. 8 Changes in the amount of residue protein during mixing of various doughs from flours of the three wheat varieties.

especially for the two stronger varieties.

The major shift in the solubility distribution on mixing is from insoluble residue to acetic acid-soluble protein and in some cases probably to the alcohol-soluble fraction. It should also be noted that the amount of acetic acid-soluble protein in the flour increased while the amount of residue protein decreased with decreasing mixing strength. Red River 68 flour contained 6.2% of the acetic acid-soluble fraction and 39.2% of the residue fraction. Analogous figures for Manitou and Talbot flours are 7.3 and 38.0 and 11.0 and 28.3% respectively. These results are consistent with those of Mullen and Smith (1965) and with those of Bushuk and coworkers (Orth and Bushuk 1972; Tanaka and Bushuk 1972).

In summary, it can be stated that the amounts of acetic acid-soluble protein and residue protein changed drastically, on mixing, both in air and in nitrogen (Figs. 7 and 8). The increases in the alcohol-soluble fraction were relatively lower. The amount of residue protein decreased with mixing while the amount of acetic acid-soluble protein increased, and the longer the mixing time the greater the change. NEMI enhanced these mixing changes considerably in both air and nitrogen. Iodate also enhanced these changes but not as strongly as did NEMI, especially when mixed in nitrogen.

Average total recoveries of protein (of all samples including flours and doughs) by solubility fractionation were 95.4, 95.0 and 92.5% for Red River 68, Manitou and Talbot respectively. These recoveries appear to be related to dough mixing strength, but were not affected by mixing condi-

tions or by the additives investigated in this study. The weaker flours apparently contain larger quantities of low molecular weight nitrogen compounds (free amino acids and peptides), so the lower recovery could be attributed to losses of such compounds during dialysis in the separation of the salt solubles from the water solubles.

In general, the results of the solubility fractionation experiment are consistent with the hypothesis that protein depolymerization occurs during dough mixing and eventually causes a decrease in dough consistency. However, the same results could also be explained on the basis of the disaggregation hypothesis if it is assumed that the disruption of the insoluble gluten complex will increase the extractability of the protein fractions that are removed by the sequence of solvents used. Presumably mixing in air or in the presence of certain chemicals such as iodate or NEMI could disrupt the gluten complex and thereby make the previously unextractable proteins more available to the extracting solvent. Accordingly, it is not possible to confirm or disprove either of the two hypotheses that were postulated on the basis of the fractionation results presented.

Gel Filtration of AUC Extracts of the Flours and Freeze-Dried Doughs

The highly dissociating solvent AUC (aqueous solution of acetic acid, urea and cetyltrimethyl ammonium bromide) solubilizes about 95% of flour protein (Meredith and Wren 1966; Bushuk and Wrigley 1971). Furthermore, it is presumed that the extracted proteins in this solvent exist as a molecular solution and not as aggregates. Fractionation of the proteins

in this solvent can be achieved by gel filtration on Sephadex (G-150 or other porosity). Accordingly, this technique was selected for the present study since it should readily distinguish between the "disaggregation" and "depolymerization" hypotheses of dough breakdown during mixing. If dough breakdown occurs by the former mechanism, then there would be no changes in the gel filtration elution profiles of extracts of doughs at different degrees of breakdown. On the other hand, if dough breakdown is due to depolymerization of proteins, then the elution profiles should show gradual changes with an increase in lower molecular weight components and a decrease in higher molecular weight components.

Figure 9 shows gel filtration elution curves for the flours of the three varieties and for various dough samples of each which were selected to represent extremes in mixing properties. The four dough samples selected for discussion were a control dough mixed for 15 min. in air, the same dough treated with the higher concentration (2.0 μeq./g.) of iodate and of NEMI, and another dough mixed for 15 min. in nitrogen with iodate (2.0 μeq./g.). Gel filtration results for the other doughs were also obtained, but are not included since they were essentially the same as the results for the control dough mixed for 15 min. in air.

The main difference between the profiles for the stronger mixing varieties (Red River 68 and Manitou) and the weaker mixing variety (Talbot) is that the former contain a considerably higher proportion of the components with molecular weights above 25,000. This is in general agreement with the solubility fractionation results if it is assumed that, for each

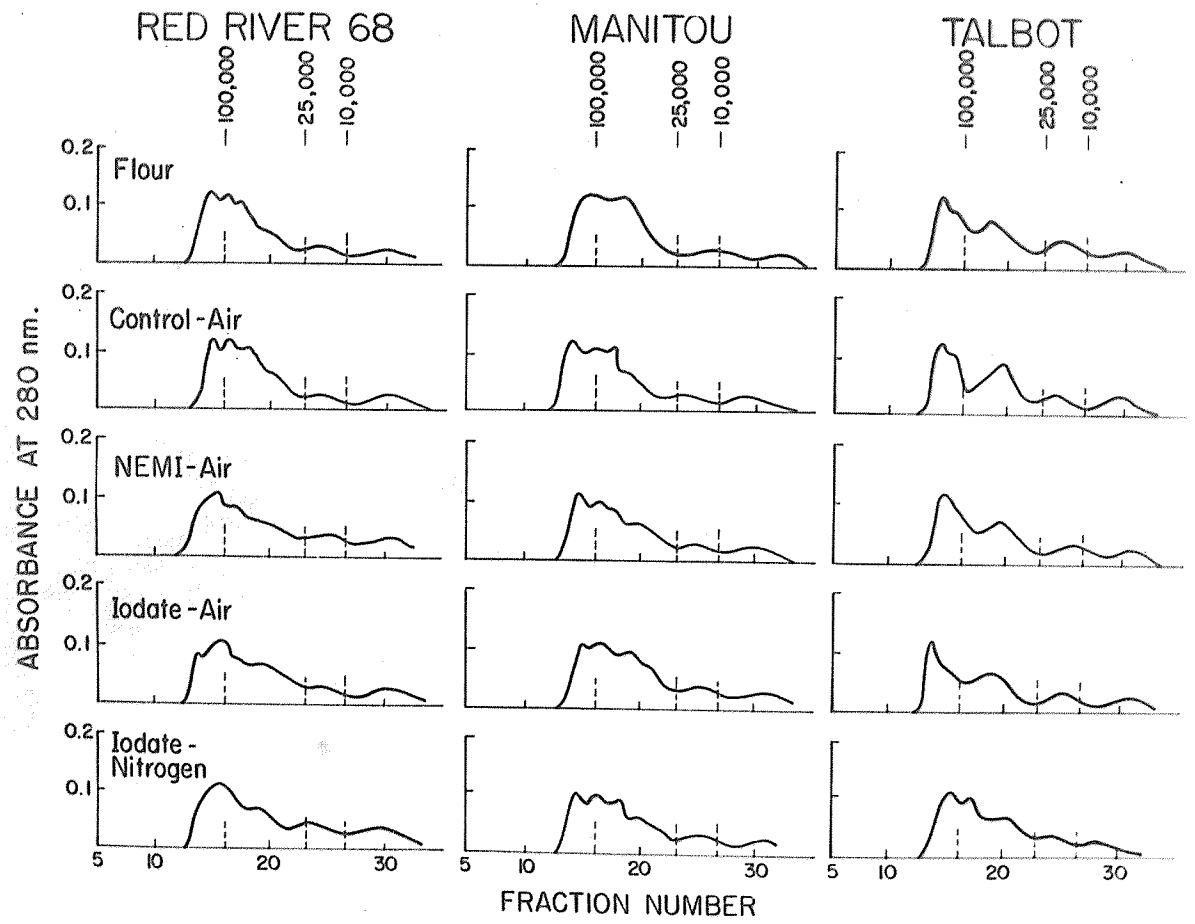

Fig. 9 Gel filtration elution curves of the AUC extracts of the flours and selected doughs for the three varieties. Figures on top indicate the elution position of proteins of given molecular weight.

variety, the solubility of the protein decreases with increasing molecular weight.

For all three varieties the elution profiles for the control doughs were essentially the same as the profiles for the extracts of the flours. Furthermore, the extracts of the 5-min. doughs gave the same profiles as the extracts of the 15-min. doughs. Accordingly, it is concluded that the difference in consistency of flour-water doughs mixed for 5 and 15 minutes in air or in nitrogen is due to disaggregation of hydrated flour (and protein) particles. As shown by Tsen (1967) and confirmed by the present study (see above), this leads to an increase in the amount of acetic acid-soluble protein but, as shown here, does not affect the molecular weight distribution as determined by gel filtration of AUC extracts.

Doughs that contained iodate and NEMI showed changes in elution profiles which suggested depolymerization of higher molecular weight components. These changes were particularly notable for the NEMI-treated doughs mixed in air and in nitrogen and for the iodate-treated doughs mixed in air. The decrease in the amount of higher molecular weight components can be visualized by comparing the elution profiles of Fig. 9, and can be expressed quantitatively in terms of the proportion of the component above 80,000 daltons in molecular weight. These data for the samples represented in Fig. 9 are given in Table 5. It is seen that, for all three varieties, 15 min. mixing of doughs containing iodate or NEMI produced a significant decrease in the proportion of the high molecular weight protein. This can only occur by some form of depolymerization.

Table 5. Changes in the Proportions of the Protein Components with Molecular Weights above 80,000 with Mixing for 15 Minutes under Various Conditions.

	Red River 68	Manitou	Talbot
Flour	0.57	0.54	0.47
Control - Air	0.54	0.52	0.45
Iodate - Nitrogen	0.50	0.47	0.44
Iodate - Air	0.43	0.44	0.40
NEMI - Air	0.43	0.45	0.40

Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) Results

Electrophoresis is an effective method for investigating qualitative and quantitative differences between complex mixtures of proteins. In most electrophoretic methods, the degree of separation is determined mainly by differences in the charge of the protein molecules. With some support media (in zone type of electrophoresis) as starch or polyacrylamide gels, molecular size is also an important factor in determining the rate of electrophoretic mobility.

In the present study polyacrylamide gel electrophoresis (PAGE) was used to examine extracts of flour and dry dough proteins complexed with sodium dodecyl sulfate (SDS) directly and after reduction with β-mercaptoethanol. This procedure, referred to as SDS-PAGE, eliminates the effects of intrinsic charge differences and separates the components according to molecular weight. If the gel is calibrated with proteins of known molecular weight complexed with SDS, the procedure then gives fairly good values for the molecular weights of the components in a complex mixture.

Various protein fractions, intact or reduced, from flour and selected doughs were examined in a separate electrophoresis experiment. The same order of protein samples was used in all the experiments. The patterns, from left to right, represent myoglobin (calibration standard) and followed by patterns of the solubility fractions in the following order:

1. Myoglobin (calibrating or marker protein; molecular weight - 17,000).
2. Protein fraction from flour.
3. Protein fraction from control dough mixed for 5 min.
4. Protein fraction from control dough mixed for 15 min.
5. Protein fraction from dough containing 2 μeq./g. NEMI mixed for 5 min.
6. Protein fraction from dough containing 2 μeq./g. NEMI mixed for 15 min.
7. Protein fraction from dough containing 2 μeq./g. iodate mixed for 5 min.
8. Protein fraction from dough containing 2 μeq./g. iodate mixed in air for 15 min.

With flour proteins, SDS-PAGE, without prior reduction, could only be used to examine the water-, salt- and alcohol-soluble fractions. The intact proteins of the acetic acid-soluble or the insoluble residue fractions are too large to enter the gel.

Water-Soluble Proteins. The SDS-PAGE patterns of the water-soluble proteins of all the flours and doughs (from the three varieties and mixed in air and in nitrogen) used in this study were essentially the same. That is, the qualitative composition of this fraction was not affected by dough mixing under normal or accentuated breakdown conditions in air or in nitrogen. Reduction of the disulfide bonds in the water-soluble proteins (if any are present) had only minor effect on the SDS-PAGE patterns. Several

minor bands became apparent after reduction. No significance is attached to this result.

By way of example, the SDS-PAGE patterns for the intact water-soluble proteins of Manitou flour and the selected doughs are shown in Fig. 10. This fraction of all samples (varieties and mixing conditions) is characterized by eight bands having the following approximate molecular weights:

1 -	18,000	5 -	36,000
2 -	22,000	6 -	46,000
3 -	24,000	7 -	48,000
4 -	30,000	8 -	65,000

Salt-Soluble Proteins. Some difficulty was encountered when the salt-soluble fractions were examined by SDS-PAGE. It seems that the SDS complexes of these proteins are not readily soluble in the solvent used in these experiments. Although the patterns were not clear (see Fig. 11 which shows the patterns for Manitou), it was possible to distinguish nine components with mobilities equivalent to approximate molecular weights as follows:

1 -	24,000	6 -	58,000
2 -	29,000	7 -	87,000
3 -	32,000	8 -	110,000
4 -	36,000	9 -	160,000
5 -	47,000		

As far as can be ascertained from the relatively poor electrophoretic

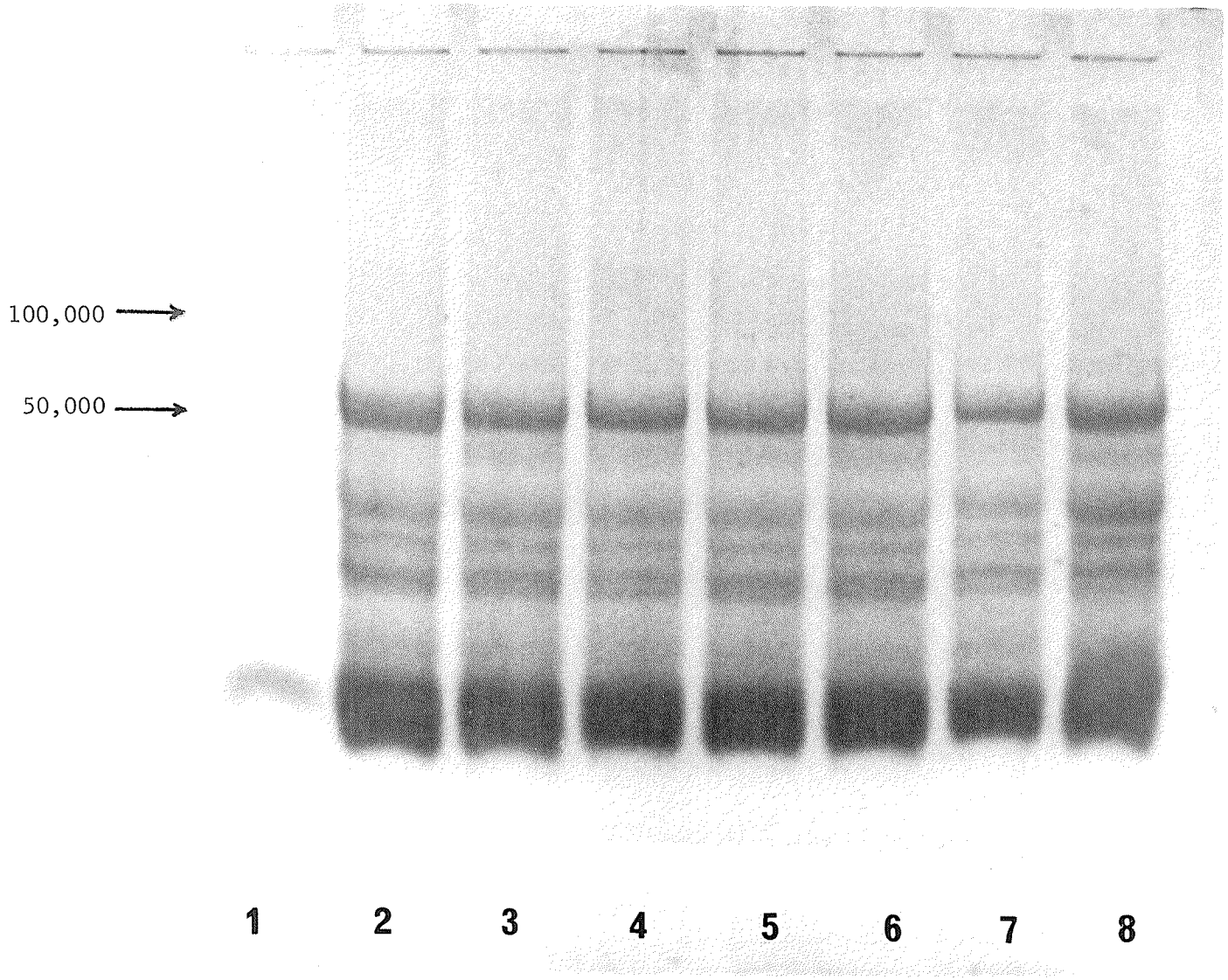

Fig. 10 SDS-PAGE patterns for intact water-soluble proteins from Manitou flour (2) and (3-8) doughs mixed in air. Identity of patterns 1-8 as listed on p. 61.

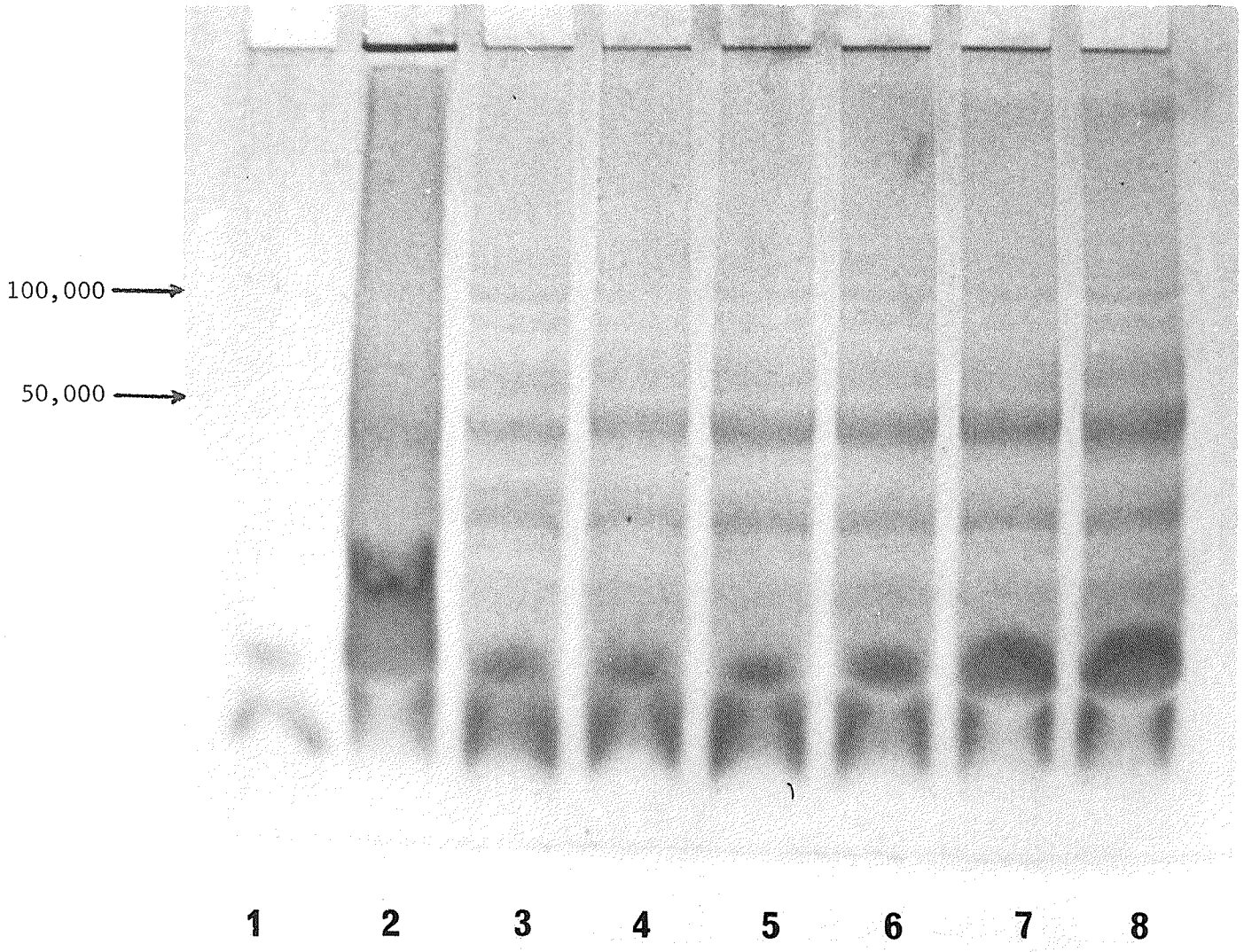

Fig. 11 SDS-PAGE patterns for intact salt-soluble proteins from Manitou flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

results for the salt-soluble fraction, dough mixing does not produce any qualitative changes in the SDS-PAGE patterns of the intact salt-soluble proteins. Several of the slow moving bands present in the patterns for the dough extracts were absent from the pattern for the flour. In view of the poor quality of these results, no significance is attached to this difference.

Reduction of the salt-soluble proteins with β-mercaptoethanol prior to treatment with SDS did not change their solubility or their PAGE patterns. Mixing in nitrogen also had not effect on the patterns. Accordingly these results are not shown.

Alcohol-Soluble Proteins. The electrophoretic patterns for the intact (non-reduced) alcohol-soluble proteins of the flours and the six selected doughs are shown in Figs. 12, 13 and 14 for Red River 68, Manitou and Talbot respectively. Examination of the results for each variety showed that dough mixing in air under the conditions investigated had no effect on the electrophoretic pattern of the alcohol-soluble proteins.

For each variety the patterns for the alcohol-soluble fractions of the doughs mixed in nitrogen were identical to those for the doughs mixed in air. By way of example, the patterns for nitrogen-mixed doughs for the variety Manitou are shown in Fig. 15. These can be compared to the patterns for the air mixed doughs in Fig. 13.

For a single variety, the patterns for the dough extracts were identical with the pattern for the flour extract. Although this may not be readily apparent from the photographs, the identity was quite obvious by

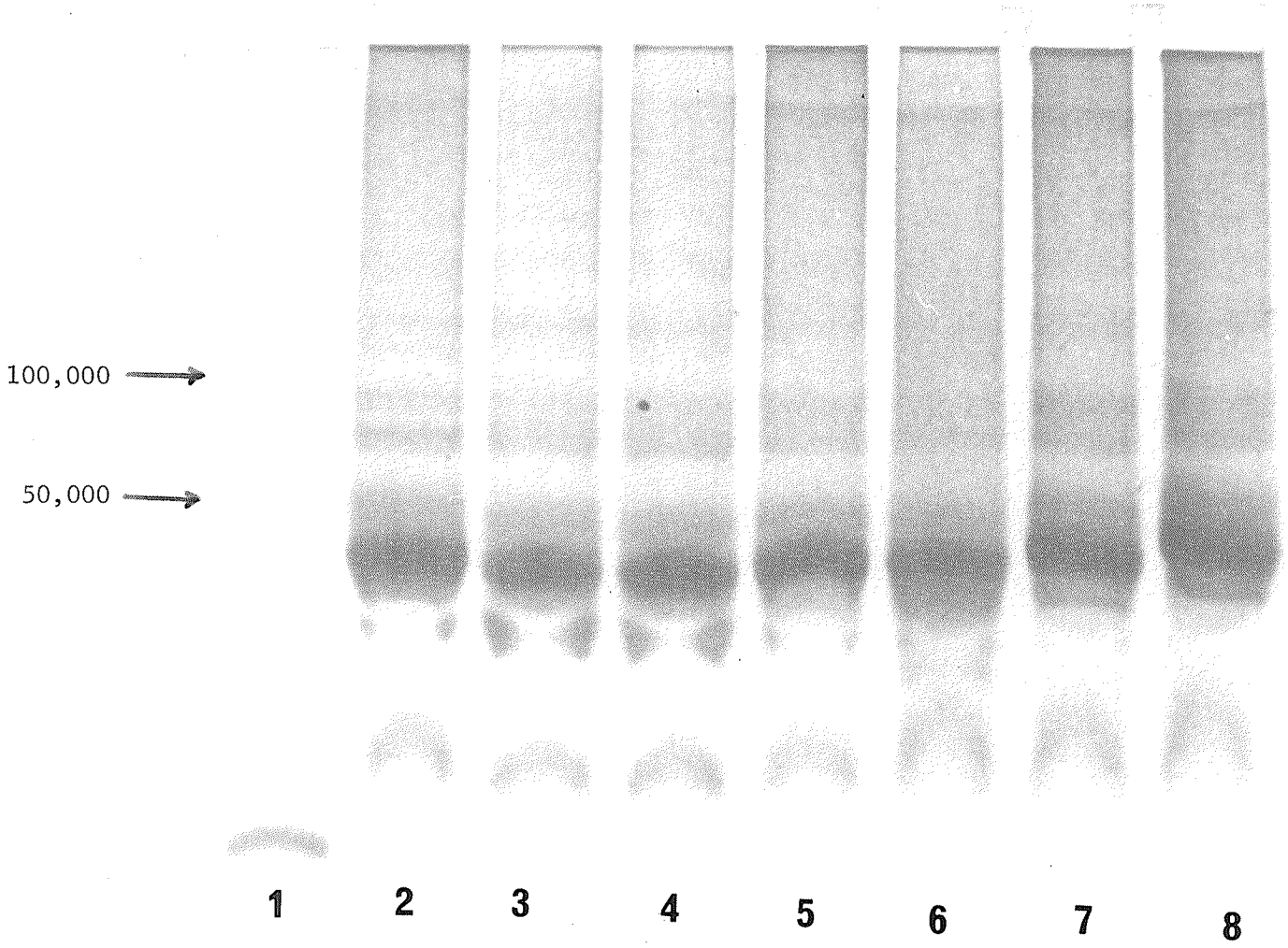

Fig. 12 SDS-PAGE patterns for intact alcohol-soluble proteins from Red River 68 flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

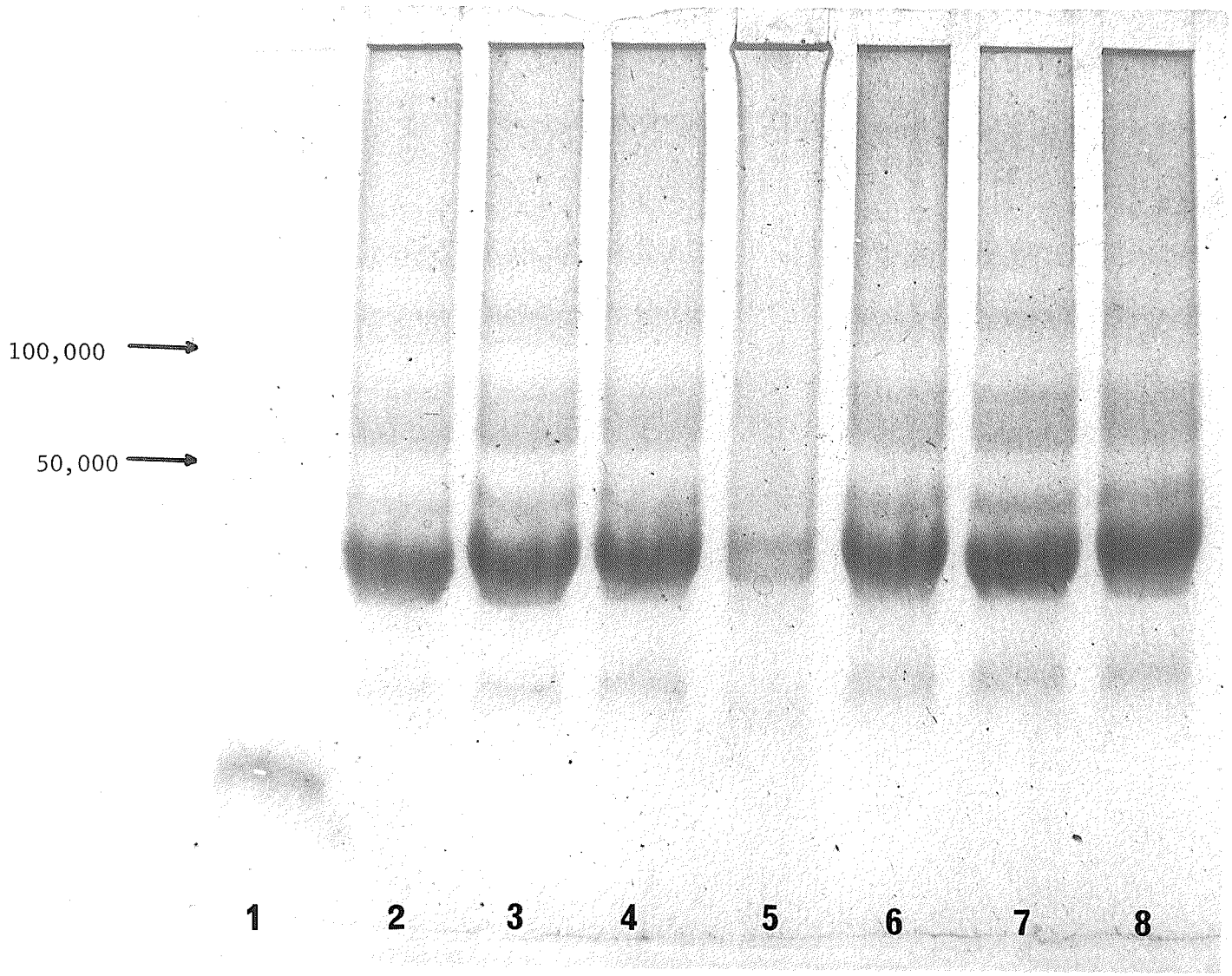

Fig. 13 SDS-PAGE patterns for intact alcohol-soluble proteins from Manitou flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

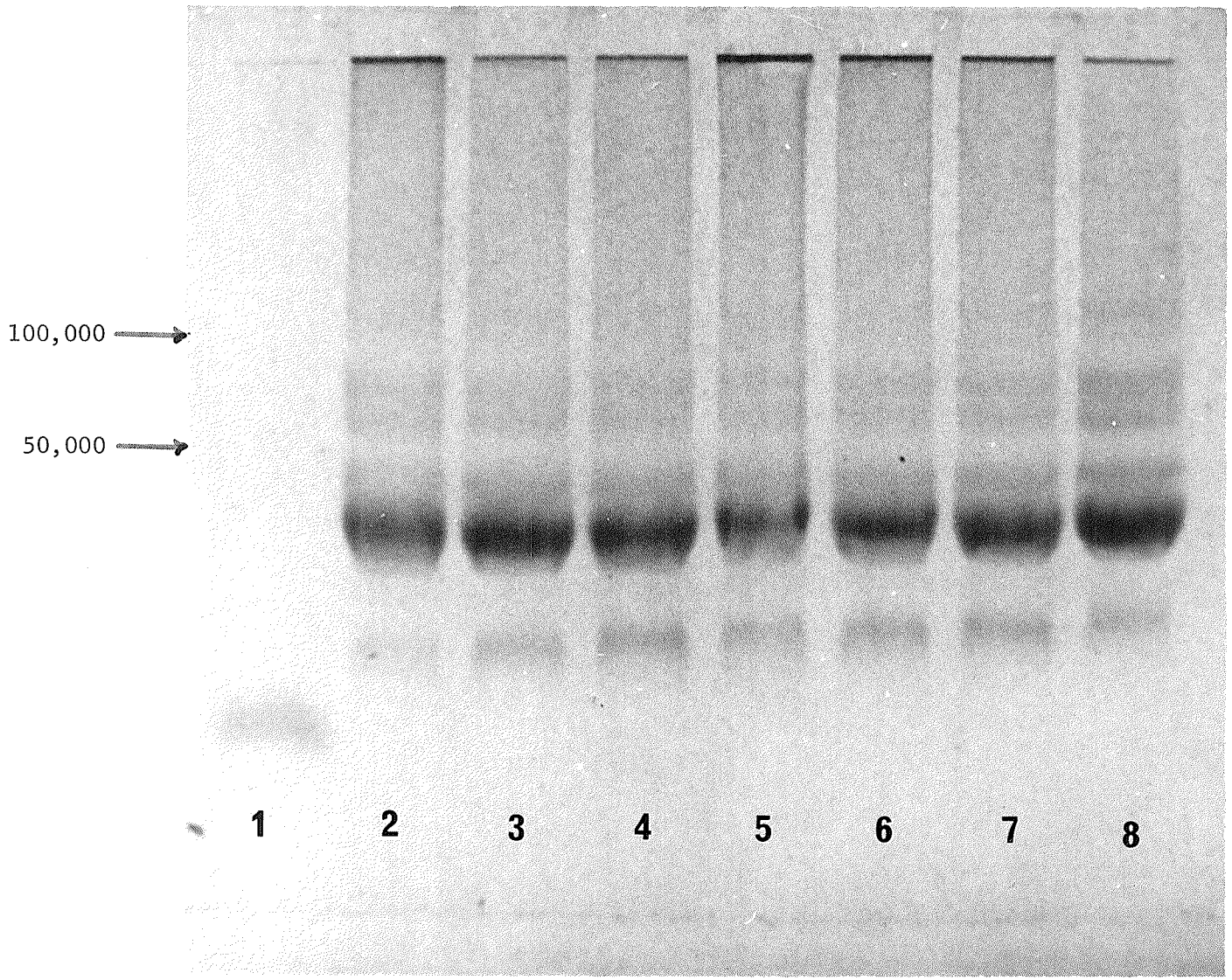

Fig. 14 SDS-PAGE patterns for intact alcohol-soluble proteins from Talbot flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

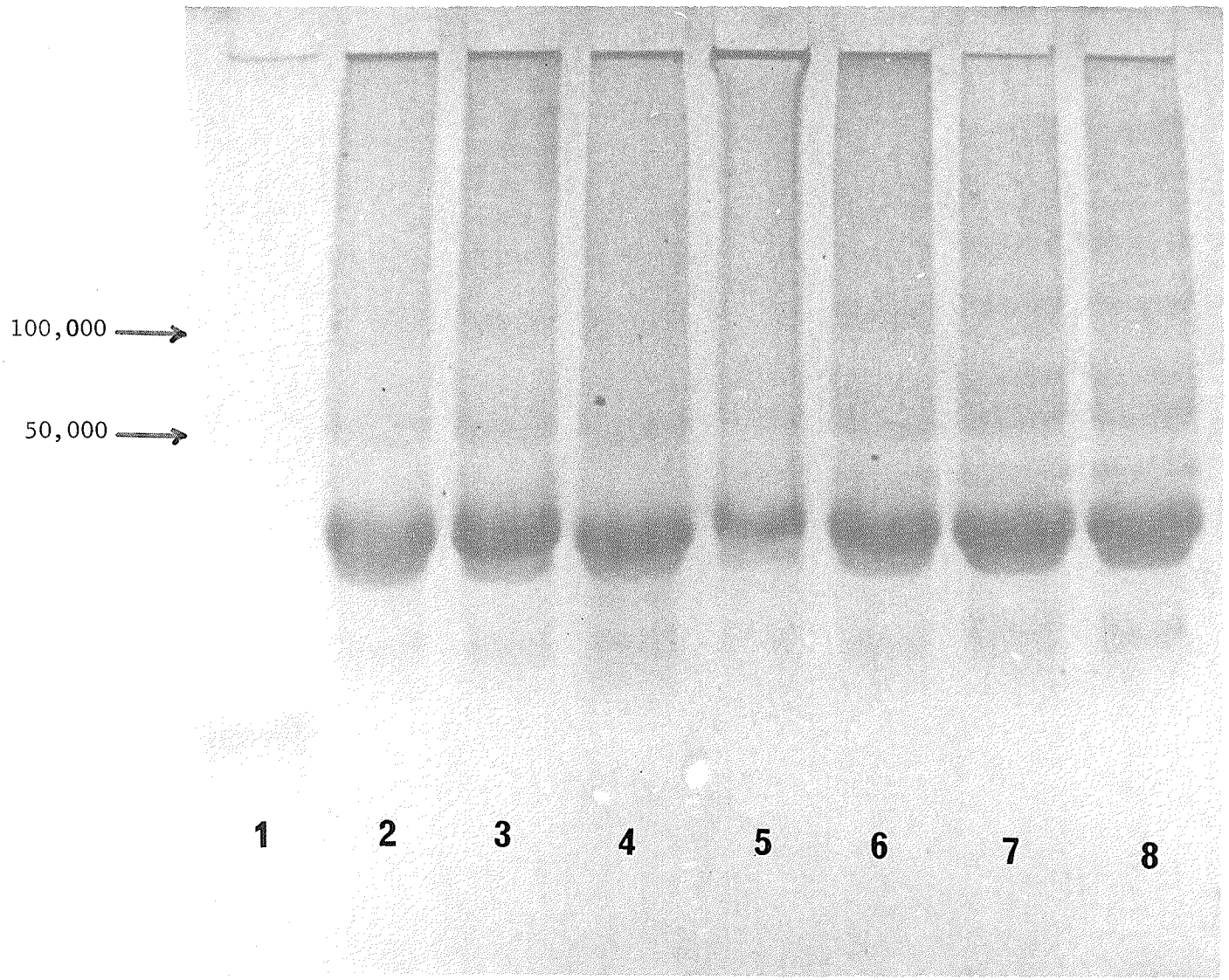

Fig. 15 SDS-PAGE patterns for intact alcohol-soluble proteins from Manitou flour and doughs mixed in nitrogen. Identity of patterns 1-8 as in Fig. 10.

comparing the gels with underlighting. Accordingly, it can be concluded that dough mixing in air or in nitrogen had no effect on the SDS-PAGE patterns of the alcohol-soluble proteins of flour. Additions of potassium iodate or NEMI also had no effect on these patterns.

To facilitate intervarietal comparisons, the electrophoretically distinguishable components for the three varieties are listed in Table 6 according to molecular weight. Red River 68 had seven components (bands) ranging in molecular weight from 20,000 to 150,000. In addition, this variety had three bands in the molecular weight region above 300,000. Manitou also had seven bands in the 20,000 - 150,000 region and three bands with molecular weights of 300,000 or higher. With technique used, it was not possible to estimate the molecular weights of components heavier than 150,000. Talbot had six bands in the measurable range and none in the 300,000 region.

Examination of the results in Table 6, shows that the SDS-PAGE pattern of the intact alcohol-soluble protein is a varietal characteristic. Only the lowest molecular weight component was common to the three varieties. Five components were common to two of the three varieties. Red River 68 had four specific components, Manitou had one and Talbot had two. It would be necessary to examine a large number of common wheat varieties in order to determine if the degree of specificity observed here is generally applicable to common wheats.

The results of Table 6 suggest that the stronger mixing varieties have more of the higher molecular-weight components in the alcohol-soluble

Table 6. List of Distinguishable Components in Non-Reduced, Alcohol-Soluble Protein for the Three Varieties.

Molecular Weight of Component	Red River 68	Manitou	Talbot
20-22,000	+	+	+
29,000	+		
36,000		+	+
43,000		+	+
48,000	+		
56,000			+
62,000		+	
65,000	+		
73,000		+	+
110,000	+	+	
120,000	+		
140,000			+
150,000	+	+	
300,000		+	
>300,000	3 bands	2 bands	

fraction. In the range above 100,000, the strong variety, Red River 68 had six components, the medium variety Manitou had five and the weak variety Talbot only one component.

The SDS-PAGE patterns were also determined for the alcohol-soluble proteins of flours and six doughs listed at the beginning of this section after reduction with β-mercaptoethanol. These results are shown in Figs. 16, 17 and 18 for Red River 68, Manitou and Talbot respectively.

The SDS-PAGE patterns of the reduced and the intact alcohol-soluble proteins showed both qualitative and quantitative differences. In general, reduction seemed to decrease the molecular weights of most of the components. For example, the fastest components for the intact proteins were in the 20,000 range, whereas in the reduced preparation they were in the 16,000 region. However, the apparent decrease in molecular weight is not sufficient to indicate that these components contain disulfide linkages combining large protein subunits. Reduction completely eliminated the components that were in the 300,000 region for the intact alcohol-soluble proteins of Red River 68 and Manitou. It is presumed that these high molecular weight alcohol-soluble proteins comprise several subunits held together by disulfide linkages and in this respect are similar to the acetic acid-soluble and the residue proteins of flour (to be discussed below).

For each variety, the patterns of the reduced alcohol-soluble proteins for the various doughs mixed in air were essentially the same except for the doughs containing NEMI and iodate (which showed considerable mixing

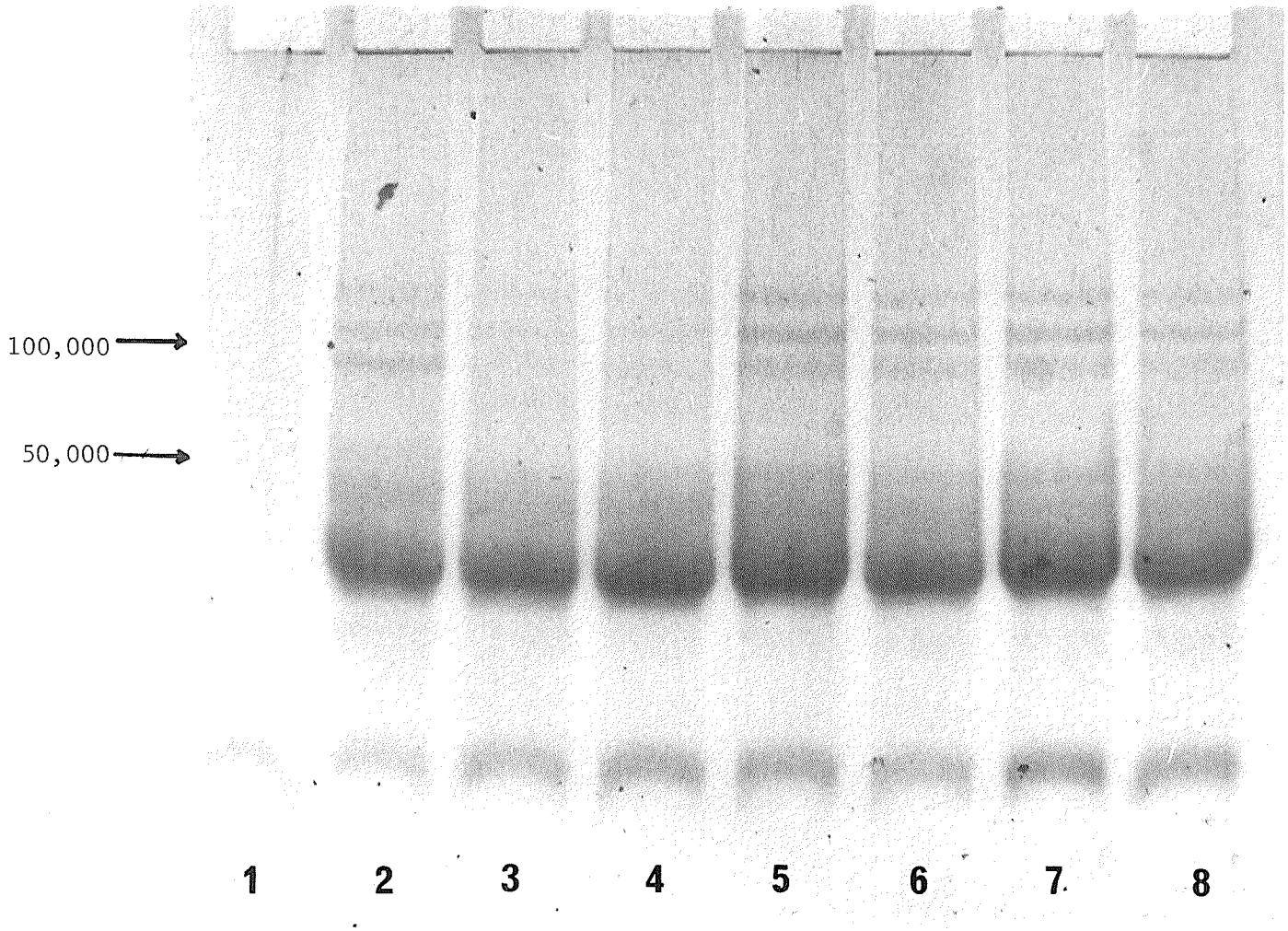

Fig. 16 SDS-PAGE patterns for reduced alcohol-soluble proteins from Red River 68 flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

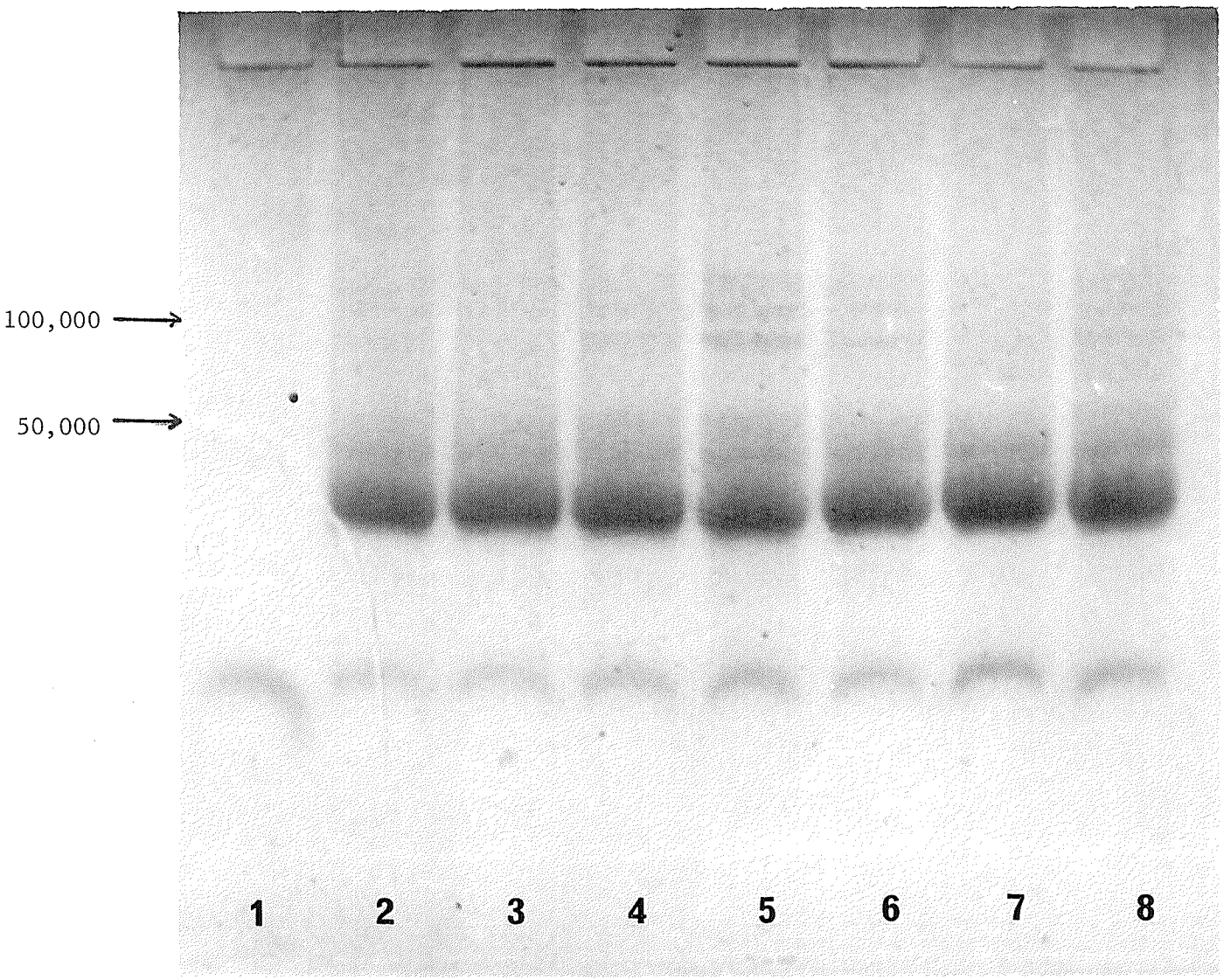

Fig. 17 SDS-PAGE patterns for reduced alcohol-soluble proteins from Manitou flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

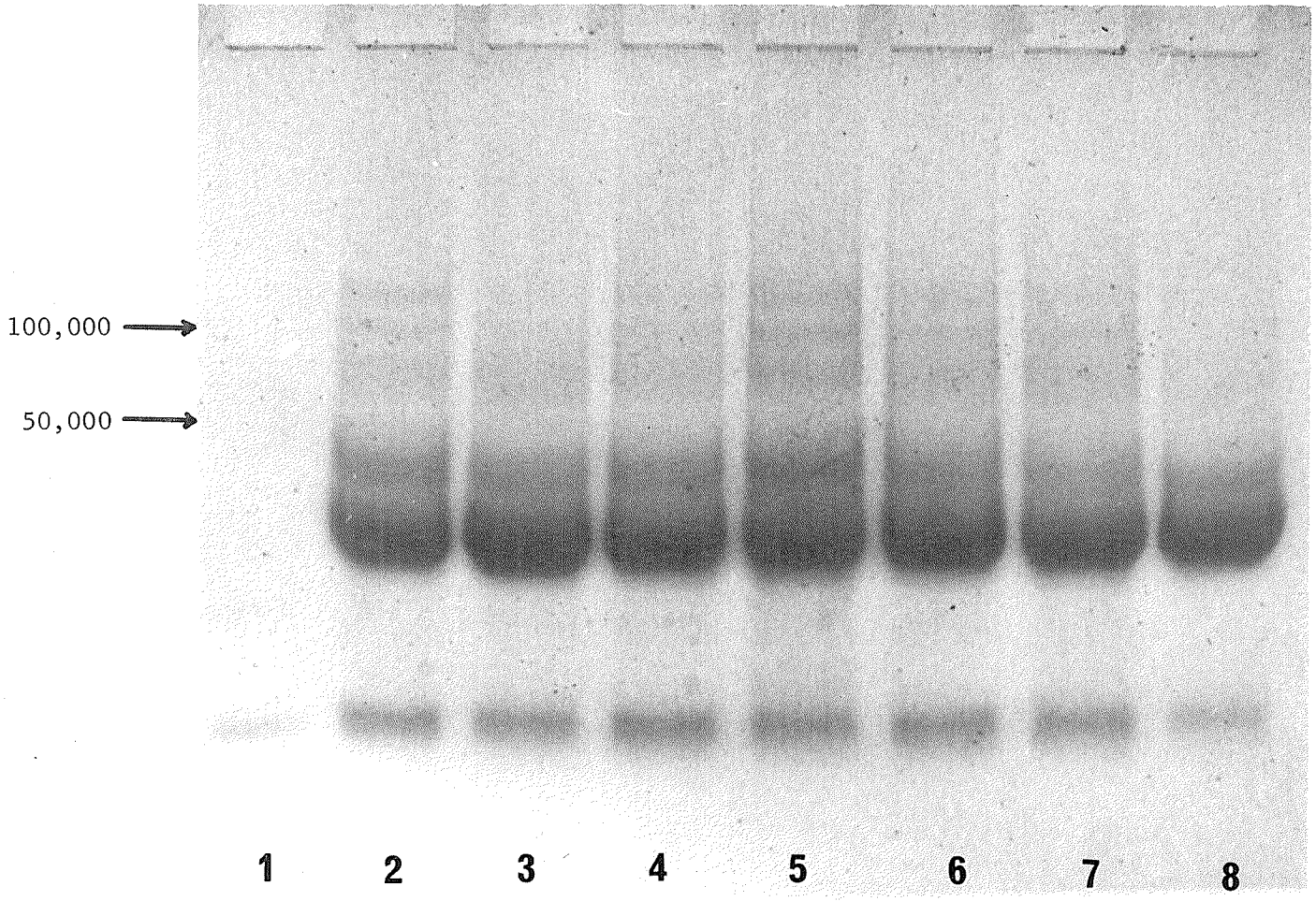

Fig. 18 SDS-PAGE patterns for reduced alcohol-soluble proteins from Talbot flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

breakdown). These doughs showed evidence of three additional "new" bands in the higher molecular weight region. These new components, identified by their approximate molecular weight, produced under "high" breakdown conditions are as follows:

Variety	Molecular Weight		
Red River 68	75,000;	100,000;	140,000
Manitou	80,000;	110,000;	160,000
Talbot	70,000;	100,000;	140,000

The appearance of these new components under conditions of extensive breakdown is taken as evidence of depolymerization of the higher molecular weight gluten proteins under these conditions. Results, to be presented in a later section, were obtained that indicate that these new components are close in molecular weight (by SDS-PAGE) to subunits of the acetic acid-soluble and the insoluble residue protein fractions.

The patterns for the doughs mixed in nitrogen were identical with those for the doughs mixed in air. By way of example, the patterns for the variety Manitou are shown in Fig. 19. Comparison of these patterns with those in Fig. 17 confirms their identity.

Acetic Acid-Soluble Proteins. Acetic acid-soluble proteins of flour can only be examined by SDS-PAGE after reduction with β-mercaptoethanol. The results for the flours and the six selected doughs mixed in air (see

Table 7. List of Distinguishable Components in Reduced Acetic Acid-Soluble Protein for the Three Varieties.

Molecular Weight of Component	Red River 68	Manitou	Talbot
19,000	+	+	+
26-27,000	+	+	+
29,000			+
33,000	+		
35-37,000	+	+	+
41,000		+	
43,000	+		+
46-47,000		+	+
50-51,000	+	+	
53,000			+
63,000	+		
80,000			+
84,000		+	
89,000	+		
110,000		+	+
120,000	+		
130,000		+	
160,000			+
170,000	+		
200,000		+	

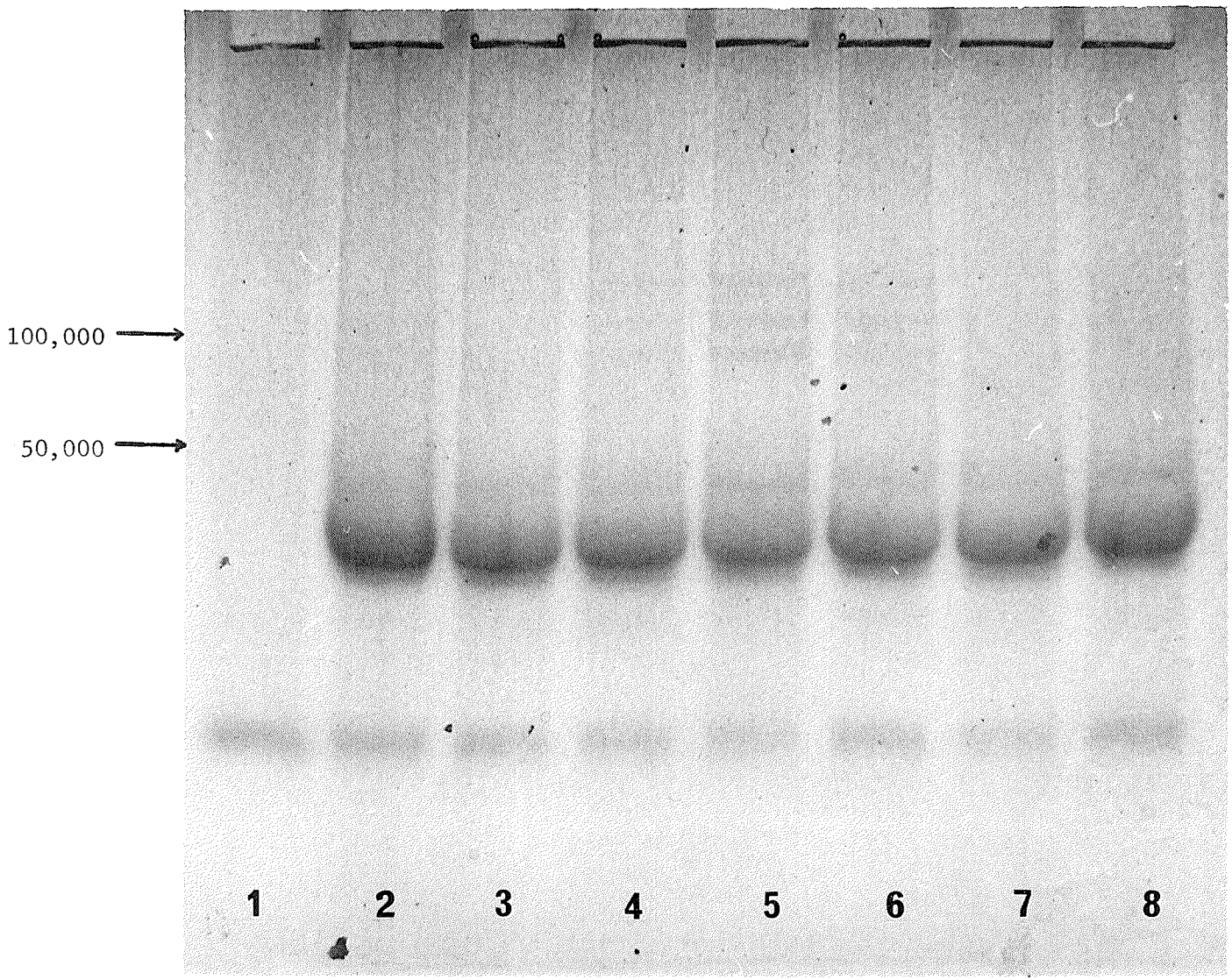

Fig. 19 SDS-PAGE patterns for reduced alcohol-soluble proteins of Manitou flour and doughs mixed in nitrogen. Identity of patterns 1-8 as in Fig. 10.

above) are shown in Figs. 20, 21 and 22 for Red River 68, Manitou and Talbot respectively.

For each variety, all the patterns are identical. Accordingly, mixing under conditions investigated did not affect the pattern of the reduced acetic acid-soluble proteins. The patterns for doughs mixed in air and in nitrogen were the same (compare results for Manitou in Fig. 23 and 21).

To facilitate intervarietal comparison, the SDS-PAGE components of the reduced acetic acid-soluble proteins are listed in Table 7 in order of increasing molecular weight.

All three varieties had 10 components. Three of the first four lower molecular weight components were common for the three varieties. There were several components that were common to each pair of varieties. However there were numerous components that were specific for each variety, especially at the high end of the molecular weight range. In the range above 51,000, Red River 68 had four specific components, Manitou had three and Talbot also had three. Presumably the varieties could be "fingerprinted" on the basis of these characteristic components. There was no apparent relationship between mixing strength and the number of the molecular weights of components of reduced acetic acid-soluble proteins of common wheat flours.

Insoluble Residue Proteins. SDS-PAGE patterns for the reduced residue proteins of flours and the six selected doughs mixed in air for the three varieties are shown in Figs. 24, 25 and 26. For each variety, all the

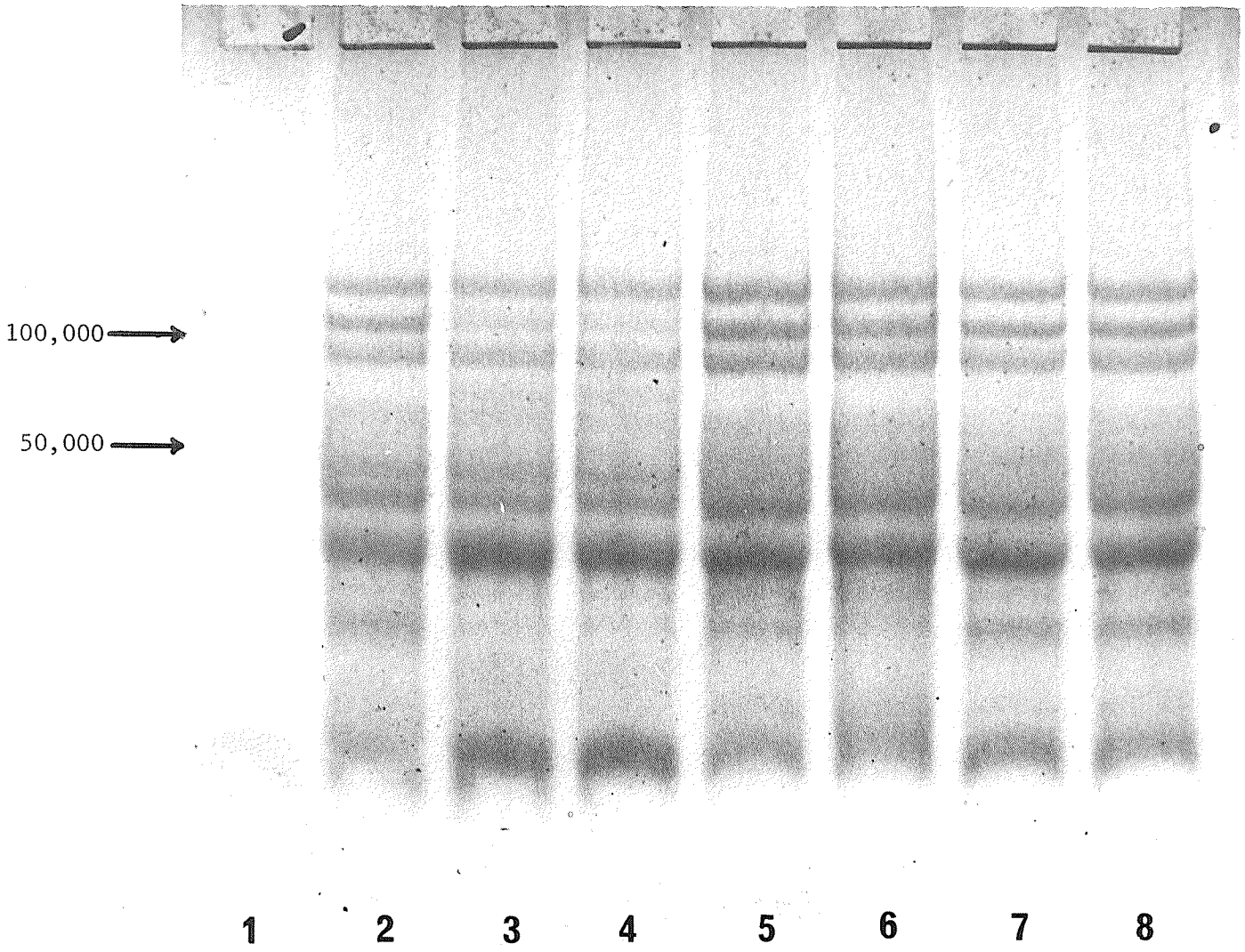

Fig. 20 SDS-PAGE patterns for reduced acetic acid-soluble proteins from Red River 68 flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

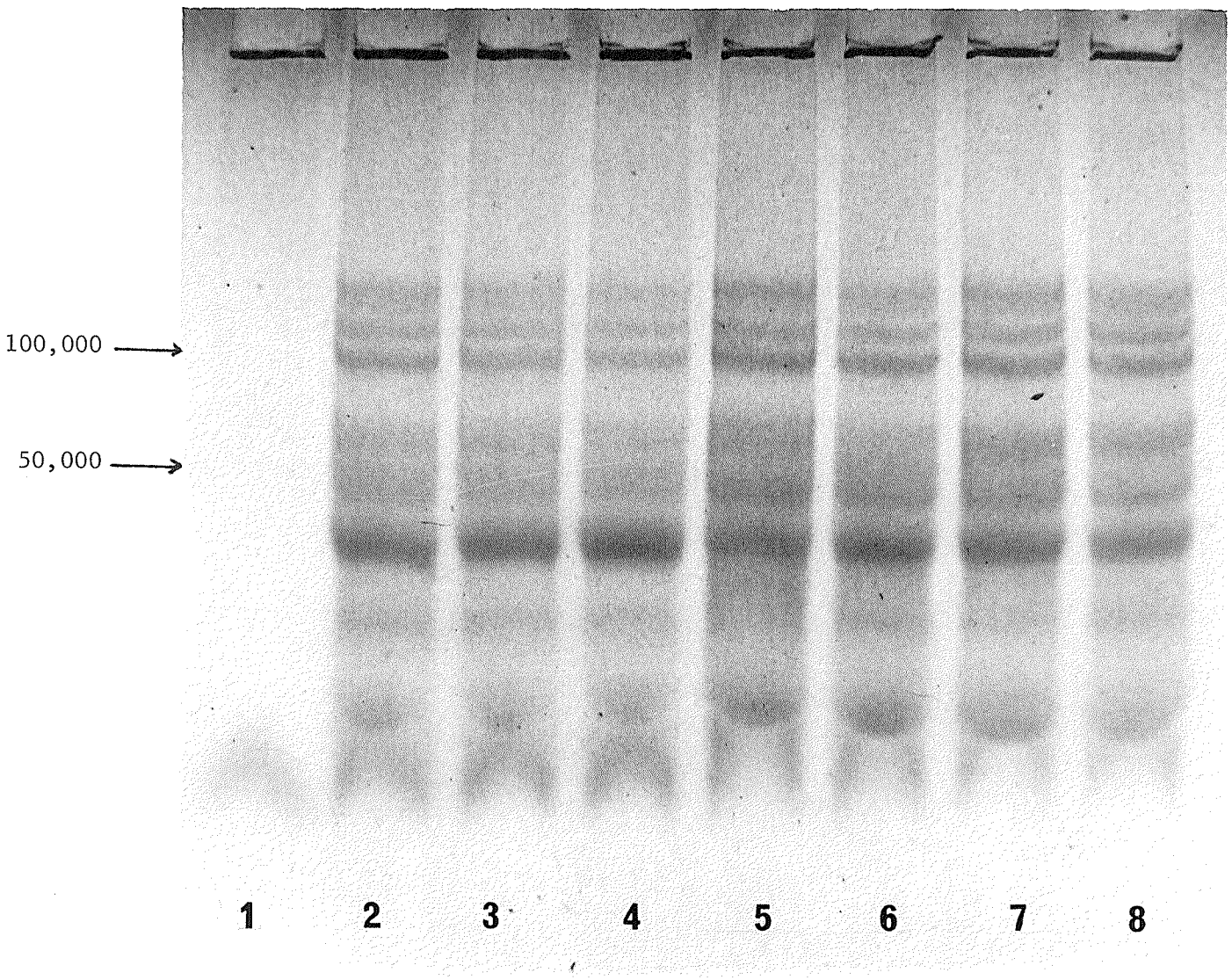

Fig. 21 SDS-PAGE patterns for reduced acetic acid-soluble proteins from Manitou flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

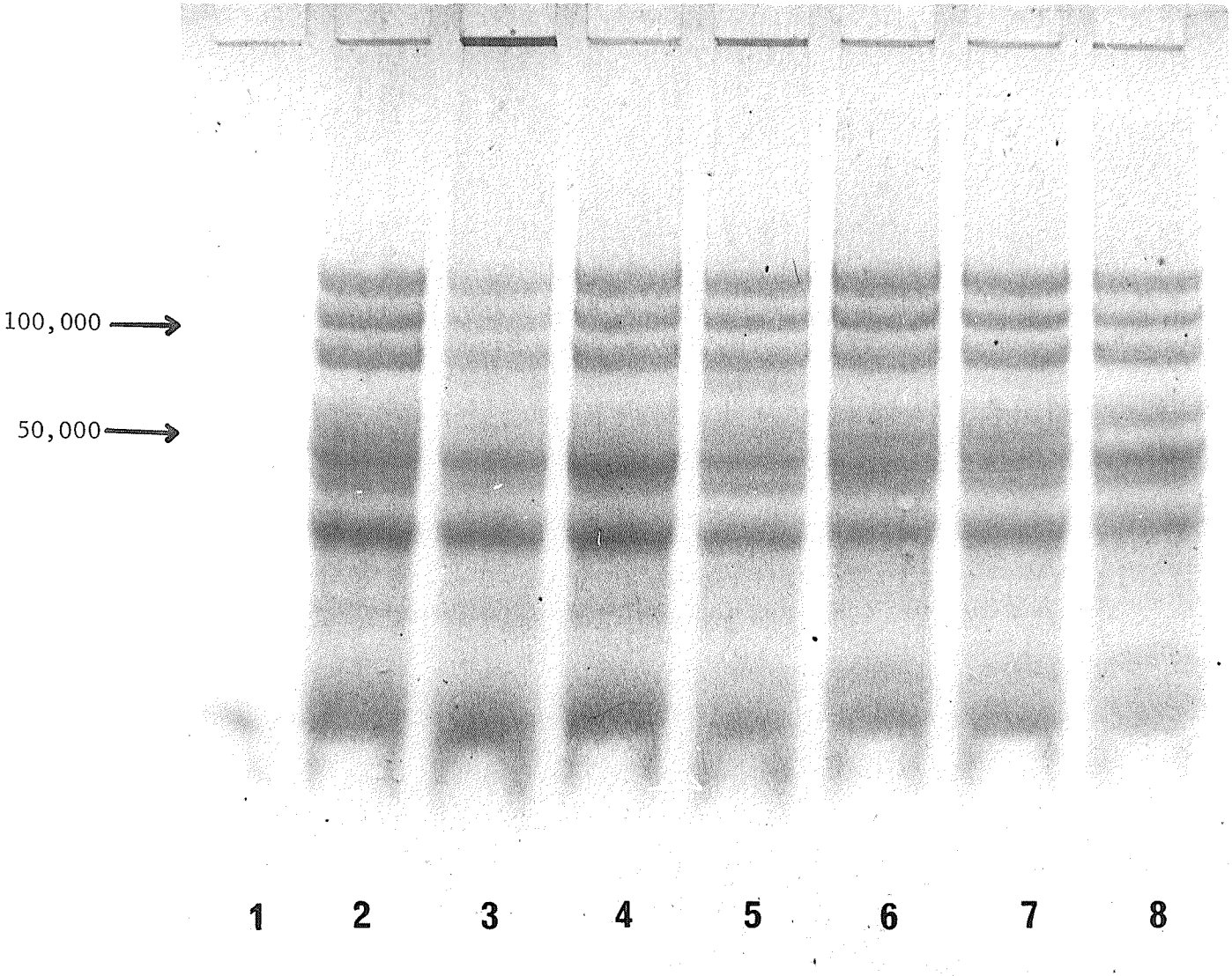

Fig. 22 SDS-PAGE patterns for reduced acetic acid-soluble proteins from Talbot flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

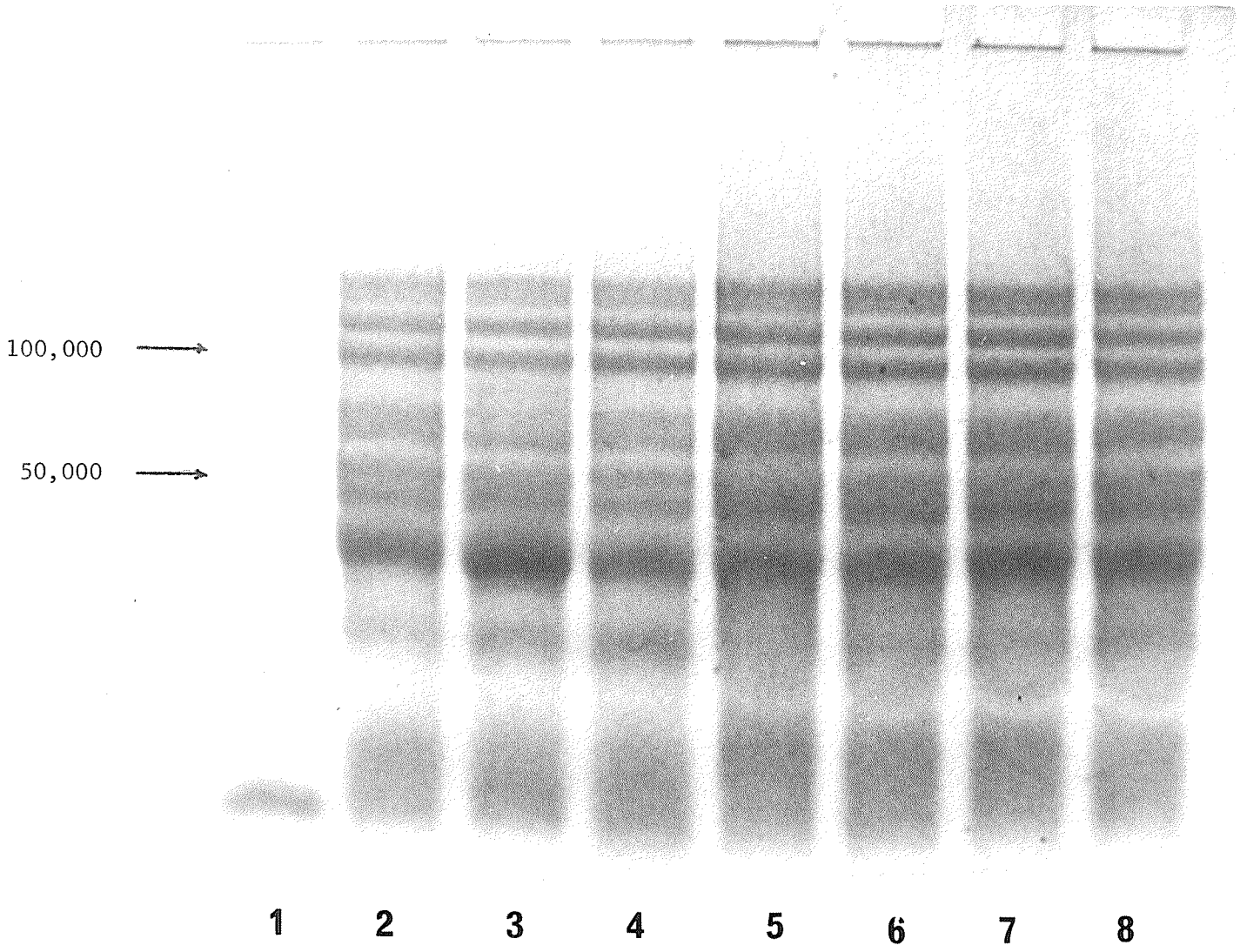

Fig. 23 SDS-PAGE patterns for reduced acetic acid-soluble proteins from Manitou flour and doughs mixed in nitrogen. Identity of patterns 1-8 as in Fig. 10.

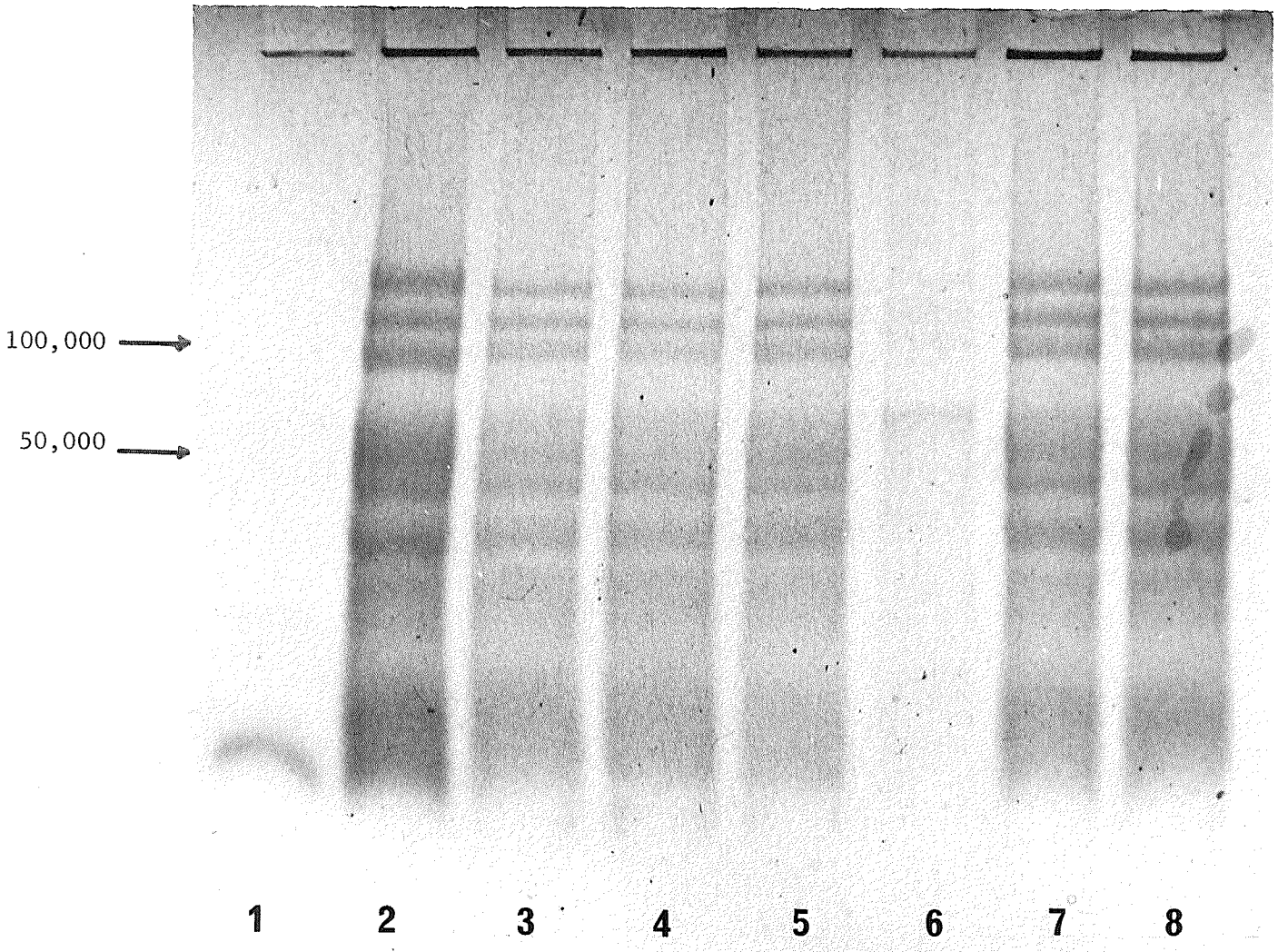

Fig. 24 SDS-PAGE patterns for reduced residue proteins from Red River 68 flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

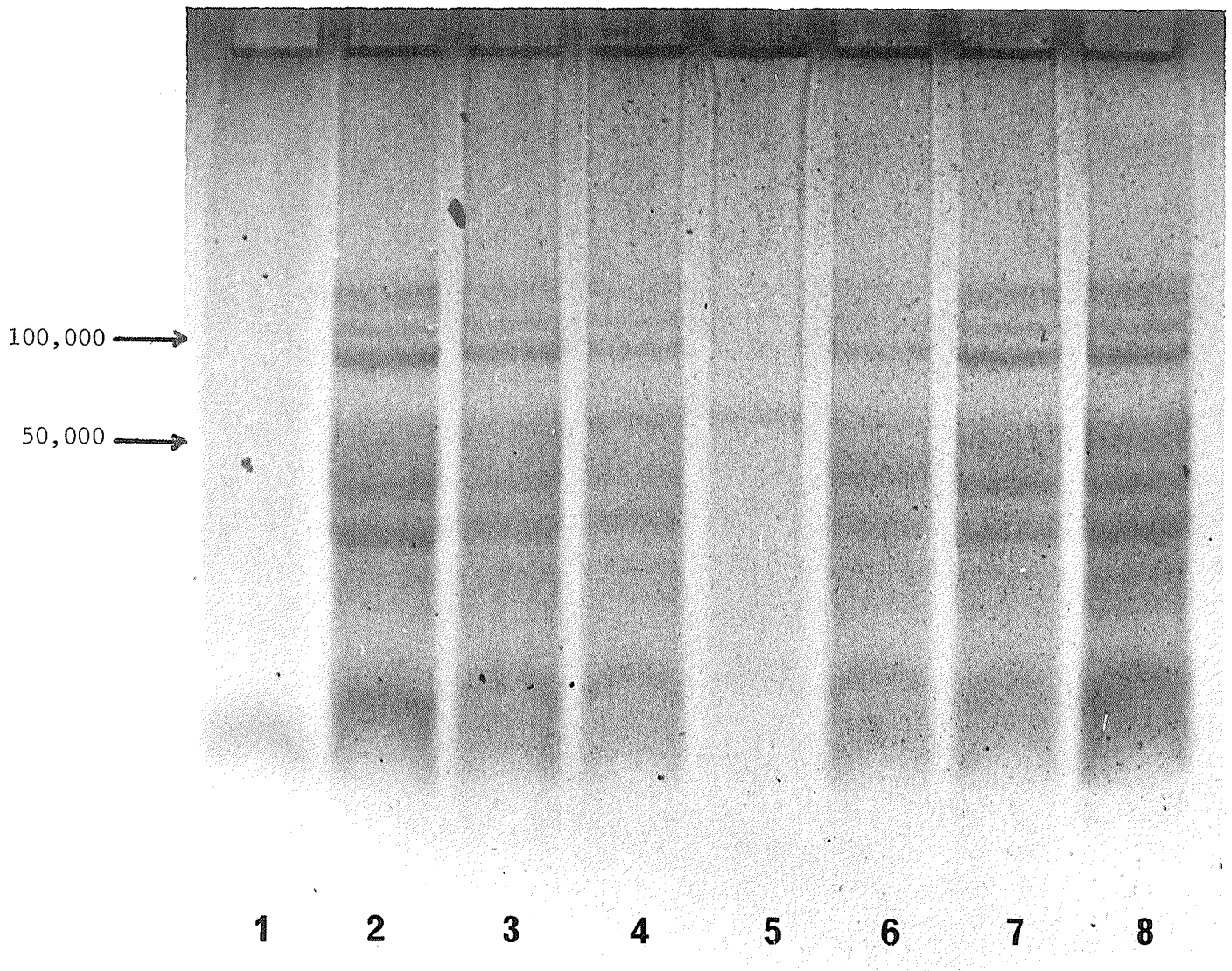

Fig. 25 SDS-PAGE patterns for reduced residue proteins from Manitou flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

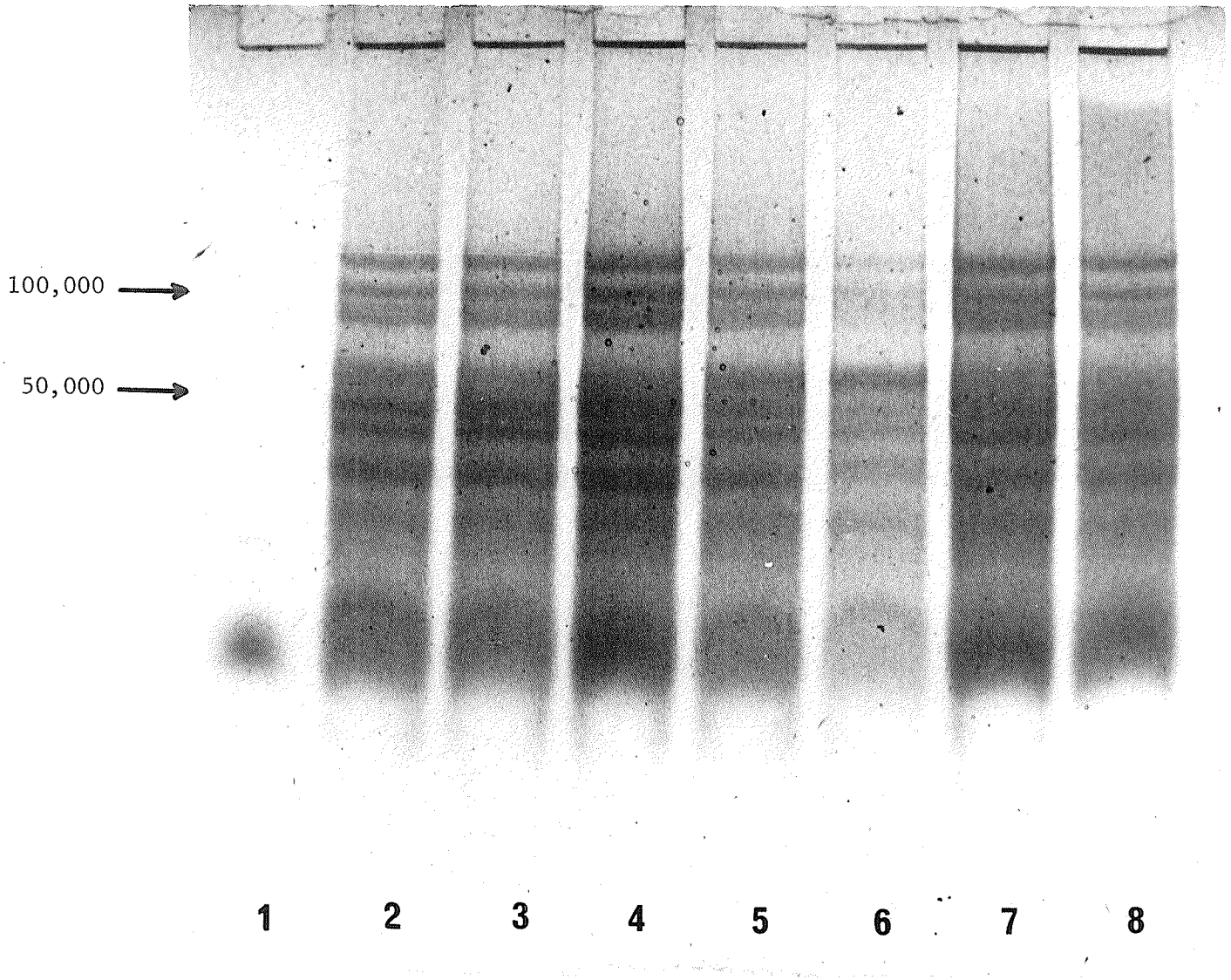

Fig. 26 SDS-PAGE patterns for reduced residue proteins from Talbot flour and doughs mixed in air. Identity of patterns 1-8 as in Fig. 10.

patterns were identical. Therefore, mixing and conditions under which doughs were mixed, investigated in the present study did not affect the SDS-PAGE patterns. The results for doughs mixed in nitrogen were the same as for doughs mixed in air (compare Figs. 27 and 25). The amount of residue protein obtained from the NEMI-treated doughs (patterns 5 and 6) was quite low and therefore the bands were too faint, in some cases, to show up in the photographs. However, the bands were sufficiently distinct for a visual comparison of the stained gels.

The SDS-PAGE patterns for the reduced residue protein were essentially the same as the patterns for the reduced acetic acid-soluble proteins. There were two minor differences which will be mentioned below. It appears that the difference in solubility of the acetic acid-soluble and residue proteins is probably due to a difference in molecular weight (e.g. the number of subunits held together by disulfide bonds).

The SDS-PAGE components in the reduced insoluble residue proteins are listed in Table 8 according to molecular weight. Each variety had 10 distinguishable components. Three of the four components of lowest molecular weight were common to the three varieties. Several components were common to two of the varieties. In the high molecular weight region, each variety had a number of characteristic components.

Comparison of the results of Tables 8 and 7 shows that the number and molecular weights of the components of the reduced residue protein are essentially the same as those of the components of the reduced acetic acid-soluble protein. The flour differences are:

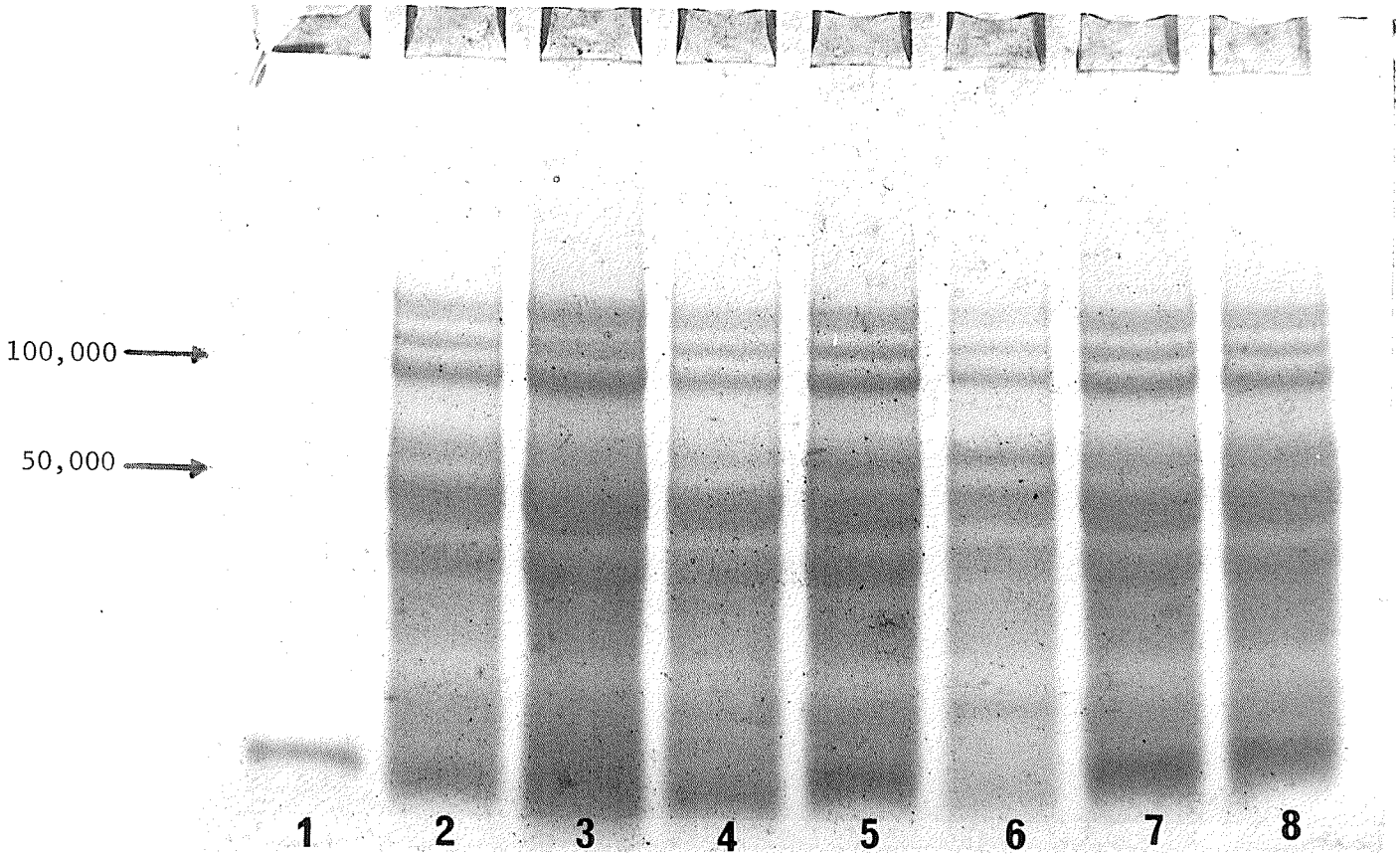

Fig. 27 SDS-PAGE patterns for reduced residue proteins from Manitou flour and doughs mixed in nitrogen. Identity of patterns 1-8 as in Fig. 10.

Table 8. List of Distinguishable Components in Reduced Residue Protein for the Three Varieties.

Molecular Weight of Component	Red River 68	Manitou	Talbot
19,000	+	+	+
26-27,000	+	+	+
29-30,000		+	+
33,000	+		
35-37,000	+	+	+
41,000		+	
43,000	+		+
47,000			+
50-51,000	+	+	
63,000	+		+
80,000			+
84,000		+	
89,000	+		
110,000		+	+
120,000	+		
130,000		+	
160,000			+
170,000	+		
200,000		+	

1. For the variety Manitou, the reduced residue protein had a component of 30,000 molecular weight which was absent in the pattern for the reduced acetic acid-soluble protein.

2. For Manitou, the reduced acetic acid-soluble protein had a component of 46,000 which was absent from the pattern for the reduced residue protein.

3. For Talbot, the reduced residue protein had a component of 63,000 molecular weight that was absent in the reduced acetic acid-soluble protein.

4. For Talbot, the reduced acetic acid-soluble protein had a component of 53,000 that was absent in the reduced residue protein.

These minor differences could have resulted from incomplete reduction and therefore no significance is attached to these observations in relation to mixing properties. As already indicated, the difference in solubility of the acetic acid-soluble protein and the insoluble residue protein is probably due to the higher molecular weight of the latter resulting from a larger number of repeating subunits held together by S-S bonds. Accordingly, the increase in the amount of acetic acid-soluble protein and decrease in the amount of residue protein during mixing, under some conditions, could only result from a depolymerization (removal of some subunits) with a concomitant decrease of molecular weight. A possible mechanism for this change will be presented in the General Discussion section.

It was suggested in the section dealing with the reduced alcohol-soluble proteins, that the three "new" components observed in the fraction for the doughs that contained NEMI originated from either the acetic acid-

soluble or the insoluble residue fractions. The patterns of the reduced proteins of these fractions had components of essentially the same molecular weight as the three "new" components of the alcohol-soluble fraction of the NEMI doughs. Further work would be required to establish complete identity of these components.

Changes in Sulfhydryl and Disulfide Contents during Dough Mixing

It was of interest to determine the sulfhydryl and disulfide contents of the doughs used in the present study because of their known involvement in the functional properties of dough. The involvement of sulfhydryl groups in the improver reaction of potassium bromate and other similar chemicals is now well established. Also, the rapid mixing breakdown of doughs in the presence of excess amounts of sylfhydryl blocking agents such as NEMI or fast acting oxidizing agents such as iodate is well known (Bushuk and Hlynka 1962).

The sulfhydryl contents of the flour and dough samples used in this study are given in Table 9. The results for the control doughs and doughs that contained the higher quantity of iodate and of NEMI are summarized in Fig. 28 which shows the loss of SH under various conditions. For dough of each variety it is seen that there was a rapid decrease in SH content during the first five minutes of mixing in air. Addition of iodate or NEMI enhanced this decrease. Mixing for an additional 10 min. produced a further but a very small decrease in SH content. The effects of atmospheric oxygen and of iodate or NEMI on SH loss appear to be additive. The effect

Table 9. SH Contents of Flours and Doughs Mixed under Various Conditions. (μeq./g. flour).

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
Flour	0.72				0.79				0.72			
Control	0.48	0.48	0.72	0.68	0.62	0.60	0.76	0.75	0.49	0.41	0.65	0.67
NEMI												
0.4 μeq./g. flour	0.47	0.34	0.43	0.40	0.47	0.39	0.44	0.47	0.38	0.35	0.39	0.42
2.0 μeq./g. flour	0.28	0.21	0.33	0.28	0.29	0.27	0.33	0.32	0.31	0.28	0.30	0.30
Iodate												
0.4 μeq./g. flour	0.39	0.39	0.47	0.50	0.48	0.43	0.58	0.60	0.37	0.34	0.50	0.52
2.0 μeq./g. flour	0.23	0.23	0.41	0.29	0.36	0.24	0.37	0.37	0.36	0.35	0.42	0.39

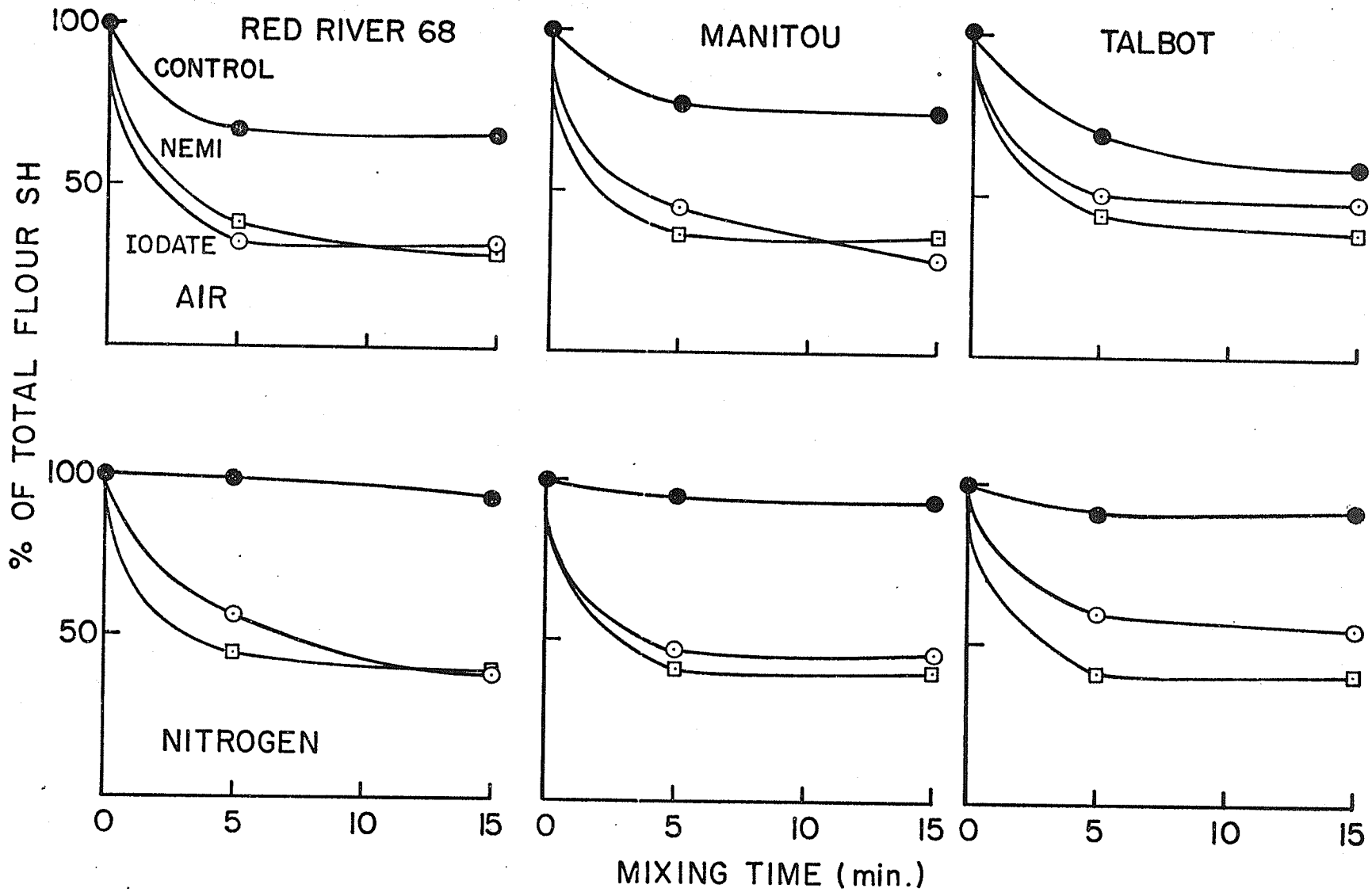

Fig. 28 The changes in SH content of doughs during mixing.

of iodate or NEMI is larger in the stronger doughs than in the weaker (Talbot) dough.

Control doughs mixed in nitrogen did not show any significant change in SH content with mixing time. Previous studies in various laboratories (Tsen and Bushuk 1963; Bloksma 1964) showed that, during mixing under nitrogen, the SH content of flour-water doughs actually increases slightly at first and then decreases to the original level in the flour. The number of determinations made in the present study was not sufficient to detect this increase in the early part of the mixing (usually at about 3-4 min. in the farinograph). Addition of iodate or NEMI produced a rapid decrease in SH content during the first 5 min. of mixing, but essentially no additional decrease during the subsequent 10 min. of mixing. The percentage loss in SH after 15 min. mixing in the presence of iodate or NEMI was greater for the air-mixed doughs than for the nitrogen-mixed doughs. Presumably this is due to the additional effect of atmospheric oxygen.

As indicated above for the air-mixed doughs, the drop in SH in doughs mixed in nitrogen due to iodate or NEMI addition was somewhat lower for the weaker dough (Talbot) than for the two stronger doughs.

The observed losses of SH during dough mixing under various conditions are analogous to results obtained by others for different flours and different mixing conditions (Sullivan et al. 1963). Absolute values for SH contents determined in the present study are consistent with published values. In making this comparison it should be kept in mind that the values here are expressed per g. of flour, and that the protein contents of the flours

used are somewhat lower than average, especially for the two hard red spring wheat flours.

On the basis of several preliminary analyses, it was decided not to analyze all of the dough samples for S-S content. In addition to the control doughs, only the doughs that showed greatest breakdown during mixing were analyzed. The results that were obtained are given in Table 10. Under the mixing conditions used in this study, the S-S content was not affected. There is a suggestion of a slight decrease in S-S or mixing when the values for flour and for the corresponding doughs containing iodate or NEMI are compared. For two of the flours the differences are just outside the experimental error. It is presumed that, if this small loss of S-S is in any way responsible for the breakdown observed in the farinograph, then these few S-S groups would have to be extremely critical in their functional (rheological) role. As suggested recently by Bloksma (1972), this could indeed be the case. However, highly precise analytical and rheological measurements would be required to verify this possibility.

The actual S-S contents obtained in the present study are consistent with published values for similar flours. Again, it should be noted that the data are expressed on a flour-weight basis, and that the protein contents of the flours used, especially the hard red spring wheat flours, are lower than the usual average values.

Table 10. Contents of Disulfide Groups of Flours and Doughs Mixed for 15 min. under Various Conditions.

		Red River 68	Manitou	Talbot
		(μeq./g. flour)		
Flour		10.6	10.4	9.8
Control	Air	10.4	10.3	9.7
	Nitrogen	10.4	10.4	9.5
NEMI (2.0 μeq./g.)	Air	10.3	10.2	9.4
	Nitrogen	10.3	10.4	9.6
Iodate (2.0 μeq./g.)	Air	10.3	10.4	9.6
	Nitrogen	10.3	10.2	9.4

Effect of Dough Mixing on Content of Free Lipids

Most cereal chemists are of the opinion that flour lipids are essential to the development of a proper bread dough. In this regard, Hoseney et al. (1970) have suggested that phospholipids form an integral part of the developed gluten. Changes in lipid extractability during dough mixing were reported by several workers (Olcott and Mecham 1947; Davies et al. 1969; Daniels et al. 1966). Under normal mixing conditions the amount of free lipid usually increased with mixing time. Accordingly, it seems reasonable to presume that a decrease in dough consistency (breakdown) could result from a gradual decrease in lipid binding by the gluten proteins. Although the possible changes in lipid binding during dough mixing do not form an integral part of the present investigation, it was felt that this question deserved some attention in that the lipid binding could be indirectly involved in the changes in the proteins. It was therefore decided to explore the possibility of the involvement of lipid binding by analysis of free lipid (lipids extractable with petroleum ether) content in the various doughs used in this study. The results that were obtained are summarized in Table 11.

For doughs mixed in air, there was a trend towards slight increase in the amount of free lipid, although most of the increases observed were within the experimental error for the analysis used. Addition of iodate or NEMI, which markedly increased the rate of dough breakdown by mixing,

Table 11. Lipid Extracted by Petroleum Ether from Flours and Ground Doughs Mixed under Various Conditions.

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
	%	%	%	%	%	%	%	%	%	%	%	%
Flour	0.91				0.87				1.04			
Control	0.35*	0.37	0.37	0.34	0.26	0.31	0.27	0.26	0.34	0.35	0.30	0.28
NEMI												
0.4 μeq./g. flour	0.38	0.41	0.34	0.36	0.26	0.30	0.29	0.28	0.30	0.33	0.30	0.29
2.0 μeq./g. flour	0.40	0.40	0.38	0.39	0.28	0.30	0.27	0.28	0.33	0.35	0.35	0.33
Iodate												
0.4 μeq./g. flour	0.36	0.38	0.41	0.39	0.29	0.29	0.30	0.33	0.36	0.37	0.35	0.32
2.0 μeq./g. flour	0.38	0.40	0.43	0.38	0.28	0.32	0.28	0.27	0.34	0.38	0.31	0.33

* Standard deviation of the lipid analysis is 0.03%.

had no effect on lipid binding in dough. Free lipid content decreased slightly in doughs mixed in nitrogen, but again this decrease was within experimental error.

The results obtained in this study are consistent with the previously published and much more extensive data of Daniels et al. (1971). The absolute quantities extracted agree with published values for doughs from similar flours. However, it is concluded that the small increases in free lipid obtained for the doughs mixed in air are not sufficient to account for the observed decrease in dough consistency.

General Discussion

When flour and water are mixed into a dough in a recording dough mixer, the recorded curve indicates the resistance to mixing at any time in the mixing process. The mixing resistance is directly related to the consistency and stickiness of the dough. The curves obtained with any one type of mixer have an initial rising part indicating an increase in resistance with mixing time, an intermediate more or less flat portion where the resistance is relatively constant followed by a falling portion indicating a decrease in resistance. These curves are usually interpreted in terms of changes in the rheological properties of doughs that are all-inclusively referred to as dough development and breakdown. The rate of dough development and subsequent breakdown depends on the type of flour and the mixing action of the mixer.

The farinograph mixer used in the present studies is one of the first recording dough mixers developed for experimental studies of mixing properties. This mixer is equipped with two Z-shaped mixing blades which rotate in opposite directions. The slower blade rotates at about 60 r.p.m. while the faster blade at approximately one and a half times faster. The farinograph mixing curve is a record of the torque on the shaft of the mixer blade.

Since its development in the early 1930's, the farinograph has become a commonplace instrument in all flour quality control and research laboratories. The rise in resistance during the initial part of mixing in the farinograph is reasonably well understood and can be explained in

terms of physical blending of dough components, hydration, and the development of a continuous gluten matrix. The reason(s) for the subsequent fall in resistance or dough breakdown have not been delineated. In general terms, it is attributed to a breakdown in the gluten complex, however it is not known whether this breakdown involves the disaggregation of gluten components of the same or different chemical makeup or the depolymerization of a functionally important component or components. For the present study, it was assumed that the breakdown of dough produced by the farinograph mixer results, in most part, from changes in the physical and/or chemical properties of the flour proteins. Accordingly, most of the study dealt with the changes in the proteins. Experiments were designed to investigate both the disaggregation and depolymerization hypotheses of dough breakdown.

Results of the exhaustive extraction experiment (Fig. 5) showed that the increase in the steady-state solubility of flour proteins in 0.05N acetic acid solution during mixing depends on the intrinsic mixing strength of the flour. Comparison of the flour and the control-air dough results, showed that the effect of mixing is greatest for the weakest flour (Talbot). With the two stronger flours, significant additional increases in the steady-state solubility were obtained only by the addition of cysteine or NEMI in doughs mixed in air or in nitrogen, or by the addition of iodate in doughs mixed in air. Iodate alone, even at the high level of 2.0 μeq./g. of flour, produced only a slight increase in protein solubility measured by the exhaustive extraction procedure.

Previous workers, especially Tsen (1967), have attributed the increase in protein solubility during dough mixing to disaggregation of protein complexes. However results of the exhaustive extraction experiment suggest that the effect may be due to some form of depolymerization. In making this conclusion, it is assumed that the action of the exhaustive extraction is sufficient to break down all of the protein and other aggregates that might be present in the hydrated flour or freeze-dried dough samples. Since the increases in protein solubility under some conditions are similar to those obtained by mixing in the presence of cysteine, it appears likely that disulfide bonds are involved in the depolymerization.

Results of the solubility fractionation and the gel filtration experiments are consistent with the depolymerization hypothesis. Furthermore, these two experiments showed that it is the amount of the high molecular weight or the most insoluble component of the gluten protein that suffers a major decrease under mixing conditions which produced the most extensive breakdown. Concomitantly increases were observed in the lower molecular weight components or those that are soluble in 70% ethanol solution or in 0.05N acetic acid solution. The marked decrease in the amount of the insoluble residue protein is particularly significant from the technological viewpoint since loaf volume appears to be directly correlated with this component (Orth and Bushuk 1972A).

The depolymerization that occurs during mixing produced only a minor change in the SDS-PAGE patterns of the various solubility fractions. The alcohol-soluble fraction of doughs that suffered extensive mixing break-

down, e.g., those containing 2.0 μeq. NEMI, had several "new" bands in the high molecular weight region. Bands of similar molecular weight were observed in the patterns of the reduced acetic acid-soluble and the insoluble residue proteins. The SDS-PAGE patterns of the reduced acetic acid-soluble proteins were identical to the patterns of the insoluble residue protein. Accordingly, it is concluded that the components of these two protein fractions are built up from the same subunits. The solubility of these proteins therefore depends on the number of subunits (molecular weight) and on their tertiary structure.

The most plausible mechanism that explains the observed results, and one that is consistent with the depolymerization hypothesis, is based on the sulfhydryl-disulfide interchange reactions originally suggested by Goldstein (1957). If the sulfhydryl (SH) compound that initiates the disulfide (S-S) interchange is a low molecular weight peptide such as glutathione (Sullivan 1940) or cysteinyl glycine (Tkachuk 1969), then the interchange would result in a decrease in the average molecular weight. This can be written in form of an equation as follows:

$$P_1SH + P_2S\text{-}S\text{-}P_3 \longrightarrow P_1\text{-}S\text{-}S\text{-}P_3 + P_2\text{-}SH$$

If P_1SH is considerably lower in molecular weight than P_2SH, then the contribution of the two components on the left of the equation to a physicochemical property such as viscosity (consistency) would be much greater than that of the two components on the right. Accordingly from macromolecular theory, viscosity of a polydisperse polymer system depends on the weight average molecular weight of the polymer components. The

disulfide interchange would be particularly facilitated by the presence in the original flour or by the addition to the dough of low molecular weight SH compounds.

The depolymerization that occurs during the mixing period does not involve the scission of peptide bonds. Although the three flours used had different proteolytic activities, there was no increase in the number of free amino groups during the 15 minutes of mixing. It could well be that the enzyme would make a notable contribution to the physical properties of dough during a much longer reaction time as for example in a 5 to 6 hr. fermentation period. Also, the free lipids do not appear to be involved in the depolymerization. Results obtained showed that there was no change in the amount of free lipid under the mixing conditions that were investigated.

The S-S interchange mechanism for the depolymerization of the high molecular weight of the insoluble flour protein is consistent with the analytical data obtained in this study in so far as it would not require any change in the number of S-S groups. The rate of this interchange would presumably depend on the concentration and the chemical nature of the low molecular weight SH compounds initially present in flour or added to the dough. In addition, the number and the nature (accessibility or availability) of the S-S groups in the flour proteins and the type of mixing action would also be important in the rate of the interchange reactions.

The S-S interchange mechanism of dough breakdown can also account for differences in mixing strength. Tsen and Anderson (1963) indicated that one of the main differences between weak and strong flours is that the former contain more "available" S-S groups that will interchange more readily. The present study suggests that there may be other factors. It was observed that the weak flour (Talbot) contained a considerably higher proportion of low molecular weight peptides which were lost in the dialysis step used in the separation of the salt-solubles from the water-solubles in the solubility fractionation. The total protein recovery for Talbot was 92.5% compared with 95.4 and 95.0% for Red River 68 and Manitou respectively. If these low molecular weight peptides contain SH groups, they would be extremely effective in initiating the S-S interchange reactions. The content of amino groups and the gel filtration results are consistent with the solubility fractionation recovery data in that both suggest that the average molecular weight of the proteins, examined by both methods, is lower for the weaker flour than for the two stronger flours. Accordingly, it is concluded that the main factor that determines mixing strength is the content of low molecular weight SH peptides. This suggestion is in general agreement with the "availability" hypothesis of Tsen and Anderson (1963), since these SH groups would be readily titratable and would be quite mobile in dough systems. By way of further work, it would be of interest to compare the chemical nature of the low molecular weight peptides from flours of different mixing strength.

The S-S interchange mechanism of mixing breakdown presents some difficulty in explaining the drastic effects of SH blocking agents (NEMI) and of strong oxidizing agents such as iodate in air-mixed doughs. Because of their ability to remove SH groups, it would appear that these agents should decrease the rate of S-S interchange and thereby slow down dough breakdown. It should be noted that the large amounts of NEMI and iodate added to doughs in the present study were selected so as to accentuate dough breakdown. These amounts are in access of the SH content. Accordingly it is presumed that under these conditions, NEMI and iodate (in the presence of air) can enhance the cleavage of S-S bonds to produce an effect that is not dissimilar from the effect of reducing agents such as cysteine. Presumably the S-S bonds involved in this action could be those that are extremely critical functionally as postulated by Bloksma (1964). Again by analogy with the effect of cysteine on dough mixing strength and flour protein solubility, it would appear that the number of S-S bonds that would have been cleaved to produce a relatively major physical effect could indeed be too small to be detected by the present analytical procedures. It will be recalled that the doughs used in the present study, even those that showed extensive breakdown, did not show any definite changes in S-S content that could be measured by the analytical procedure used.

Previous measurements of S-S content in doughs mixed under various conditions by Tsen and Bushuk (1963) also did not show any significant trends with mixing time. NEMI could enhance the cleavage of S-S bonds in

flour proteins by the mechanism advanced by Spackman et al. (1960) to explain the results obtained with oxidized glutathione. In this case, the suggested reactions are:

$$G\text{-}S\text{-}S\text{-}G + OH^- \rightleftharpoons G\text{-}S^- + GSOH \qquad (1)$$

$$G\text{-}S^- + NEMI \longrightarrow GS\text{-}NEMI \qquad (2)$$

The equilibrium constant for reaction (1) is quite low so that under slightly acidic conditions the amount of GS^- is extremely small. However, in the presence of NEMI, the mercaptide ion would be removed as soon as it is formed and this would lead to a gradual decrease of S-S. It is presumed that iodate and oxygen, if present in amounts that are in access of the SH content, could react similarly by an oxidation mechanism. If this suggestion is correct, then it should be possible to detect a loss of S-S groups under extreme breakdown conditions in the presence of NEMI or iodate. The perplexing feature is that as yet no one has succeeded in obtaining confirmatory analytical evidence of the suggested loss of S-S. Accordingly, the mechanism of the extensive dough breakdown by mixing in the presence of relatively large quantities from technological viewpoint of SH blocking or fast oxidizing agents remains unresolved.

The results obtained in the present study extend previously published work, particularly that of Mecham (1959) and Tsen (1967). These workers emphasized the disaggregation mechanism of dough breakdown during mixing whereas the present study places a greater emphasis on the depolymerization mechanism. Perhaps both mechanisms are important. Further work is required to determine the contribution and the technological significance of each.

SUMMARY OF RESULTS AND CONCLUSIONS

1. The changes in some physicochemical properties of the proteins of flour during dough mixing in a farinograph under various conditions were investigated using flours of three wheat varieties of different mixing strength but of similar protein content. The three varieties and their mixing strength classification are: Red River 68 - strong, Manitou - medium, and Talbot - weak.
2. Additions of NEMI and potassium iodate and mixing for 15 minutes in air or in nitrogen were used to produce accentuated dough breakdown. The effect of air, NEMI and iodate on the rate of dough breakdown was more evident in doughs from weak flour than in doughs from strong flour.
3. The proteolytic activity of flours of the three varieties was low and did not have any effect on dough breakdown during the mixing period examined.
4. The strong wheat (Red River 68) had the lowest, the medium wheat (Manitou) intermediate, and the weak wheat (Talbot) the highest proteolytic activity. That is, the order on the basis of mixing strength was consistent with the order based on proteolytic activity.
5. The number of free amino groups in doughs for each variety remained constant during mixing for all the conditions investigated.
6. For the three varieties used, the content of free amino groups in flour increased in the following order: Red River 68, Manitou, and Talbot. This order is the same as the order of proteolytic activities.

7. The exhaustive extraction showed that the increase in the steady-state solubility of flour proteins in 0.05N acetic acid solution during mixing depends on the intrinsic mixing strength of the flour. Comparison of the flour and the control-air dough results, showed that the effect of mixing on protein solubility was greatest for the weakest flour (Talbot).

8. With the two stronger flours Red River 68 and Manitou, significant additional increases in the steady-state solubility in 0.05N acetic acid were obtained during mixing by the addition to the dough of cysteine or NEMI and mixing in air or in nitrogen, and by the addition of iodate and mixing in air. In doughs mixed in nitrogen, iodate produced only a slight increase in protein extractability.

9. Results of the solubility fractionation showed that the amount of the alcohol-soluble and the acetic acid-soluble proteins increased with mixing, while the amount of insoluble residue protein decreased.

10. Gel filtration results showed that the high molecular weight component (residue component) suffered a major decrease during mixing under conditions which produced the greatest amount of breakdown. Concomitantly increases were observed in the low molecular weight components (alcohol- and acetic acid-soluble components).

11. Mixing produced only a minor change in the SDS-PAGE patterns of the protein fractions obtained by solubility fractionation. The patterns of the alcohol-soluble fraction of doughs that suffered extensive mixing breakdown, e.g., those containing 2.0 μeq. NEMI, had several

"new" bands in the high molecular weight region. Bands of similar molecular weight were observed in the patterns of the reduced acetic acid-soluble and the insoluble residue proteins.

12. The SDS-PAGE patterns of the reduced acetic acid-soluble proteins were identical to the patterns of the reduced insoluble residue protein and were not affected by mixing.

13. The most plausible explanation for the observed changes in the flour proteins that occur during mixing, is that the high molecular weight insoluble component in gluten depolymerizes to form components of lower molecular weight which are soluble in 70% ethanol solution or 0.05N acetic acid solution. This depolymerization could occur through the disulfide interchange reactions initiated by low molecular weight sulfhydryl peptides or proteins. According to this depolymerization hypothesis, mixing strength of a wheat flour would be largely dependent on the content of low molecular weight sulfhydryl peptides. The results obtained for the three wheat varieties used in this study are consistent with this hypothesis.

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APPENDIX

Table 1. Exhaustive Extraction of Red River 68 Flour and Doughs Mixed for 15 Min. under Various Conditions.

Homogenizing Time (min.)	5	10	30	60	120
	(% of Total Flour Protein)				
Flour	45.6	49.0	52.4	57.7	62.9
Control, air	57.2	61.4	62.0	63.2	64.0
NEMI*, air	81.2	86.3	89.9	92.1	94.6
Iodate*, air	67.3	86.5	88.8	90.3	92.9
Iodate*, nitrogen	61.7	65.7	66.3	67.5	70.0
Cysteine*, air	88.7	93.0	93.2	94.7	94.1

*Amount of reagent = 2.0 μeq./g. flour

Table 2. Exhaustive Extraction of Manitou Flour and Doughs Mixed for 15 Min. under Various Conditions.

Homogenizing Time (min.)	5	10	30	60	120
	(% of Total Flour Protein)				
Flour	47.1	56.8	59.9	67.1	66.0
Control, air	72.0	76.2	81.2	80.6	82.2
NEMI*, air	90.4	91.8	94.0	94.1	96.2
Iodate*, air	86.7	88.9	90.5	91.7	90.6
Iodate,* nitrogen	72.0	77.6	83.2	85.3	85.3
Cysteine*, air	89.1	90.4	92.0	93.2	94.3

*Amount of reagent = 2.0 μeq./g. flour

Table 3. Exhaustive Extraction of Talbot Flour and Doughs Mixed for 15 Min. under Various Conditions.

Homogenizing Time (min.)	5	10	30	60	120
	(% of Total Flour Protein)				
Flour	55.4	60.9	65.6	67.9	69.4
Control, air	76.1	82.4	88.4	88.8	89.6
NEMI*, air	87.2	90.2	91.9	93.3	94.1
Iodate*, air	89.6	91.6	89.9	91.8	91.5
Iodate*, nitrogen	89.7	89.5	89.3	90.4	91.9
Cysteine*, air	88.2	91.8	93.5	92.5	94.1

*Amount of reagent = 2.0 μeq./g. flour

Table 4. Distribution of Protein of Water-Soluble Fraction of Flours and Doughs Mixed under Various Conditions.

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
	%	%	%	%	%	%	%	%	%	%	%	%
Flour	12.6				11.5				12.3			
Control	11.5	11.1	12.4	11.4	12.8	12.4	10.7	10.0	11.3	12.1	12.0	12.6
NEMI												
0.4 µeq./g. flour	11.9	10.9	11.4	13.0	11.5	12.0	10.4	10.0	12.8	11.5	11.8	11.3
2.0 µeq./g. flour	12.9	13.4	11.1	10.2	12.1	12.4	10.8	11.5	10.9	12.0	9.4	10.7
Iodate												
0.4 µeq./g. flour	10.8	10.5	10.2	11.7	12.0	11.9	12.2	11.6	12.3	10.0	11.4	11.3
2.0 µeq./g. flour	14.1	12.3	11.1	10.4	12.1	12.5	11.0	9.4	11.0	12.0	10.9	9.9

Table 5. Distribution of Protein of Salt-Soluble Fraction of Flours and Doughs Mixed under Various Conditions.

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
	%	%	%	%	%	%	%	%	%	%	%	%
Flour	2.7				2.4				2.1			
Control	2.5	1.7	2.9	3.0	2.4	1.8	2.6	2.0	2.2	1.6	1.7	1.8
NEMI												
0.4 μeq./g. flour	3.0	2.2	2.3	2.6	2.4	2.1	2.8	2.1	2.2	2.0	1.5	2.0
2.0 μeq./g. flour	2.9	2.3	2.6	2.8	2.6	2.2	3.1	1.9	2.2	2.0	2.2	2.3
Iodate												
0.4 μeq./g. flour	2.7	2.5	2.7	2.8	2.4	2.2	1.9	1.6	1.9	2.2	1.7	2.0
2.0 μeq./g. flour	2.6	1.9	3.1	2.5	1.9	2.5	1.6	2.0	1.7	2.2	1.6	2.2

Table 6. Distribution of Protein of Alcohol-Soluble Fraction of Flours and Doughs Mixed under Various Conditions.

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
	%	%	%	%	%	%	%	%	%	%	%	%
Flour	33.1				35.5				37.3			
Control	33.6	35.4	31.4	32.5	35.4	36.1	33.8	33.9	38.4	40.8	38.2	40.3
NEMI												
0.4 μeq./g. flour	36.5	38.8	32.2	30.4	35.8	41.1	36.6	38.6	37.9	42.2	37.1	40.4
2.0 μeq./g. flour	40.5	41.4	32.5	37.8	41.5	49.3	37.1	45.7	41.5	44.1	39.2	44.8
Iodate												
0.4 μeq./g. flour	29.6	32.6	36.2	32.9	21.6	32.1	33.8	34.5	34.9	34.7	34.3	37.2
2.0 μeq./g. flour	38.3	41.5	31.6	32.6	41.7	43.5	35.0	41.8	40.3	43.0	36.0	43.1

Table 7. Distribution of Protein of Acetic Acid-Soluble Fraction of Flours and Doughs Mixed under Various Conditions.

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
	%	%	%	%	%	%	%	%	%	%	%	%
Flour	6.2				7.3				11.0			
Control	11.1	12.7	10.6	11.8	10.4	12.2	10.2	12.3	17.5	19.8	11.1	12.3
NEMI												
0.4 μeq./g. flour	15.4	28.7	13.2	15.9	16.6	22.2	10.2	15.3	28.6	32.2	22.2	26.1
2.0 μeq./g. flour	17.9	31.8	30.0	38.5	19.5	26.0	22.4	29.4	30.3	26.0	30.1	28.7
Iodate												
0.4 μeq./g. flour	13.5	17.5	8.5	12.3	13.7	17.5	9.4	12.0	24.7	36.1	19.1	21.0
2.0 μeq./g. flour	18.3	23.5	14.0	19.1	13.0	17.7	10.3	13.4	27.6	25.9	28.2	26.5

Table 8. Distribution of Protein of Residue Fraction of Flours and Doughs Mixed under Various Conditions.

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
	%	%	%	%	%	%	%	%	%	%	%	%
Flour	39.2				38.0				28.3			
Control	37.6	36.2	38.4	38.0	36.0	33.5	36.5	34.9	24.3	21.0	27.3	26.4
NEMI												
0.4 μeq./g. flour	25.3	12.5	36.2	32.6	27.5	18.0	33.6	30.2	11.6	6.0	19.4	9.3
2.0 μeq./g. flour	20.4	5.8	20.6	5.3	21.9	5.7	21.6	6.1	6.2	5.0	13.1	6.8
Iodate												
0.4 μeq./g. flour	37.0	33.2	38.6	35.0	37.0	31.6	37.5	34.0	18.5	8.3	27.0	22.2
2.0 μeq./g. flour	22.2	17.9	36.5	31.7	26.8	18.4	35.1	28.1	13.8	7.4	16.3	9.5

Table 9. Recovery of Nitrogen from Five Solubility Fractions of Flours and Doughs Mixed under Various Conditions.

	Red River 68				Manitou				Talbot			
	Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
Mixing Time (min.)	5	15	5	15	5	15	5	15	5	15	5	15
	%	%	%	%	%	%	%	%	%	%	%	%
Flour	93.2				94.7				91.0			
Control	96.3	97.1	95.7	96.7	97.0	96.0	93.8	93.1	93.7	93.3	90.3	93.4
NEMI												
0.4 µeq./g. flour	92.1	93.1	95.3	94.5	93.8	95.4	93.6	96.2	93.1	93.9	92.0	90.1
2.0 µeq./g. flour	94.6	94.7	96.8	94.6	97.6	95.6	95.0	94.6	91.1	90.3	94.0	93.3
Iodate												
0.4 µeq./g. flour	93.6	96.3	96.2	94.7	96.7	95.3	94.8	93.7	92.3	91.3	93.5	93.7
2.0 µeq./g. flour	95.5	97.0	96.3	96.3	95.5	94.6	93.0	94.7	94.4	90.5	93.0	91.2