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UCTURE AND GENETICS OF GLUTENIN IN RELATION TO BREADMAKING QUALITY

by

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ABSTRACT

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Three methods for preparing glutenin, namely, Osborne fractionation, gel-filtration chromatography, and pH precipitation with subsequent purification using SE-Sephadex C-50 were evaluated. On the basis of electrophoretic and amino acid analyses, the third method was found to yield glutenin free of low-molecular weight proteins, the major contaminant of glutenin prepared by other procedures.

Subunits obtained by reduction of glutenin from 26 hexaploid wheats of diverse baking quality were examined by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE). The subunit composition of these glutenins was genetically controlled. The composition does not appear to be related to breadmaking quality or to location of growth.

Glutenins from four extracted (AABB) tetraploid wheats, their hexaploid (AABBDD) common wheat counterparts, a synthetic hexaploid (AABBDD), its parents and seven accessions of Aegilops squarrosa (donor of D genome of common wheat) were isolated, reduced and analyzed by SDS-PAGE. The glutenin subunits of the synthetic hexaploid were simply inherited from its tetraploid (AABB) and diploid (DD) parents. Each extracted tetraploid lacked three glutenin subunits and showed a decrease in the intensity of a fourth electrophoretic

band present in the hexaploid parent. Three of the affected subunits were of the same molecular weight for each hexaploid-tetraploid pair, the fourth being different for one of the four pairs. The four subunits affected in each pair were present in the Aegilops squarrosa samples studied. SDS-PAGE patterns of the glutenin subunits of Aegilops squarrosa varieties anathera, strangulata, and typica were identical whereas the two highest-molecular weight subunits of a fourth variety, meyeri, were of slightly lower molecular weight than the analogous units of the first three varieties. Common (bread) wheats, contained glutenin subunits of high-molecular weight which were absent in durum wheats. Their presence appears to be a necessary condition for breadmaking quality.

SDS-PAGE patterns of reduced glutenins of nullisomic-tetrasomic lines of Chinese Spring showed that four glutenin subunits are coded by genes on chromosome 1D.

Similar analysis of glutenins of ditelocentric lines showed that these genes are located on the long arm of this chromosome. The four subunits assigned to chromosome 1D were absent in the durum LD 222 and present in its (LD 222) 1D-1B substitution line. Repression of the synthesis of subunits coded for by chromosomes of the A or B genome was observed in lines tetrasomic for chromosomes 2B, 3B, and 6B.

Scanning electron microscopy of glutenins of Canthatch, Manitou (bread wheats), Stewart 63 (durum), a synthetic hexaploid and its parents, and Prolific (rye) showed characteristic structures that could be related to the rheological properties of the flour-doughs of these cereals. Reduction of Manitou glutenin resulted in complete loss of its fibrous structure.

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INTRODUCTION

Gluten, the complex viscoelastic substance responsible for the dough-forming ability of bread wheat flour, is composed largely of the proteins gliadin and glutenin. Determination of the structure of these proteins is required in order to understand dough rheology and breadmaking quality at the molecular level.

Gliadin proteins, which are soluble in a variety of solvents, have been quite extensively characterized. On the other hand, only recent developments in biochemical techniques have made it possible to study the structure of glutenin. Ironically, the properties that make glutenin functionally important, i.e. its large molecular size and insolubility in aqueous solvents, have hindered its physicochemical study. This, in turn, has delayed progress in understanding its role in wheat protein quality with respect to breadmaking.

One of the most useful new techniques used in this study was sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE). This method, coupled with improved preparative methods employing recently developed solvents, has resulted in a rapid accumulation of information on the structure of glutenin. The complexity and importance of the functional role of glutenin make the determination of its structure and genetic inheritance an extremely challenging and worthwhile study.

SDS-PAGE allows the determination of the molecular weights of individual components of very complex protein mixtures. It is particularly useful in the study of glutenin which comprises many different polypeptide subunits joined by disulfide bonds. This experimental approach forms the basis of the major portion of this thesis.

Another technical development that is extremely important to studies of the structure of biological materials, is the scanning electron microscope (SEM). The perfection of this instrument, and procedures for its use, was a major advance in research on the ultrastructure of a wide range of materials. When glutenins from various sources were viewed with the SEM, very detailed photographs with a three dimensional quality were obtained. The structures observed can also be related to the functional (breadmaking) properties of the flours from which the glutenins were prepared.

Along with studies of the structure of glutenin, this project presented an opportunity to examine some aspects of the genetics of this protein. Cereals, particularly wheats, are ideal materials for a study of the inheritance of proteins and the location of the genes that control their synthesis. The availability of a variety of aneuploid lines and synthetic amphiploids allowed us to determine that glutenin subunits are simply inherited and to locate the chromosomes containing the genes responsible for the synthesis of those subunits.

This structural and genetic information leads to a better understanding of the properties of glutenin and its functional role, and provides "protein markers" that indicate the presence of a particular chromosome in the wheat.

The results are presented in this thesis as a series of five papers. They form part of a series entitled "Studies of Glutenin". Two of these papers have already been published in Cereal Chemistry and three others have been submitted to this journal for publication.

The format adopted for this thesis deviates somewhat from the standard doctoral thesis. It has a number of distinct advantages. Firstly, it allows early publication of the results without extensive rewriting. Secondly, each paper includes its relevant literature review, materials and methods section, and discussion which results in a clearer presentation. Finally, the practice gained in writing manuscripts for publication is much more valuable to a student than that gained from writing a standard thesis, since most research results are published in form of scientific papers.

One criticism of the format adopted was that sometimes it creates discontinuities in the presentation of the results and difficulties in the formulation of an overall conclusion. To overcome this, a general discussion and conclusion section has been included. The author-date system for listing references is used and thereby only one bibliography is necessary at the end of the thesis, rather than a separate one for each paper, the literature review, and the general discussion. This method eliminates the repetition of references, another minor criticism of the present type of format.

REVIEW OF THE LITERATURE

Chemical Properties, Structure, and Cellular Location of Glutenin

Glutenin, as defined originally by Osborne (1907) is the acetic acid-soluble glutelin of wheat-flour. This protein is primarily responsible for the elasticity of the viscoelastic gluten complex whose properties, to a large extent, determine the breadmaking potential of a bread wheat flour. The other major protein in gluten is gliadin which contributes viscous flow to the gluten. A fractionation procedure developed recently by Meredith and Wren (1966), permits classification of the flour proteins on the basis of their molecular weights. Gel-filtration chromatography on Sephadex G-200 in the solvent AUC (0.1M acetic acid, 3M urea, 0.01M hexadecyltrimethylammonium bromide) produces four major, fairly well resolved, peaks; glutenins (high-molecular weight proteins), gliadins, albumins, and non-protein nitrogen.

When dissolved in AUC and applied to a Sephadex column glutenin prepared by the Osborne fractionation procedure was shown to comprise both glutenin and gliadin according to the gel-filtration classification (Hwang, 1972). Conversely when gliadin prepared by the Meredith and Wren procedure was subjected to electrophoresis on a 7 1/2% polyacrylamide gel, a significant proportion of the protein did not enter the gel and therefore should not be referred to as gliadin. There is an urgent need for a more precise classification of flour proteins.

The functional importance of gluten in breadmaking has led to great interest in its structure. Since gliadin and glutenin combine to form gluten, their similarities and differences have been extensively studied.

Woychik et al. (1964) found that reduced glutenin subunits migrated readily into starch gels and had mobilities similar to those of reduced gliadins. They suggested that glutenin may be a polymer of gliadin components joined by disulfide bonds. This hypothesis was supported by Ewart (1966) on the basis of electrophoretic fingerprints of gliadin and glutenin. He found many similarities between the fingerprints and concluded that gliadin and glutenin molecules had considerable portions with the same amino acid sequences.

Elton and Ewart (1966) reduced glutenin obtained from four different wheat varieties and observed that their starch-gel electrophoretic patterns contained a number of components of the same mobility as those of the corresponding reduced gliadins. Varietal differences were apparent in the electrophoretic patterns of the reduced glutenins.

Evidence for distinct differences in enzymically-produced peptides from gliadin and glutenin was recently reported by Bietz and Rothfus (1970). The peptides produced by peptic hydrolysis had average molecular weights of 1,990 and 1,150 for glutenin and gliadin respectively. Larger peptide segments occurred between pepsin susceptible bonds in glutenin than in gliadin, but many of the polypeptide segments from the proteins were identical. Further work by these authors (Bietz and Rothfus, 1971) showed that several

peptides were common to peptic digests of gliadin and glutenin, but most were unique. Glycine occurred more frequently in the peptides of glutenin, and proline more in those of gliadin.

Bietz and Wall (1972) studied the molecular weight distribution of the components of reduced gliadin and glutenin using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. They found most gliadins were single chained. The three major components had molecular weights of 36,500, 44,200, and 11,400. Molecular weights of 69,300 and 78,100 were obtained for Ω-gliadins. Glutenin, on the other hand, comprised subunits with molecular weights ranging from 11,600 to 133,000. Two glutenin subunits had the same molecular weights as the major gliadin polypeptides. The marked differences in molecular weights observed for other glutenin subunits and gliadin suggests that the region of similar sequence in gliadins and glutenins must constitute only part of these proteins.

Jones et al. (1961) used the analytical ultracentrifuge and obtained a weight-average molecular weight of 2-3 million for wheat glutenins. Despite this high value, their preparation also contained a significant quantity of small molecules (below 50,000). The weight-average molecular weight did not vary greatly in aluminum lactate and guanidine thiocyanate indicating that the larger molecules were not loosely aggregated polymers but represented stable molecules.

Gliadin and glutenin contain a very high proportion of the amino acids glutamine and proline (Wu and Dimler, 1963a, 1963b). About one in every three amino acids is glutamine, and one in every seven is proline.

This high content of glutamine results in a high degree of association of peptide chains through hydrogen bonds. Both proteins contain low proportions of arginine, lysine and histidine (amino acids capable of carrying a positive charge at acid pH) and so have low ionic character. The relatively high proportion of hydrophobic amino acids in these proteins permits apolar binding between the polypeptide chains and contributes to their general insolubility in aqueous solvents of high ionic strength.

The isoelectric points of the gluten proteins generally fall in the pH range from 6 to 9 (Wrigley, 1968a,b). At, or near, their isoelectric points they are quite insoluble, but are reasonably soluble at pH's below 4 or 5.

Gliadin and glutenin differ in their amino acid composition (Ewart, 1967). Glutenin is higher in lysine, tryptophan, and glycine whereas gliadin is higher in proline, glutamic acid plus glutamine, cystine, isoleucine, phenylalanine, and amide nitrogen.

In addition to the physicochemical studies of glutenin components, there have been a number of interesting electron microscopy studies of its ultrastructure. Seckinger and Wolf (1970) obtained transmission electron micrographs of particles and surface dispersions of proteins from hard and soft wheats. A purified gliadin fraction contained particles 20 to 80 A° in diameter whereas the glutenin fraction did not form small particles.

The transmission electron microscope was used by Simmonds (1972) to demonstrate the presence of endoplasmic reticulum and other membraneous residues in mature endosperm cells. He concluded that part of the glutenin

fraction in wheat flour and dough originated from the endoplasmic reticulum. The remainder of the glutenin in dough was derived from:

a) The high-molecular weight fraction present in the storage protein of the endosperm (Bushuk and Wrigley, 1971) and,

b) from the association of protein and lipid components on the addition of water to flour during dough preparation.

The identification of protein bodies in the developing wheat endosperm by Buttrose (1963), Graham et al. (1962) and Jennings et al. (1963) was a major step towards understanding the biosynthesis and the morphological origin of flour proteins. The number and size of these protein bodies increased with the development of the wheat kernel. This increase was paralleled by an increase in the amount of acetic acid-soluble protein in the endosperm (Jennings, 1968; Jennings and Morton, 1963; Jennings et al., 1963). These proteins, which are the major components of gluten, are deposited as storage proteins and are subsequently available as nutrients for the new plant during germination.

In contrast to the findings of Simmonds (1972), it had been suggested earlier that the gluten and cytoplasmic proteins were distinctly different. Morton and Raison (1963) and Morton et al. (1964) observed that protein bodies were formed in proteoplasts in the endosperm cells. On the other hand Buttrose (1963) concluded that the cytoplasmic proteins were most likely synthesized independently on ribosomes associated with the endoplasmic reticulum.

Density-gradient centrifugation (Graham et al., 1963) was used to prepare relatively small protein bodies. Although gel electrophoresis of

these proteins showed mostly slow-moving components, their amino acid compositions were similar to albumins and globulins (Jennings and Morton, 1963). Larger protein bodies had an amino acid composition comparable to that of the gluten proteins. There may be two different types of protein bodies, each composed of a distinct type of storage protein (Jennings, 1968).

Glutenin and Breadmaking Quality

Glutenin plays an extremely important role in the determination of breadmaking quality. Pomeranz (1965) suggested that the proportion of flour protein dispersible in 3M urea be used as an index of baking potential. Flours of poor breadmaking quality contained a greater proportion of urea-soluble protein. Conversely, the greater the proportion of high-molecular weight insoluble protein (mostly glutenin) the better the breadmaking potential.

In two reports Mullen and Smith (Mullen and Smith, 1965; Smith and Mullen, 1965) demonstrated that the mixing time to maximum consistency in the farinograph was correlated with the ratio of high- to low-molecular weight protein for two flours of widely different mixing requirements. The factors accounting for the large differences in the rheological properties of the two flours were found in the gluten-starch fraction. Addition of this fraction to doughs mixed in a farinograph increased mixing requirement, whereas addition of the water-soluble gliadins decreased this requirement.

Gluten proteins dispersed in 0.005N lactic acid were fractionally precipitated by a gradual increase in pH (Shogren et al., 1969). As the pH

was increased glutenin precipitated whereas the gliadins remained in solution. Fractions collected by this procedure, and added to the base flour, indicated that mixing time and baking absorption increased with an increase in glutenin, whereas oxidation requirement and loaf volume increased with an increasing proportion of gliadin.

Lee and Mac Ritchie (1971) used reconstituted flours to examine the affect on rheological properties of protein fractions soluble in urea solutions and in 0.1M sodium hydroxide (NaOH). Early urea extracts decreased mixing stability in the mixograph and gave weaker, more extensible doughs. Late urea and NaOH extracts increased mixed stability and produced stronger doughs. Gel-filtration profiles showed that the proportion of high-molecular weight protein in the extract increased with successive extractions.

Much research effort has been directed at relating the protein solubility distribution, as determined by a modified Osborne fractionation, with breadmaking quality. Chen and Bushuk (1970) observed that the protein solubility distribution of a hard red spring wheat (HRS) differed from a variety of triticale and the durum and rye parents of the triticale. The HRS wheat had a lower proportion of water-soluble protein and a higher proportion of insoluble or high-molecular weight protein. This appeared to be the major reason for the superior breadmaking quality of this wheat compared with the durum wheat and the triticale.

In a study of three hexaploid bread wheats and their AABB extracted tetraploid wheats, Dronzek et al. (1970), found that two of the tetraploids were of inferior baking quality to, and contained a much lower proportion

of residue protein than, their corresponding hexaploids. The third tetraploid was comparable in baking quality and contained approximately the same proportion of residue protein as its hexaploid parent.

The flour proteins of twenty-six wheat varieties of diverse baking quality grown in four locations in Western Canada were fractionated by the Osborne technique in an attempt to relate the protein solubility distribution to breadmaking quality (Orth and Bushuk, 1972; Orth et al., 1972). The proportion of acetic acid-soluble glutenin in the total flour protein was negatively correlated with protein quality. On the other hand the proportion of residue protein was significantly correlated with Zeleny sedimentation value, farinograph dough development time, farinograph mixing tolerance index, remix loaf volume and loaf-volume per unit protein. The proportion of residue protein was shown statistically to be the best index of breadmaking quality.

Mecham (1968) demonstrated a definite role for glutenin in dough mixing. Mixed doughs contained more protein soluble in dilute acetic acid than did the original flours. The proportion of residue or "gel" fraction quickly decreased with increased mixing time. This observation indicates that one important role of mixing is the breakdown of the highest molecular weight glutenin into acetic acid - soluble protein. The breakdown of residue protein during mixing has been confirmed by Tanaka (1972) who has also postulated a depolymerization mechanism for this process.

Another important factor governing the solubility of glutenin is its association with lipid. Simmonds and Wrigley (1972) observed that much less protein was extractable from gluten than from storage protein prepared

using organic solvents, and thus depleted in lipid. Presumably, lipid protein association in gluten during hydration and dough formation causes this marked solubility change.

From the research reports reviewed above, and much other work, it is evident that the acetic acid - soluble and insoluble or residue flour proteins (mostly glutenins) are essential to breadmaking quality.

Recent Advances in Studies of the Structure of Glutenin

Because of their insolubility in many aqueous systems, and their large molecular size, the glutenins of bread wheats have not been extensively studied. In recent years solvents and biochemical techniques applicable to this group of proteins have been developed and have led to renewed interest in their physicochemical nature.

Most techniques used in studies of the structure of macromolecules depend on the availability of suitable solvents. Meredith and Wren (1966) introduced the highly dissociating solvent AUC (aqueous acetic acid, urea and hexadecyltrimethylammonium bromide) which solubilizes 95% of the total flour protein. Flour extracts with this solvent, applied to Sephadex G-200 columns, were fractionated into four main classes; glutenins, gliadins, albumins, and non-protein material absorbing at 280 nm. The glutenin peak obtained by this gel-filtration fractionation, represents both the acetic-soluble glutenin and most of the insoluble residue protein of the classical Osborne fractionation. Although complete resolution was not obtained, gel filtration in this solvent is useful, as almost all the flour protein can be solubilized by AUC and an initial separation can be attained.

Solubilization of the residue protein was accomplished in the solvent hydrochloric acid-2-chloroethanol (Cluskey and Dimler, 1967). Electrophoresis showed that the protein dissolved in this solvent comprised components of similar mobility to acetic acid-soluble glutenins as well as some faster moving components. They concluded that the residue protein was a mixture of high-molecular weight constituents consisting of polypeptides linked through disulfide bonds.

The best information on the structure of glutenin has been obtained by initially reducing its disulfide bonds and thereby producing soluble subunits. Reduction of glutenin, followed by aminoethylation of its subunits, allowed separation into four groups of polypeptides by fractional precipitation with cupric nitrate (Rothfus and Crow, 1968). Material precipitated by 0.0283 M cupric nitrate accounted for 22% of the glutenin and consisted of slow-moving electrophoretic components with amino acid compositions different from the other gluten proteins. These peptides were particularly high in glutamic acid, glycine, and tyrosine. Gel-permeation chromatography indicated a molecular weight of approximately 80,000 for these glutenin subunits.

Glutenins from eleven wheat varieties, of five different classes, were compared in terms of protein composition, carbohydrate composition, intrinsic viscosity and sensitivity to salt precipitation (Huebner, 1970). Starch-gel electrophoresis of reduced and alkylated glutenins showed significant differences among varieties of the same class, but greatest differences were noted for wheats of different classes. Intrinsic viscosities of the glutenins were generally unrelated to the genetic background

of the wheats. The response of glutenins to salt precipitation suggested that gluten quality may be directly related to the sensitivity of its glutenin proteins to changes in ionic strength.

An unusual solvent for glutenin was recently reported by Mecham et al. (1972). The residue remaining after repeated extractions of flour with 0.01M acetic acid, was readily solubilized in 0.01M acetic acid containing 0.04M mercuric chloride. The authors concluded that rupture of disulfide bonds was not involved in this solubilization as the glutenin retained its rubbery cohesiveness.

The application of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to flour proteins has enabled detailed study of the high-molecular weight glutenins (Bietz and Wall, 1972). Without reduction, glutenins are too large to enter standard polyacrylamide gels and consequently their study by this technique is impossible. Reduction and alkylation of glutenin, followed by complexing with SDS, produces subunits that can be readily separated by electrophoresis in a 5% polyacrylamide gel.

SDS-PAGE separates polypeptides on the basis of their molecular weights (Shapiro et al., 1967). The normal dependence of electrophoretic mobility on both charge and size is reduced to a dependence on size alone after the protein is complexed with SDS. Reacting soluble proteins with SDS results in an extended polypeptide chain surrounded by negatively charged SDS molecules (at or near neutral pH) (Reynolds and Tanford, 1970).

The reliability of molecular weights determined by this technique has been investigated by a number of researchers. Weber and Osborn (1969) determined molecular weights with an accuracy of ± 10% for forty proteins, ranging in molecular weight from 15,000 to 100,000 daltons. Slightly better accuracy was achieved by the use of internal standards (Dunker and Rueckert, 1969). Using this technique molecular weights obtained agreed within ± 6% of literature values.

SDS-PAGE studies of collagen peptides uncovered one possible source of inaccuracy in molecular weights determined by this method (Furthmayr et al., 1971). These peptides, with up to 22 mole percent proline or hydroxyproline, moved slower than expected for their known molecular weights. Their high content of imino groups could affect the flexibility of the SDS-protein complexes and thereby decrease their mobility. The overall effect of this would be an apparent increase in molecular weight.

Another factor to consider when employing this technique is the amount of SDS bound to the protein. Nelson (1971) found that this value depended on the charge on the protein. The relative mobility of the SDS-protein complex, and therefore the calculated molecular weight, varied significantly with the specific amount of detergent bound. Tung and Knight (1972) observed that the log of molecular weight was proportional to mobility for a series of proteins only when they had the same hydrodynamic shape and charge to mass ratio. Many proteins met this requirement but there were a few exceptions.

Correlation of Chromatographic and Electrophoretic Results to Breadmaking Quality

The development of chromatographic and electrophoretic techniques capable of resolving flour proteins initiated research aimed at relating the presence or absence of specific proteins to breadmaking quality.

Elton and Ewart (1962), using starch-gel electrophoresis, observed significant intervarietal differences amongst the gluten proteins from eight varieties of wheat, but little difference in the albumins and globulins. They concluded that the former differences were sufficiently marked to give rise to variations in protein quality for breadmaking.

Starch-gel electrophoresis of the flour proteins from fifteen wheats representing six wheat types was used to distinguish hard red spring, hard red winter and durum wheats (Kelley and Koenig, 1963). A greater number of slower moving components was noted in the patterns for hard red winter and club than in those for hard red spring wheats.

The proteins extracted from fourteen bread wheats with pyrophosphate buffer and 0.05M acetic acid, were subjected to electrophoresis on polyacrylamide gels and chromatography on CM-cellulose (Lee and Wrigley, 1963). Electrophoretic and chromatographic patterns of the proteins from the primitive and current commercial wheats studied were generally similar. Marked differences in the patterns of tetraploid and hexaploid varieties were noted. However, no one particular electrophoretic or chromatographic pattern could be related to baking quality.

Distinct intervarietal differences in flour proteins separated by electrophoresis on starch gels were obtained by Coulson and Sim (1965). There was no apparent relationship between electrophoretic patterns and rheological properties. The regions of intermediate mobility were similar for all varieties but there were major differences evident in the region of the slow-moving components.

Doekes (1968) employed starch-gel electrophoresis in a study of the water-soluble gliadins of eighty flours. Five types of electrophoretic patterns could be distinguished. Wheats placed in a class on the basis of their patterns were of the same general type. There was a suggestion that genetically related wheats had similar gliadin electropherograms. Although a possible relationship between baking quality and electrophoretic pattern was suggested by this work, many wheats were extremely difficult to classify and the method has not been generally accepted.

Flour proteins of twenty-six wheat varieties, each grown at four stations in Western Canada, were studied by solubility fractionation and polyacrylamide disc-gel electrophoresis (Orth and Bushuk, 1972). The protein distribution in relation to baking quality was discussed earlier in this review. Minor intervarietal differences in the electrophoretic patterns of the albumins and globulins, and major qualitative differences amongst the gliadins, were observed. Although a broad range of baking quality was represented by the varieties, the electrophoretic patterns could not be related to breadmaking quality.

Information on a possible relationship between the gel-filtration profile of flour proteins and baking quality was recently published by

Bushuk and Wrigley (1972). These workers fractionated the proteins extracted with AUC on a Sephadex G-150 column. The flour from rust infected Marquis wheat lacked one of the low-molecular weight glutenin peaks present in the flour from the healthy sample. This could explain the observed decrease in baking quality resulting from the infection. The rust did not affect the electrophoretic patterns of the proteins separated on 7 1/2% polyacrylamide gels (below 100,000 in molecular weight). Perhaps the differences observed by gel-filtration chromatography were not detected by electrophoresis since in the latter technique the larger proteins did not enter the gel. The presence of a low-molecular weight glutenin in bread wheats and its absence in durum wheats (Bushuk and Wrigley, 1971) gives further support to the hypothesis that this glutenin may be related to baking quality.

The SDS-PAGE technique discussed earlier was first applied to flour proteins by Bietz and Wall (1972). Reduction of glutenin allowed resolution of its subunits by electrophoresis whereas intact glutenin remained as one band at the origin. Distinct differences in the patterns of the reduced glutenins from durum, hard red winter, hard red spring, club, soft white winter, and soft red winter wheats were noted but no relationship to quality could be formulated from these results.

Wall et al. (1972) studied the reduced glutenin from durum, triticale, rye and hard red spring wheat. The durum glutenin lacked the two highest molecular weight components found in glutenin from hexaploid wheats. The triticale studied contained glutenin components typical of both rye and durum wheats, but also a high molecular-weight band associated with bread

wheats. They suggested that the latter was introduced during crossing of the triticale with hexaploid wheats. The triticale was obtained by further crossing of the original durum wheat by rye amphiploid with common wheat in an attempt to introduce baking quality into the synthetic species.

Chromosomal Location of Quality Factors

The hexaploids (AABBDD) appear to have emerged as a result of amphiploidy between a cultivated tetraploid (AABB) and a species of Aegilops (DD) (Johnson et al., 1967). Genetic material has been developed and can be used to determine the genomic and chromosomal location of the genes responsible for baking quality or for the synthesis of specific proteins. In this manner, breadmaking quality may be attributed to a particular protein or group of proteins. Furthermore the association of a protein with a specific chromosome can be used as an indication of the presence of that chromosome in a wheat. Such "chromosome markers" are very useful in cytogenetic studies.

Dronzek et al. (1970) studied the electrophoretic patterns of the soluble proteins from three hexaploids and their AABB extracted tetraploids. Although significant qualitative differences occurred between varieties the patterns for the albumins, globulins, and gliadins were essentially the same within each hexaploid - tetraploid pair. Under the electrophoretic conditions used, only proteins of molecular weight less than 100,000 entered the gel.

In a subsequent study using the same hexaploid-tetraploid pairs as Dronzek et al., as well as synthetic hexaploids and their tetraploid (AABB) and diploid (DD) parents, Konarev et al. (1972) associated two slow-moving gliadins with the D genome. The electrophoretic technique used in this work allowed greater resolution of components and this may explain the disagreement of these results with those of Dronzek et al. (1970).

Shepherd (1968) used starch-gel electrophoresis of the urea-soluble proteins of aneuploid lines of Chinese Spring to determine the chromosomal location of the genes coding for nine of the seventeen major bands in the pattern of the parent. The remaining eight proteins were not affected by any of the chromosome deletions. The synthesis of these must be controlled by two or more chromosomes or by one of the nine chromosomes not tested.

Boyd et al. (1969) studied the starch-gel electrophoretic patterns of the 2M urea-soluble proteins from Canthatch (a hexaploid bread wheat), Tetracanthatch (AABB component of Canthatch) and two synthetic (AABBDD) hexaploids produced using different D-genome donors. In each case the hexaploid pattern was the sum of that of the Tetracanthatch and D-genome donor. Previously Boyd and Lee (1967) demonstrated a dramatic change in the electrophoretic pattern of these proteins on removal of the short arm of chromosome 1D. Two slow-moving bands were completely deleted.

The application of a two dimensional technique involving electrofocusing and starch-gel electrophoresis allowed greatly increased resolution of gliadins (Wrigley, 1970). This protein group could be resolved into

over forty components. Major intervarietal differences in the mobility of components was evident. Removal of the D genome from the three hexaploid bread wheats, Prelude, Rescue and Thatcher, caused the complete deletion of a number of gliadins. Further studies using this technique (Wrigley and Shepherd, 1973) resulted in the location of genes responsible for the synthesis of approximately 30 of these gliadins. All of the proteins identified so far are controlled by genes on the homoeologous chromosomes of group 1 and group 6.

Welsh and Hehn (1964) determined the breadmaking quality of the 21 monosomic lines of Kharkof MC 22, a hard red winter wheat. The absence of chromosome 1D resulted in a dramatic decrease in the doughball-fermentation time and an extreme weakening of the farinograph curve. Morris et al. (1966) and Schmidt et al. (1966) studied 21 lines derived from the common wheat variety Chinese Spring by successive substitution of single chromosomes from the bread wheat Cheyenne. Mixograph and baking tests indicated chromosomes 1B, 4B, 7B and 5D were primarily responsible for the high baking quality of Cheyenne.

Welsh et al. (1968) studied the baking properties of the complete set of substitution lines of 3 hard red spring varieties with Chinese Spring as the common background variety. Many of these lines showed marked improvement over Chinese Spring indicating that the genes for breadmaking quality are located on a number of different chromosomes.

Johnson (1972) employed gliadin protein electrophoretic patterns of Triticum and Aegilops varieties in a study of the evolution of breadwheats. All subspecies of the hexaploid T. aestivum (AABBDD) showed a very uniform

profile that could only be simulated by the pattern associated with a uniform mixture (2:1) from specific profile types of the ancient tetraploid cultivar T. dicoccum (AABB) and the wild diploid Aegilops squarrosa (DD).

The marked influence of the D genome on breadmaking quality has been demonstrated by a number of workers. Kerber and Tipples (1969) determined the baking quality of Canthatch, a hard red spring wheat, Tetracanthatch, its AABB component, the durum Stewart 63, and five synthetic hexaploids produced by crossing Tetracanthatch with five varieties of Aegilops squarrosa. Compared with Canthatch, Tetracanthatch had extremely poor baking characteristics. The baking performance of the synthetic hexaploids was intermediate between that of Tetracanthatch and Canthatch. Their studies also showed that the two synthetic hexaploids that received the D genome from the variety strangulata of Aegilops squarrosa had superior breadmaking quality compared to the other synthetic hexaploids. Morphologically these two resembled common bread wheat varieties. The authors speculated that strangulata could be either the original donor of the D genome to hexaploid bread wheat or, through later introgression, the source of its distinguishing characteristics.

RESULTS AND DISCUSSION

The results are presented as a series of five papers that have been submitted for publication in Cereal Chemistry. Their order of presentation is based on the logical development of the project through the following stages. Development of preparative and analytical techniques; relation of SDS-PAGE patterns of reduced glutenins to breadmaking quality; genomic and chromosomal location of genes coding the synthesis of specific glutenin subunits; and finally, investigations of glutenin ultrastructure using the scanning electron microscope.

Studies of Glutenin. I. Comparison of Preparative Methods[1]

Abstract

Three preparative methods for glutenin, viz. Osborne fractionation, gel-filtration chromatography, and pH precipitation with subsequent purification using SE-Sephadex C-50 were evaluated. Amino acid analyses and disc-gel electrophoresis of the products showed that the third method yields glutenin that is free from low-molecular weight contaminants. The dissociating solvent AUC (acetic acid, urea, CTAB) which dissolved 93% of the flour protein was used for the gel filtration and pH precipitation methods. Two other detergents, sodium dodecyl sulfate and Brij 35, in combination with acetic acid and urea, were less effective than CTAB and solubilized 60% and 89% of the flour protein respectively. Because of its relatively high solvent efficiency, and the fact that it is neutral, and so does not migrate on electrophoresis at pH 3.2, Brij 35 is preferable over CTAB as a protein dispersant for electrophoretic studies.

Introduction

The relative insolubility of wheat glutenins has made their isolation and study extremely difficult. An increased awareness of their importance in breadmaking (Orth and Bushuk, 1972), and the fact that they represent up to 50% of the total flour protein, made the development of methods for their isolation and purification imperative. Introduction of the AUC solvent

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[1]Accepted for publication in Cereal Chemistry under the co-authorship of R. A. Orth and W. Bushuk.

(water solution containing-acetic acid-urea and hexadecyltrimethylammonium bromide CTAB) (Meredith and Wren, 1966) was a major advance in this field as it can solubilize up to 98% of the total flour protein.

The aim of the present work was to determine the best method of obtaining pure glutenin (free of gliadin and other low-molecular weight impurities) for subsequent molecular and structural characterization. Polyacrylamide gel electrophoresis and amino acid analyses were used as test of purity.

Materials and Methods

Flour Sample. The flour used was milled from a pure variety of hard red spring wheat (Manitou) on a Buhler experimental mill after overnight tempering to 15.5% moisture. It contained 12.9% protein (N x 5.7 on a 14.0% moisture basis), 0.40% ash, had a starch damage of 28.2 Farrand Units and gave a loaf volume by the remix baking test of 860 c.c. for 100 g. of flour.

Amino Acid Analysis. Protein samples (10 mg.) were weighed into hydrolysis tubes and hydrolyzed with 4 ml. of 6N hydrochloric acid under vacuum at 110°C. for 24 hr. The hydrolysate was dried over sodium hydroxide pellets in a vacuum desiccator. The residue was dissolved in 8 ml. of citrate buffer solution of pH 2.2 and centrifuged. A Beckman Model 121 amino acid analyzer was used for the analyses. Since most of the protein fractions analyzed contained nitrogenous compounds such as urea, the amino acid data were reported as mole percent on an ammonia-free basis.

Polyacrylamide Gel Electrophoresis (PAGE). Electrophoresis was performed at pH 3.2 by the method of Davis (1964) as modified by Chen and Bushuk (1970b). The gels were stained with Coomassie Brilliant Blue according to the directions of Koenig et al. (1970).

Results and Discussion

Modified Osborne Fractionation. For many years the major solvent used in the preparation of wheat glutenin has been dilute acetic acid, originally proposed by Osborne (1907). This procedure suffers from two major disadvantages. The most serious is that a large fraction of the flour protein is insoluble in the acetic acid solution. Accordingly this insoluble or residue protein has received considerably less attention than the readily soluble fraction (Cluskey and Dimler, 1967; Inamine et al., 1967; Cole et al., 1972; Mecham et al., 1972). The second disadvantage of the Osborne method is that it yields a glutenin fraction that appears to be highly contaminated with gliadin, albumin, and globulin proteins. To illustrate these points, 10 g. of Manitou flour was fractionated by the modified Osborne procedure (Chen and Bushuk, 1970a). The acetic acid-soluble glutenin accounted for 10.9% of the total flour protein and 26.2% remained as insoluble residue after 3 hr. extraction with acetic acid. PAGE electrophoresis of the acetic acid-soluble glutenin gave a pattern with relatively high concentrations of bands of high mobility (Fig. 1). These appear to be albumin, globulin and gliadin impurities representing a significant proportion of total protein.

Amino acid composition of the acetic acid-soluble glutenin (Table 1, column a) shows that this preparation has lower lysine, arginine, aspartic

Fig. 1. PAGE pattern of glutenin prepared by the Osborne fractionation

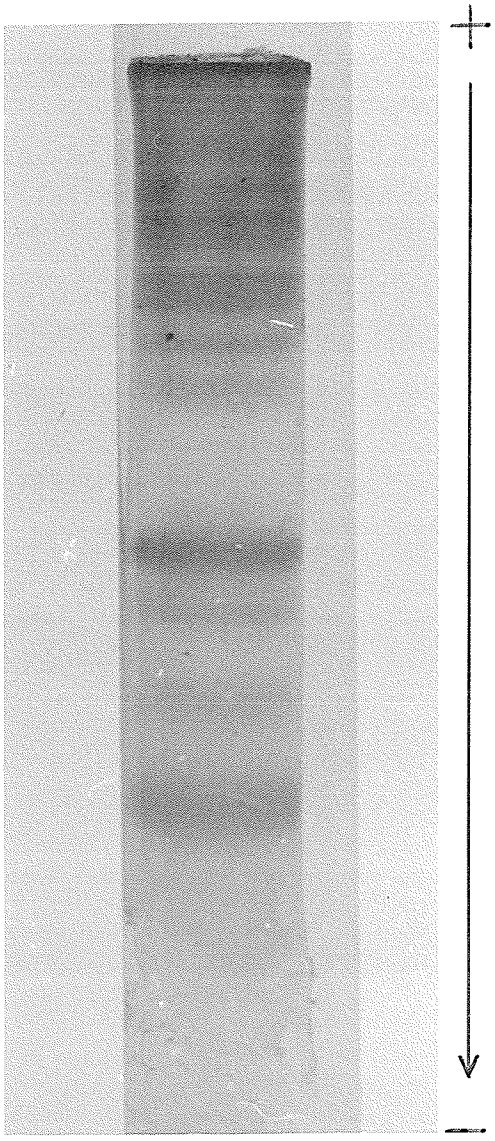


Table 1. Amino Acid Composition of Protein Fractions

	Mole percent on an ammonia free basis*						
	a	b	c	d	e	f	g
Lysine	1.41	1.52	1.10	1.04	2.82	0.82	2.19
Histidine	1.71	1.78	1.53	1.90	1.85	1.57	1.81
Arginine	2.43	2.43	2.87	2.14	3.54	2.08	3.01
Aspartic acid	2.85	2.66	2.36	3.32	6.41	2.55	4.42
Threonine	2.91	2.93	2.39	2.13	3.67	2.26	3.01
Serine	6.37	6.68	5.53	5.30	6.81	5.46	6.10
Glutamic acid	35.12	35.72	37.78	36.32	29.73	38.14	30.36
Proline	13.35	12.89	17.06	15.98	5.14	18.10	12.46
Glycine	7.71	7.33	4.80	3.09	6.01	3.02	9.27
Alanine	3.65	3.39	2.96	3.52	5.74	3.07	4.49
Valine	4.31	4.22	3.71	4.70	5.82	4.11	4.66
Methionine	1.23	1.37	1.16	1.31	1.56	1.24	1.43
Isoleucine	3.25	3.26	3.39	4.32	4.70	3.75	3.17
Leucine	6.87	7.02	6.30	7.45	8.56	6.87	7.02
Tyrosine	2.86	2.36	1.77	2.46	2.60	1.75	3.26
Phenylalanine	3.97	4.43	5.30	5.04	5.04	5.20	3.35

a. Osborne glutenin

b,c,d,e. Peaks I ⟶ IV G-100 Sephadex

f. pH 6.4 70% EtOH soluble protein

g. pH 6.4 70% EtOH insoluble after SE-Sephadex cleanup

* tryptophan, cysteine(and cystine) were not determined

acid, threonine, glycine, alanine, and tyrosine contents, and higher glutamic acid, proline, and phenylalanine contents than the purest glutenin preparation obtained by pH precipitation (compare columns a and g, Table I). These differences confirm the electrophoretic results that the acetic acid-soluble glutenin is highly contaminated with other flour proteins.

Extraction with Water-Acetic Acid-Urea-Detergent Solvents. Meredith and Wren (1966) introduced the AUC solvent (0.1M acetic acid, 3M urea and 0.01M CTAB) to studies of wheat protein. Their studies, and work in this laboratory (Bushuk and Wrigley, 1971), showed that AUC can dissolve as much as 98% of the flour protein of some bread wheats. Duplicate determinations of the extraction efficiency of protein of Manitou flour used in the present study were performed according to the method of Bushuk and Wrigley (1971). Because flour was used instead of ground grain, as used by Bushuk and Wrigley (1971), only 5-min. homogenization time was employed. An extractability of 93 $\pm$ 1% was obtained. This value was somewhat lower than the value for ground wheat with 1-hr. homogenization (Bushuk and Wrigley, 1971) but is essentially the same as that reported by Meredith and Wren (1966) using 2-min. homogenization for flour.

Identical extractions with solvents comprising 0.1M acetic acid, 3M urea and either 0.01M Brij 35 $\left[C_{12}H_{25}(OCH_2CH_2)_{23}OH\right]$ from Calbiochem (AUB) or 0.01M sodium dodecyl sulfate, removed 89% and 60% of the flour protein, respectively. The AUB extraction was 4 percentage units lower than the AUC extraction. However Brij 35 is preferred over CTAB for electrophoretic work because it is electrically neutral and therefore does not migrate during electrophoresis.

Figure 2 gives the PAGE patterns of the proteins extracted by AUB and AUC and also shows the effect of dialysis (at room temperature) on AUC extracts. The nondialyzed AUC extract gave a very strongly stained, broad band of relatively high mobility. The AUC-dialyzed and AUB nondialyzed extracts do not show this band. The broad high-mobility band was assumed to be CTAB. These results showed that dialysis for five days against distilled water is sufficient to remove all of the free CTAB from the protein solution. Use of the nonpolar Brij 35 as the detergent in the solvent is therefore recommended over CTAB or sodium dodecyl sulfate when electrophoresis is planned and lengthy dialysis is impractical.

Comparison of PAGE patterns for proteins extracted using AUC and AUB showed qualitatively similar patterns. Variation in the mobility of some fast-moving proteins solubilized by AUB and AUC can be explained by formation of a detergent-protein complex whose net charge depends upon the charge of the native protein and the detergent involved. Use of uncharged Brij 35 produced a decrease in the mobility of these proteins compared to those complexed with the cationic CTAB.

Gel Filtration. Gel filtration was performed on a 10.0 x 74.0 cm. column of Sephadex G-100 (particle size 40-120μ) using AUC as the solvent. Upward flow, as recommended by Bushuk and Wrigley (1971), allowed 2-months continuous use of the column at a flow rate of 130 ml./hr. The eluant was monitored at 280 nm. by an ISCO U.V. recorder and collected at the rate of 12 fractions per hour. Seventy milliliters of AUC-protein solution, clarified by centrifugation at 100,000 g. was applied and chromatographed. The elution profile obtained is shown in Fig. 3. The tubes containing the frac-

Fig. 2. PAGE patterns of Manitou flour protein dissolved in (left to right) AUB, AUC (dialyzed) and AUC (nondialyzed).

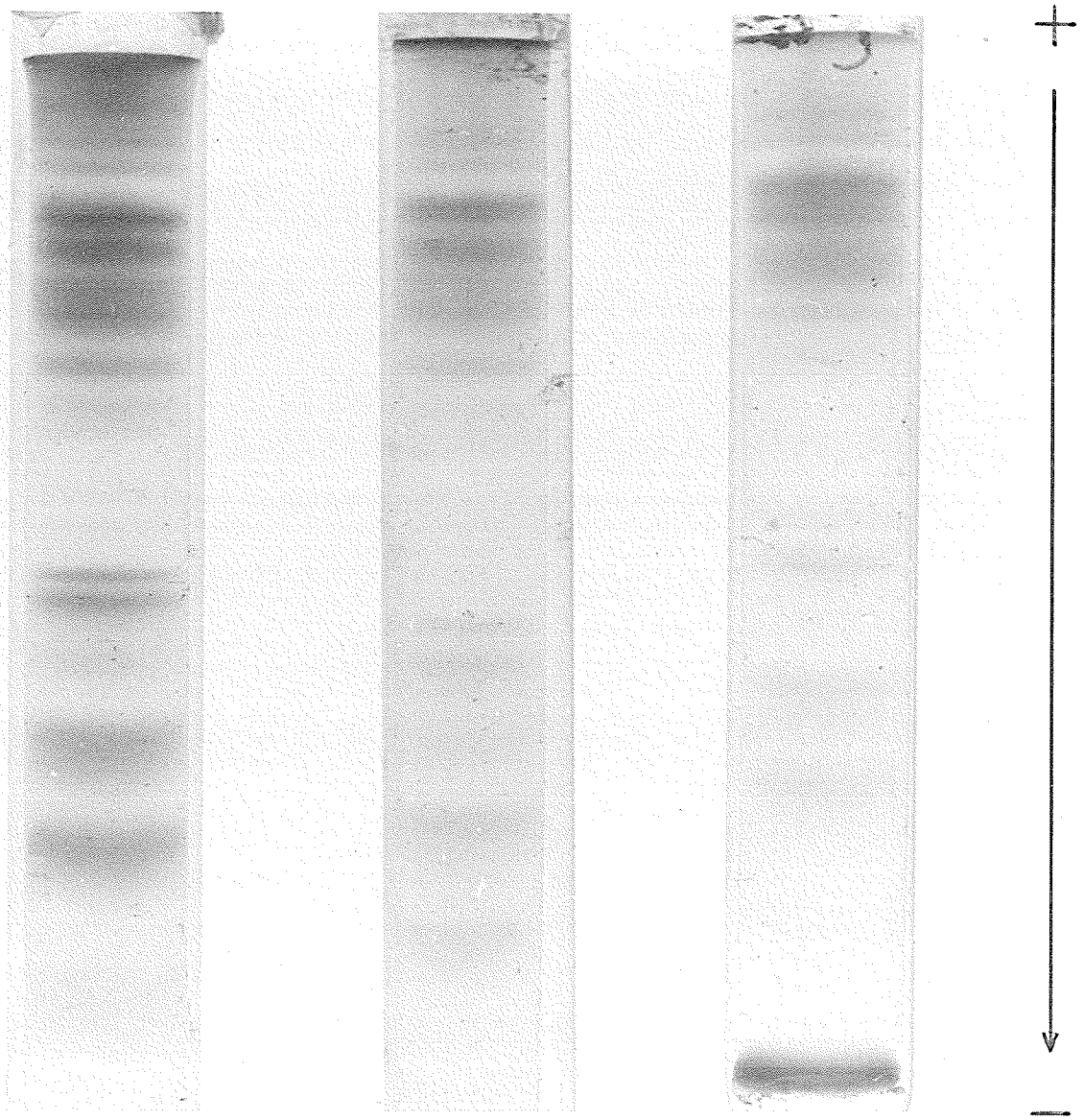
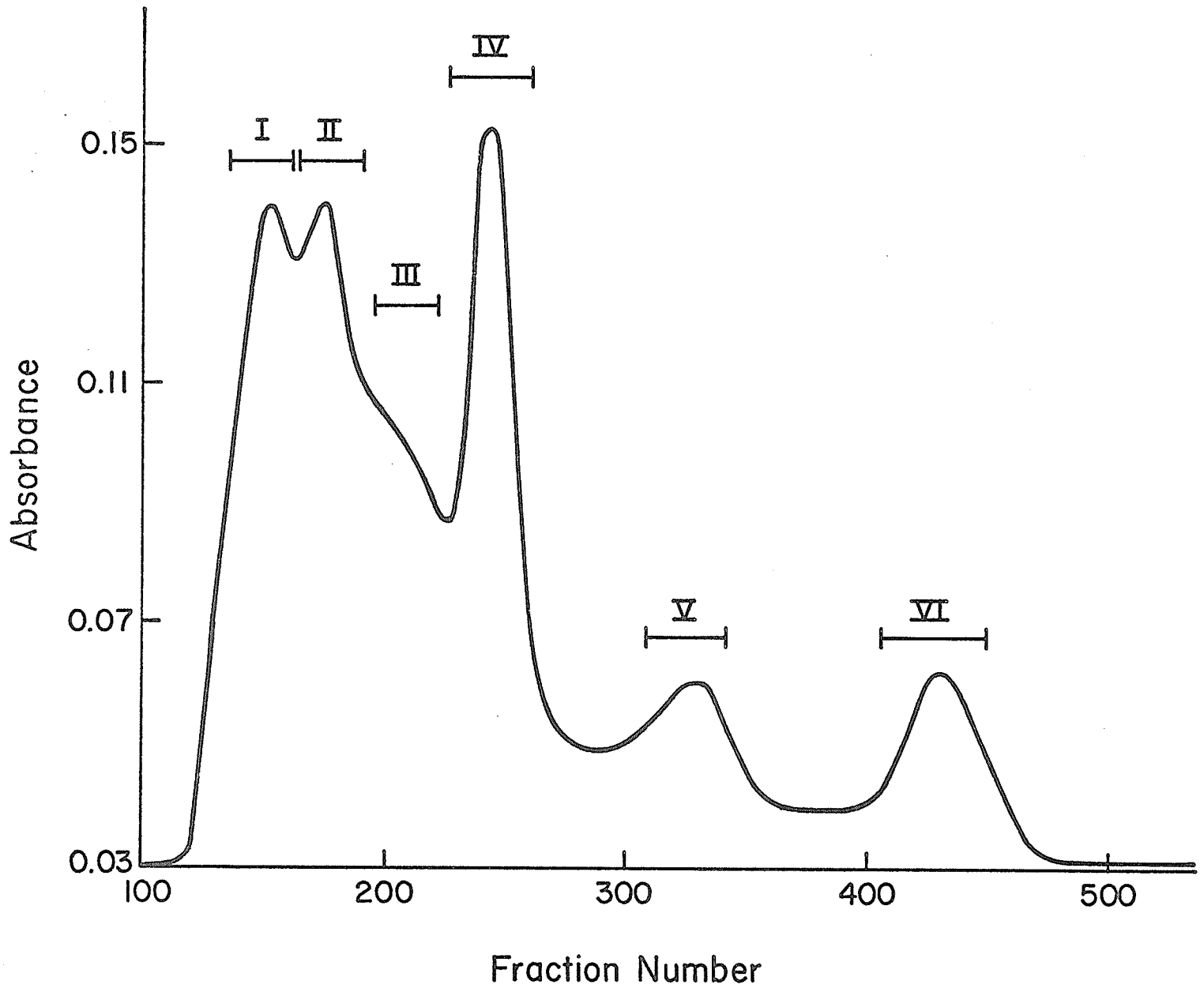


Fig. 3. Sephadex G-100 chromatography of Manitou flour protein in AUC.

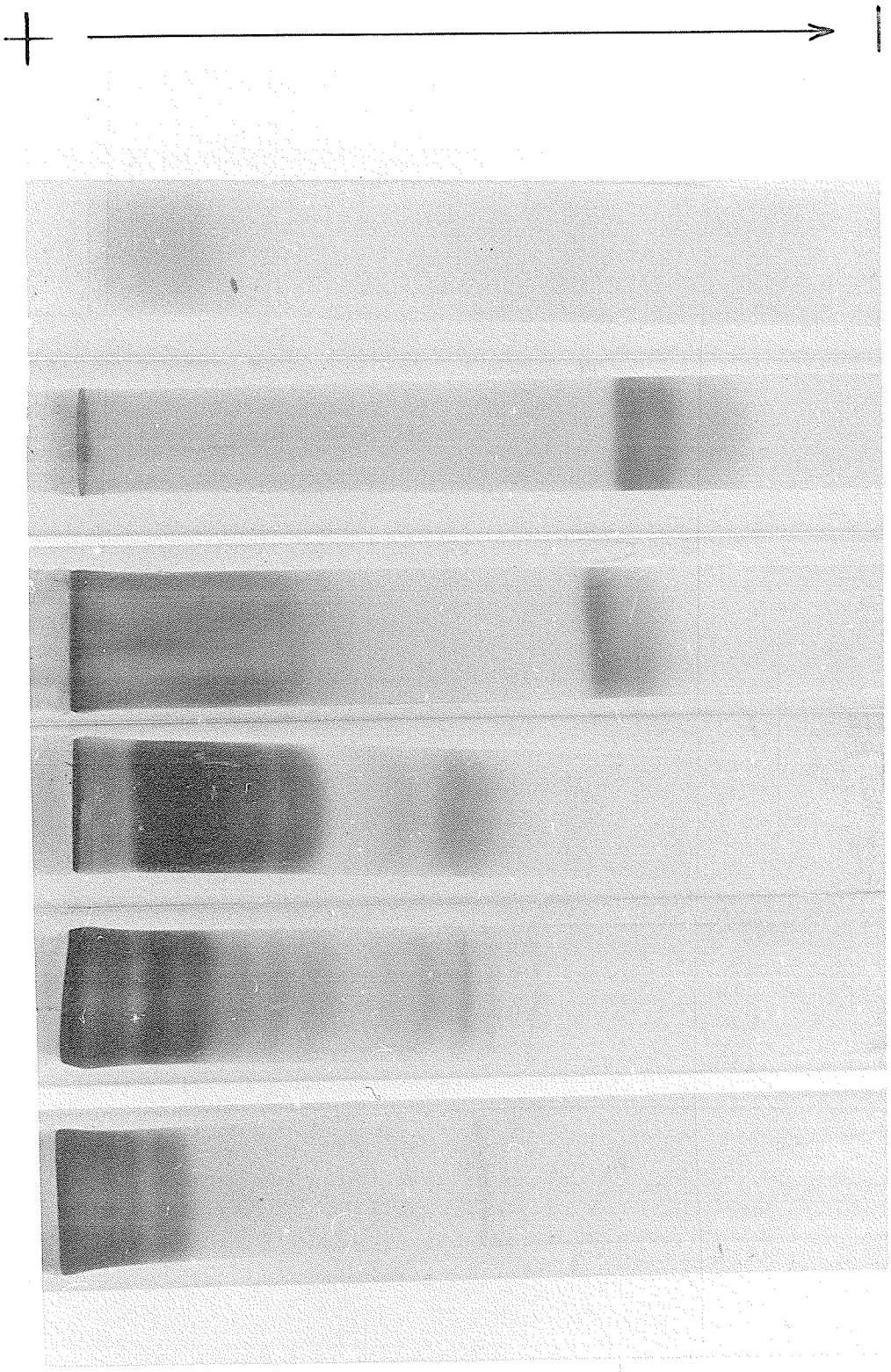

IV
I
II
III
0.15
0.11
0.07
0.03
Absorbance
V
VI
100
200
300
400
500
Fraction Number

tions indicated were pooled and dialyzed against distilled water for five days. The dialyzed solutions were freeze dried.

The electrophoretic patterns in Fig. 4 indicate considerable overlap of chromatographic peaks. Peaks I and II appear similar in their components, although peak II has more of the faster moving components (R_f 0.3 to 0.6) and more bands in the gliadin region (R_f 0.1 to 0.3). Peak III contains mostly gliadins with very little glutenin protein (mobilities between the origin and 0.1). Peak IV contains a significant amount of the gliadins of peak III and a large amount of a fast moving doublet with an R_f of about 0.7. This doublet is also evident in peak V which contains very little of the slowest-moving components. Peak VI contains a very small amount of protein of high and low mobilities and may represent amino acids and peptides and higher-molecular weight proteins still eluting at the end of the chromatography.

Amino acid analyses of peaks I to IV confirmed their nature as determined by electrophoresis (Table 1, columns b,c,d and e). The proteins of peak IV are particularly rich in the basic amino acids, arginine and lysine. This explains in part their high mobility at acid pH (Lawrence et al., 1970). These proteins are also very high in aspartic acid but have much less glutamic acid and proline than peaks I, II or III. Amino acid compositions of proteins represented by peaks I and III correspond to glutenin and gliadin respectively when compared with published amino acid compositions (Ewart, 1967). Peak II has an amino acid composition that does not fit into either gliadin or glutenin classification but appears to be a mixture of both. The major feature of the proteins of peak I is their very high glutamic acid,

Fig. 4. PAGE patterns of Manitou flour protein fractionated on Sephadex G-100 using AUC solvent. Left to right, peaks I to VI as in Fig. 3.



proline, and glycine content. Peak III is even higher in glutamic acid and is also characteristic of gliadins, having a low glycine and high proline content compared with data for peak I.

pH Precipitation and Ion Exchange Purification. Gliadins are known to be soluble in 70% aqueous ethanolic solutions whereas glutenins are insoluble in this solvent at pH 6.4 (Woychik et al., 1964). The applicability of this preparative method in conjunction with AUC solvent was tested by the following procedure: Ten grams of flour, in dough form, was washed under a gentle stream of distilled water until a gluten ball was obtained. This gluten was then dissolved in 170 ml. of AUC by overnight magnetic stirring. The solution was centrifuged at 20,000 g. for 1/2 hr. and the supernatant was made 70% (V/V) in ethanol and adjusted to pH 6.4 by the dropwise addition of 1N NaOH. The resulting precipitate was allowed to settle overnight at 2°C. and separated by centrifugation. This precipitate (crude glutenin) was then dispersed in 0.01M acetic acid, dialyzed against distilled water for 5 days, and freeze dried. An aliquot of the supernatant from the centrifugation after the pH precipitation was also dialyzed and freeze dried.

The freeze dried crude glutenin (0.2 g.) was dispersed by overnight magnetic stirring in 50 ml. of AUC containing 0.1 g. of SE-Sephadex C-50 and centrifuged. The supernatant was dialyzed against water and freeze dried to yield purified glutenin. Ten grams of flour yielded 0.154 g. of purified glutenin, representing 10% of the total protein. This unusually low yield resulted because of losses in the purification.

Samples of the protein that dissolved in ethanolic AUC of pH 6.4, and the purified glutenin, were subjected to amino acid analysis and PAG electro-

phoresis. An electrophoretic pattern was also obtained for the initial crude precipitate (see Fig. 5).

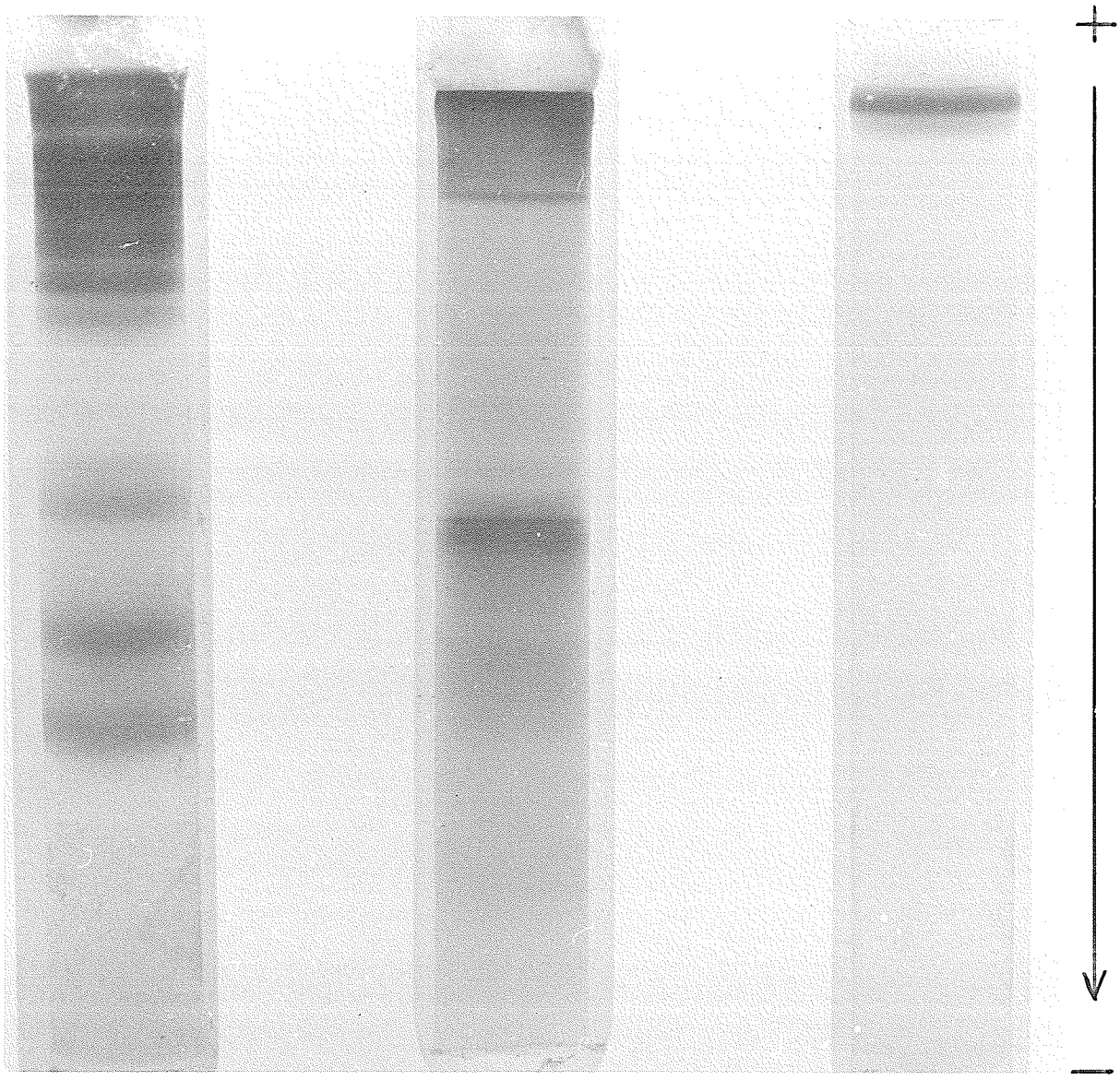
The effectiveness of the SE-Sephadex C-50 in removing impurities of low molecular weight from the crude precipitate is demonstrated strikingly in Fig. 5. No fast-moving bands were evident for this protein, whereas the initial precipitate (centre gel), contained a high proportion of gliadin, globulin, and albumin bands. Likewise, the ethanol-soluble protein contained a large number of fast-moving components. Since at pH 3.5 (in AUC) most of the flour proteins would be positively charged, the selective binding of the low molecular weight components to SE-Sephadex probably involves molecular sieving action as well as charge. Glutenins, because of their large molecular size, would not penetrate the beads and so would not be adsorbed very strongly. These preliminary results indicate that SE-Sephadex may be extremely useful as a support material for chromatographic separation of flour proteins.

When compared with peak I protein from Sephadex G-100 fractionation (Table I, column b), the SE-Sephadex-purified glutenin is higher in lysine, arginine, aspartic acid, threonine, glycine, alanine, and tyrosine and lower in glutamic acid, proline and phenylalanine, indicating that it is a purer form of glutenin. Peak I protein was higher in the amino acids that characterize gliadin and lower in those that characterize glutenin when compared with the purified glutenin.

Summary

A modified Osborne fractionation, gel filtration chromatography on Sephadex G-100 and pH precipitation, followed by further purification using

Fig. 5. PAGE patterns of (left to right) pH 6.4-70% ethanolic AUC soluble protein, insoluble protein, purified by SE-Sephadex.



an ion exchanger were compared as preparative methods for glutenin. The third method is recommended as it is simple, effective, and provides pure glutenin in good yield.

Studies of Glutenin. II. Relation of Variety, Location of Growth and Baking Quality to Molecular Weight Distribution of Subunits[1]

Abstract

Subunits obtained by reduction of glutenin from 26 hexaploid wheats of diverse baking quality were examined by SDS-polyacrylamide gel electrophoresis. The number of observable bands, which varied from ten to fourteen, was not characteristic of baking quality. Intervarietal differences in the number and molecular weight of the subunits cannot be used to characterize the baking potential amongst these hexaploid wheats. Genetically related varieties contained common glutenin subunits, the closeness of their electrophoretic patterns being dependent on their genetic similarity. Location of growth had no effect on the subunit composition of four varieties each grown at four locations.

Introduction

Solubility fractionation of wheat flour proteins from many varieties has led to the conclusion that the amounts of acetic acid-soluble and the insoluble residue protein (glutenin) are correlated with breadmaking quality (Orth and Bushuk, 1972; Orth et al., 1972). Because of their large particle sizes and general insolubility in aqueous systems, it has not been possible to determine their structure in the hope of relating it to the functional properties of dough.

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[1]Accepted for publication in Cereal Chemistry under the co-authorship of R. A. Orth and W. Bushuk.

A marked increase in the solubility of glutenin can be obtained by reductive cleavage of its disulfide bonds (Woychik et al., 1964). Starch-gel electrophoresis (Huebner, 1970) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Shapiro et al., 1967; Bietz and Wall, 1972) of subunits obtained by reduction of glutenin from wheats of different varieties and classes indicated marked differences.

The present study, which was in progress at the time the work of Bietz and Wall (1972) was reported, examines the SDS-PAGE patterns of the reduced glutenin of 26 bread wheats grown at four locations in Western Canada. The aim of this work was to determine if the subunits of glutenin were related to baking quality and whether they were affected by environment or simply genetically controlled.

Materials and Methods

Materials. The varieties used, their parentage, loaf-volume per unit protein and flour protein are the same as used in a previous study (Orth and Bushuk, 1972). These varieties are ideal for this study because of their diverse baking qualities and the genetic similarity of a number of the samples.

Molecular weight markers for the SDS-PAGE, and their source, are given in Table I.

Preparation of Glutenins. A modified Osborne fractionation scheme, as described by Chen and Bushuk (1970a), was used to prepare the glutenin proteins. Traces of gliadin and low-molecular-weight proteins were removed from these preparations by dissolving 50 mg. of crude glutenin in 12.5 ml. of solvent

Table 1. SDS-PAGE Molecular Weight Markers

Protein	Molecular weight (daltons)	Source
γ-Globulins	160,000	Sigma
Bovine serum albumin - dimer	132,000	Calbiochem
Bovine serum albumin - monomer	66,000	Calbiochem
Ovalbumin	45,000	Sigma
Pepsin	35,000	Nutri. Biochem.
α -Chymotrypsin	21,600	Calbiochem
Myoglobin	17,000	Calbiochem
Trypsin inhibitor	14,300	Calbiochem
Cytochrome C	12,400	Calbiochem

comprising 0.1M acetic acid, 3M urea and 0.01M hexadecyltrimethylammonium bromide (pH 3.5), stirring overnight with 25 mg. SE-Sephadex and centrifuging (Mita and Yonezawa, 1971; Orth and Bushuk, 1973a). The supernatant containing purified glutenin was dialyzed for three days against distilled water and freeze-dried.

Preparation of SDS-Subunits. The solutions used for the reduction of glutenin and SDS-PAGE are listed in Table 2. Glutenin (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. After reduction and complexing with SDS, the protein solutions were made 10% w/v in sucrose, and 20 µl. of a 0.3% aqueous solution of bromophenol blue was added to each sample. Molecular weight markers were complexed with SDS at a concentration of 1 mg per ml. Since reduction of the standard proteins was not required, 0.002M N-ethylmaleimide replaced the β-mercaptoethanol for all marker proteins.

Preparation of Gels. The 5% gel was prepared by dissolving 11.0 g. of acrylamide and 0.29 g. of bisacrylamide cross-linking agent in 210 ml. of electrode buffer of pH 7.3 and 0.33 ml. of N', N', N,N-tetramethylethylenediamine. This solution was deaerated, and 100 mg. of ammonium persulfate in 10 ml. of buffer was added. The gel slab was polymerized in an E-C vertical gel electrophoresis unit using 6-mm. spacers and an eight-slot mold.

Electrophoresis. After addition of the buffer and removal of the slot former, the gel was conditioned for 30 min. using the voltage required for the electrophoretic experiment. Fifty microliters of the SDS-glutenin solution, or 10 µl. of the standard protein solution, was added to each slot and allowed to settle for 10 min. with the buffer pump turned off. The protein-

Table 2. Solutions for SDS-PAGE

Stock Buffer: 1 liter containing:
7.8 g. NaH_2PO_4 H_2O
20.4 g. Na_2HPO_4
10.0 g. SDS
Electrode Buffer:
Dilute the stock buffer to one-tenth original strength. Final pH 7.3
Protein Solvent:
The electrode buffer containing 1% (w/v) of SDS and 1% (v/v) of β-mercaptoethanol

SDS complexes were then run into the gel at 130 v. (120 ma.) and, after 10 min., the buffer pump was turned on. The run was continued at 130 v. until the bromophenol blue marker had traveled 7 cm. (approximately 3 hr.). On completion of electrophoresis, the gel was stained with Coomassie Brilliant Blue according to the method of Koenig et al. (1970). After de-staining, the gels were photographed and the mobilities of the protein bands were measured relative to the bromophenol blue marker. A plot of log of molecular weight versus mobility for the proteins of known molecular weight gave a calibration curve that was used to determine the molecular weights of the glutenin subunits. This plot showed a slight downward curvature as found by Bietz and Wall (1972) for the same (5%) gel concentration. SDS-PAGE yields molecular weights with an accuracy of better than $\pm$ 10% for polypeptides with molecular weights between 15,000 and 100,000 (Weber and Osborn, 1969).

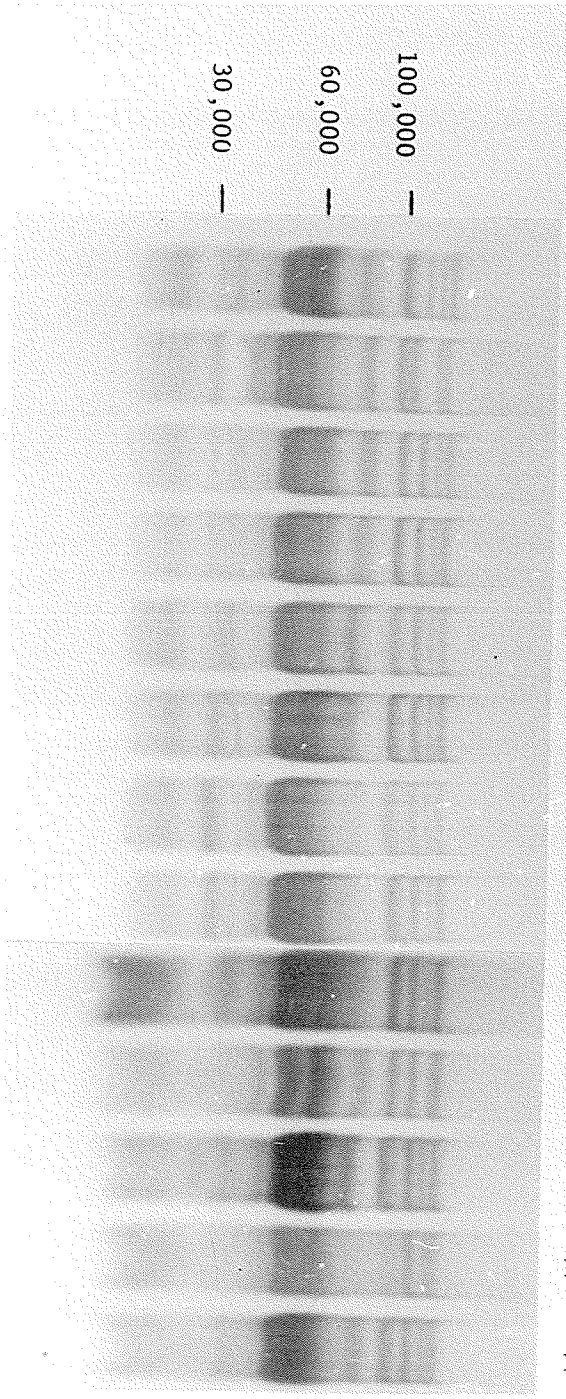
Results and Discussion

Varietal Variation in the Subunit Molecular Weight Distribution of Glutenin.

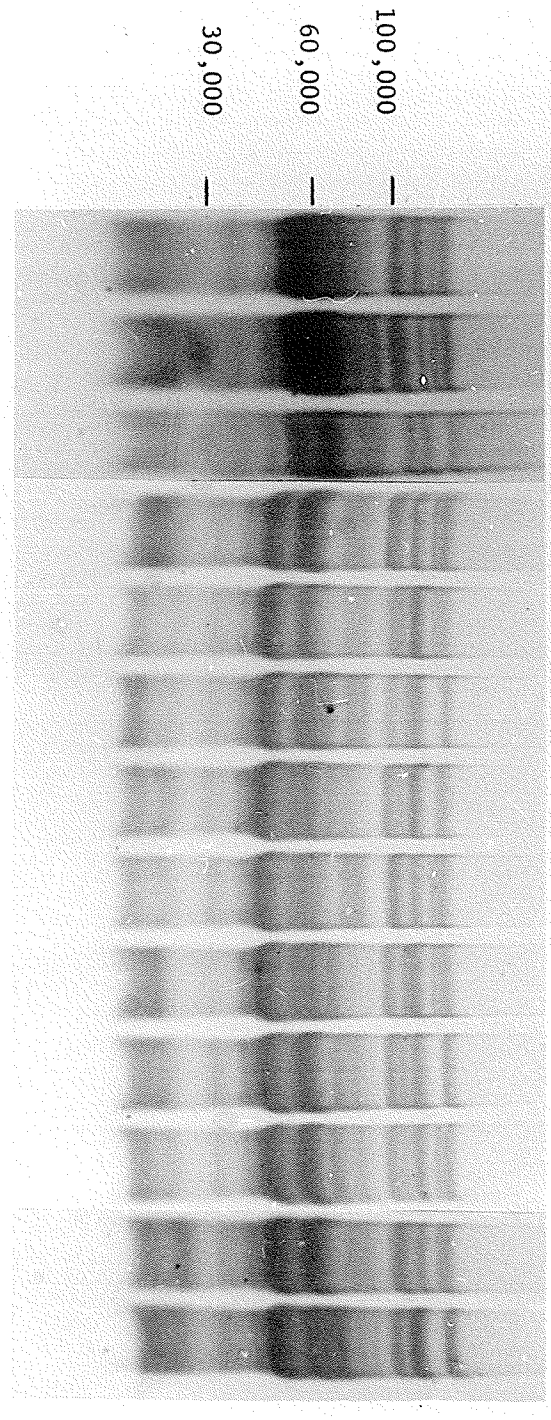
Since the major objective of our study was to determine if any relationship existed between breadmaking quality and the glutenin subunits, the first electrophoretic runs were on reduced glutenins from the 26 flours arranged in order of decreasing loaf-volume per unit protein (see Table 2, Orth and Bushuk, 1972). The electropherograms are shown in Fig. 1.

Two facts are apparent from the results of Fig. 1. Firstly, there is no obvious characteristic SDS-PAGE pattern of glutenin subunits for a particular level of baking quality. Secondly, although there are obvious differ-

Fig. 1. SDS-PAGE patterns of the glutenin subunits of 26 bread wheats. The varieties are arranged in order of decreasing baking quality from 1 to 26.



1. RUSHMORE
2. SONORA 64
3. THATCHER
4. MANITOU
5. MARQUIS
6. PEMBINA BACKCROSS
7. 6704
8. 6702
9. FORTUNA
10. OPAL
11. KOTA
12. PITIC 62
13. JUSTIN RESELECTION



14. COMANCHE X CT736
15. SVENNO
16. GABOTO
17. NAPO 63
18. ANIVERSARIO
19. MAGNIF ENTRERRIANO
20. MAGNIF 41
21. THATCHER BACKCROSS
22. LERMA ROJO 64A
23. CARAZINHO
24. GABO CROSS
25. E931 CROSS
26. R37

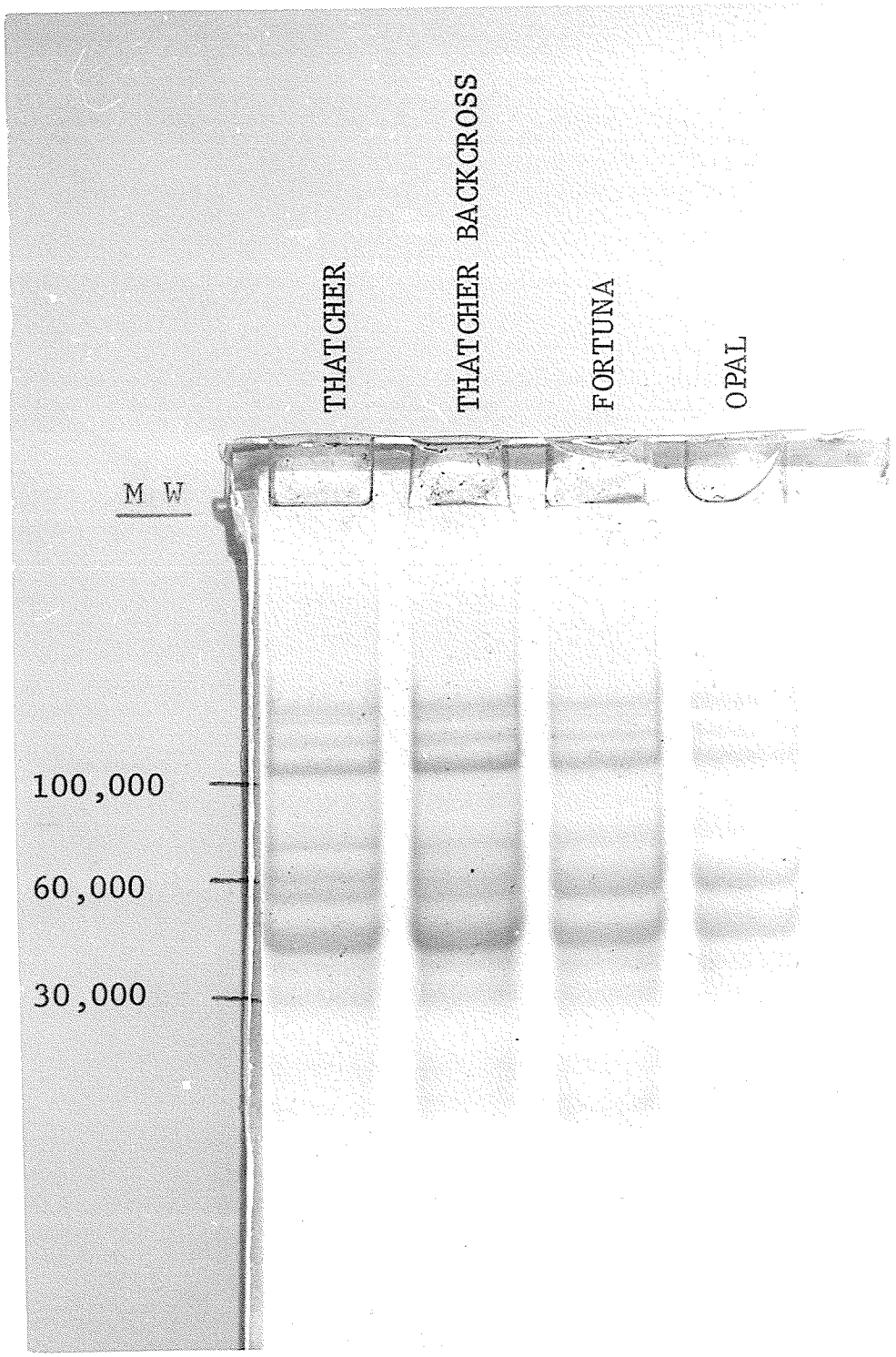
ences between varieties, all showed the characteristic pattern of hexaploid bread wheats as found by Bietz and Wall (1972). These patterns can be divided into three fairly distinct regions: - high-molecular weight (greater than 100,000 daltons), intermediate (60,000), and low-molecular weight subunits (less than 30,000). All glutenins examined in the present study contained a number of subunits in each of these regions.

Most variation was noted in the number and molecular weight of the slow-moving or high-molecular weight subunits. The number of bands varied from 3 to 5 in this region. Variation was also evident in the fast-moving subunits, but this was not as pronounced as in the high-molecular weight subunits.

Since the glutenins from these wheats do not have a subunit molecular weight distribution characteristic of their breadmaking quality, it was concluded that additional factors are involved in the determination of the overall quality. The absence of high-molecular weight glutenin subunits in durum wheats (Huebner, 1970; Bietz and Wall, 1972) indicates that their presence in bread wheats may be a necessary condition for quality. However, the results obtained in the present study indicate it is not a sufficient condition.

To illustrate this conclusion, the reduced glutenins of two closely related varieties of very different baking quality (Thatcher and Thatcher Backcross) and two genetically unrelated varieties of similar quality (Opal and Fortuna) were compared by SDS-PAGE on the same gel slab (Fig. 2). Although Thatcher and its backcross were distinctly different in baking quality, their patterns were qualitatively identical. On the other hand, Fortuna and

Fig. 2. SDS-PAGE patterns of the glutenin subunits of Thatcher, Thatcher backcross, Fortuna and Opal.


THATCHER
THATCHER BACKCROSS
FORTUNA
OPAL
M W
100,000
60,000
30,000

Opal, of essentially equal quality, had very different patterns.

The 26 varieties used in this study included many that are related to the variety Marquis. These varieties, with their number referring to their order in Fig. 1 given in parentheses, are: Rushmore (1), Thatcher (3), Manitou (4), Marquis (5), Pembina backcross (6), 6702 (7), 6704 (8), Fortuna (9), Justin reselection (13), and Thatcher backcross (21). The glutenin subunits of all these varieties were qualitatively identical by SDS-PAGE indicating that the subunits of glutenin are genetically controlled.

Effect of Location of Growth on the Subunit Molecular Weight Distribution of Glutenin. The reduced glutenins of four varieties of widely different baking quality (Rushmore, Manitou, Pitic 62 and R37), each grown at four stations, were examined by SDS-PAGE. No qualitative or obvious quantitative inter-station differences were found for any of the varieties. It was therefore concluded that the molecular weight distribution of glutenin subunits of healthy bread wheats are not affected by location of growth.

Summary

The number of distinct subunits and the molecular weight distribution of subunits of reduced glutenin are genetically controlled and are not affected by location of growth. There is no apparent relationship between the SDS-PAGE patterns of the reduced glutenins and breadmaking quality.

Studies of Glutenin. III. Identification of Subunits Coded by the D Genome and Their Relation to Breadmaking Quality[1]

Abstract

Glutenins from four extracted AABB tetraploid wheats, their hexaploid (AABBDD) common wheat counterparts, a synthetic (AABBDD) hexaploid, its parents, and seven accessions of Aegilops squarrosa were isolated, reduced, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The glutenin subunits of the synthetic hexaploid were simply inherited from its tetraploid (AABB) and diploid (DD) parents. Each extracted tetraploid lacked three glutenin subunits and showed a decrease in the amount of one electrophoretic band present in its hexaploid parent. Three of the affected subunits were of the same molecular weight for each hexaploid-tetraploid pair, the fourth being different for one of the four pairs. In each pair the four deleted (or diluted) subunits were of the same molecular weight as subunits present in the glutenin of Ae. squarrosa samples studied. The electrophoretic patterns of the reduced glutenin of the varieties typica, anathera, and strangulata were almost identical. The two highest molecular weight glutenin subunits of a fourth variety, meyeri, were smaller than the analagous subunits in the other three varieties.

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[1] Submitted for publication in Cereal Chemistry under the co-authorship of R. A. Orth and W. Bushuk.

Common (bread) wheats contained glutenin subunits of high-molecular weight which were absent in durum wheats; their presence appears to be a necessary condition for breadmaking quality.

Introduction

Previous work (Kerber and Tipples, 1969; Dronzek et al., 1970) demonstrated that the D-genome, and particularly chromosome ID (Welsh and Hehn, 1964), was extremely important in the determination of the breadmaking properties of wheat flour. The baking quality of synthetic and natural AABBDD hexaploid wheats was generally superior to that of extracted and natural AABB tetraploids (Kerber and Tipples, 1969; Dronzek et al., 1970). Subsequently (Morris et al., 1966; Schmidt et al., 1966) chromosomes 1B, 4B and 5D were found to be critical to the baking potential of the bread wheat variety Cheyenne.

Since quality is related to the protein solubility distribution (Dronzek et al., 1970; Orth and Bushuk, 1972; Koenig et al., 1964; Pomeranz, 1965; Mullen and Smith, 1968; Chen and Bushuk, 1970) and in particular to the relative proportions of acetic acid-soluble and insoluble protein fractions (both are glutenins), analysis of glutenin is an essential step in the understanding of the molecular basis of breadmaking quality.

Until recently electrophoresis of glutenins was very difficult because of their high molecular weights and insolubility in aqueous systems. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as first applied to flour protein by Bietz and Wall (1972), allows electrophoresis of glutenin subunits obtained by reduction of disulfide bonds. Apart from

providing excellent resolution of the glutenin subunits this technique also allows determination of the molecular weight (MW) of each subunit with an accuracy of ± 10% (Weber and Osborn, 1969). Using this technique Bietz and Wall (1972) found that the reduced glutenins of a number of classes of hexaploid bread wheat had very similar patterns. The one durum wheat used in this study was deficient in two high MW subunits and a subunit of MW 32,600. The absence of these high MW subunits in the durum wheat suggests their possible relationship to breadmaking quality.

Using 26 hexaploid bread wheats of diverse baking quality, Orth and Bushuk (1973b) showed that there was no obvious relationship between quality differences and the SDS-PAGE patterns of their reduced glutenins. The glutenins from all of these wheats contained high MW subunits, regardless of quality. They suggested the presence of these subunits may be a necessary, but insufficient, condition for the breadmaking quality of common wheats.

The present study examines the inheritance of glutenin subunits and their relation to baking quality using varieties in which the D-genome has been added or removed cytogenetically. Glutenins from four hexaploid - extracted (AABB) tetraploid pairs, a synthetic hexaploid (AABBDD), its tetraploid (AABB) and diploid (DD) parents and six other accessions of Ae. squarrosa were analyzed by SDS-PAGE.

Materials and Methods

Wheat Samples. Canthatch, a Canadian hard red spring wheat of excellent milling and breadmaking properties, Tetracanthatch, the AABB component of Canthatch, and the synthetic hexaploid produced by crossing

Tetracanthatch with Aegilops squarrosa var. strangulata (RL5271), were used for the first part of this study. The hexaploids and the extracted tetraploid were grown at one location in Winnipeg in 1967. The grain of the D-genome donor Ae. squarrosa var. strangulata was obtained from plants grown under similar conditions. The same samples were used by Kerber and Tipples (1969) in their study on the effect of the D-genome on baking quality. An additional six samples of four varieties of Ae. squarrosa were obtained from the University of Manitoba seed stocks. These comprised two accessions of variety strangulata, one of typica, two of anathera, and one of meyeri.

The other extracted tetraploids that were used were derived by Kaltsikes from the hard red spring varieties Prelude, Rescue and Thatcher. The three pairs were grown together on an experimental plot at the University of Manitoba. A detailed description of the genetic derivation of the tetraploids, together with pertinent quality data were reported by Kaltsikes et al. (1968).

All chemicals used were reagent grade.

Preparation of Glutenins. Glutenins were prepared by the pH precipitation method described previously (Orth and Bushuk, 1973a). Glutenins of Canthatch, Tetracanthatch, the synthetic hexaploid and the samples of Ae. squarrosa were extracted directly from ground grain since the quantities available were insufficient to mill into flour. The starting material for the preparation of glutenins of the hexaploids and extracted tetraploids of Prelude, Rescue and Thatcher was flour milled on the Buhler experimental mill.

SDS-PAGE. SDS-PAGE was performed by the method of Koenig et al. (1970) with slight modifications. The modified method was described previously (Orth and Bushuk, 1973b). Electrophoresis was carried out at 130 V. in a 5% acrylamide gel using a phosphate buffer of pH 7.3. It was terminated when the bromophenol blue marker had migrated 7.0 cm.

The proteins used to calibrate the gels, with their MW and source in parenthesis, were as follows: γ-globulin (160,000; Sigma), bovine serum albumin (132,000 and 66,000; Calbiochem.), ovalbumin (45,000; Sigma), chymotrypsinogen A. (23,000; Schwarz/Mann), myoglobin (17,000; Calbiochem) and ribonuclease (13,000; Schwarz/Mann). A plot of log MW versus mobility produced a line with the characteristic, but as yet unexplained, slight downward curvature found for SDS-PAGE of other proteins in 5% acrylamide gels (Bietz and Wall, 1972).

The term baking quality as used in this article refers to breadmaking quality.

Results and Discussion

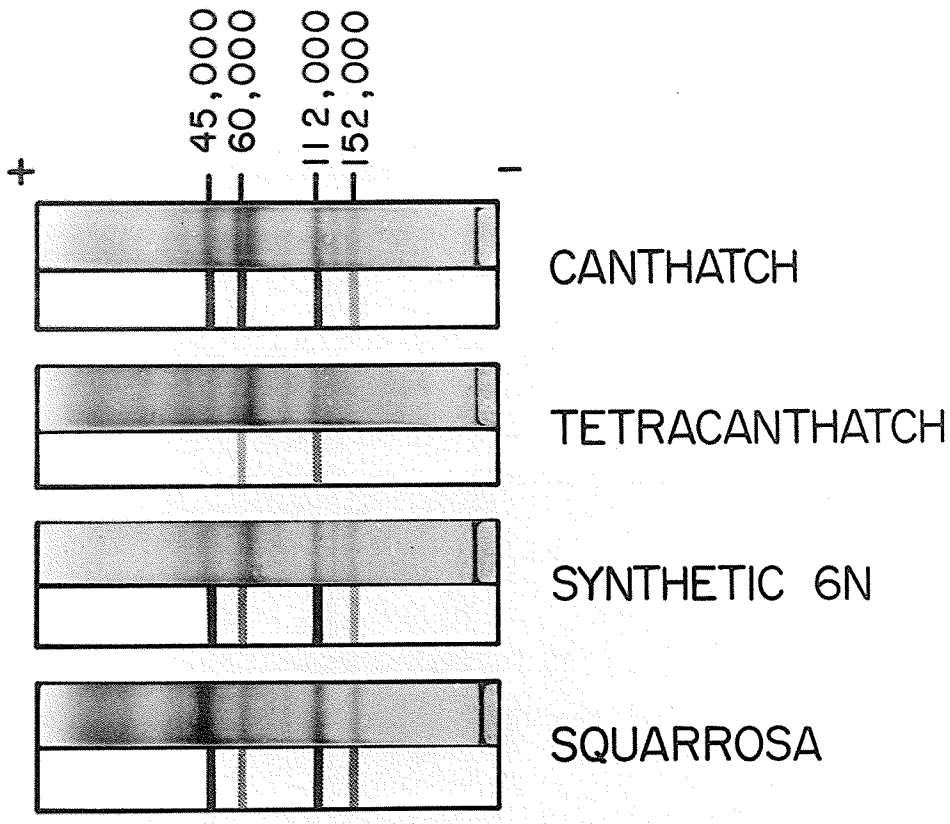
Synthetic Hexaploid and Its Parents. Figure 1 gives the SDS-PAGE patterns of the reduced glutenin of the following genetically related grain species:

Canthatch - a natural AABBDD hexaploid wheat

Tetracanthatch - tetraploid AABB component of Canthatch

Synthetic 6N - synthetic AABBDD hexaploid derived from Tetracanthatch and *Ae. squarrosa* var. *strangulata* (DD)

Fig. 1. SDS-PAGE patterns of reduced glutenins of Canthatch, Tetracanthatch, a synthetic hexaploid and Ae. squarrosa var. strangulata.


45,000
60,000
112,000
152,000
+
−
CANTHATCH
TETRACANTHATCH
SYNTHETIC 6N
SQUARROSA

Squarrosa - Ae. squarrosa var. strangulata (diploid DD)

Because photographic reproduction of these patterns results in a loss of detail, and in order to highlight the bands important to this discussion, both the photographs of the gels and schematic representations of the bands under discussion are shown in this figure and in Figure 2.

Reduced glutenin from Canthatch gave an SDS-PAGE pattern typical of bread wheats (Bietz and Wall, 1972; Orth and Bushuk, 1973b). Eleven bands were evident with molecular weights ranging from 13,000 to 152,000. It was reported by Weber and Osborn (1969) that SDS-PAGE yields molecular weights with an accuracy of better than $\pm$ 10% for polypeptides with molecular weights between 15,000 and 150,000. Although the molecular weights quoted in this article may be subject to this error they can still be used to identify the bands, since the major error involved in this technique is not its reproducibility for a particular polypeptide, but the inherent error in the assumption that the polypeptide-SDS complexes will migrate solely according to MW (Weber and Osborn, 1969).

Tetracanthatch lacked the highest MW subunit when compared to Canthatch. This subunit, MW 152,000, has been found in all hexaploid bread wheats studied (Bietz and Wall, 1972; Orth and Bushuk, 1973b) and is absent in all the durum wheat glutenins studied thus far. The second and third components affected by removal of the D-genome are those of MW's 112,000 and 60,000. Both appear to be diluted rather than absolutely deleted. The fourth change is a deletion of the 45,000 band.

The dilutions that were observed could result from a decrease in the amount of the same subunit, coded by the same gene in different genomes (redundancy), or from a deletion of a subunit of different amino acid composition and sequence but of the same molecular weight. If the latter is true, then the bands representing the 112,000 and 60,000 subunits which showed a dilution upon removal of the D-genome would comprise two or more different polypeptides.

The synthetic hexaploid contained the same number of bands at mobilities corresponding to the same molecular weights as those of Canthatch. A band of moderate intensity is present at 60,000 for Canthatch whereas the corresponding band in the synthetic hexaploid is weak. Otherwise corresponding bands are of comparable intensity.

Reduced glutenin from the squarrosa contained seven subunits at MW's of 152,000; 112,000; 80,000; 60,000; 45,000; 39,000 and 14,000. Five of these subunits appear to be the same as those affected by removal and addition of the D-genome.

The glutenin from the synthetic hexaploid contained the subunits present in its parents. No new bands were evident and all the major bands of both parents were present in the progeny. Accordingly, it is concluded that the glutenin subunits of the synthetic hexaploid are simply inherited from its parents.

Boyd et al. (1969) used starch-gel electrophoresis to compare the grain proteins soluble in 2M urea for the same four species used in this part of the present study. There was no difference in the patterns of Canthatch and the

synthetic hexaploid; the patterns of the tetraploid and diploid parents were themselves different and also differed from the two hexaploids.

Extracted AABB Tetraploids and Their Hexaploid Parents. The effect of removal of the D-genome was studied further by analyzing the reduced glutenins of three hexaploid varieties and their extracted AABB tetraploids. Previous workers (Dronzek et al., 1970) found differences in the protein solubility distribution between these two groups but could find no qualitative differences by conventional polyacrylamide gel electrophoresis of their water-, salt-, ethanol- and dilute acetic acid-soluble proteins.

Figure 2 shows the SDS-PAGE patterns of the reduced glutenins of the three hexaploid - tetraploid pairs. For each variety, extraction of the D-genome removed three of the high MW bands present in each hexaploid and decreased the intensity of a fourth. The three deleted subunits are characterized by the following molecular weights: 152,000; 112,000 and 45,000. The dilution occurred in the 80,000 subunit. These MW's are the same as those for four of the seven bands present in the reduced glutenin of the Ae. squarrosa var. strangulata (see Fig. 1).

The results presented here differ slightly from those obtained for the Canthatch - Tetracanthatch pair (see above). The 112,000 subunit was deleted in the former but diluted in the latter. Also the conversion of Canthatch to Tetracanthatch produced a dilution in the 60,000 subunit whereas in the other three pairs the dilution was in the 80,000 subunit.

The SDS-PAGE patterns of the reduced glutenins of seven accessions of Ae. squarrosa are shown in Figure 3. Four varieties are represented;

Fig. 2. SDS-PAGE patterns of reduced glutenin of Prelude, Tetraprelude, Rescue, Tetrarescue, Thatcher and Tetrathatcher.

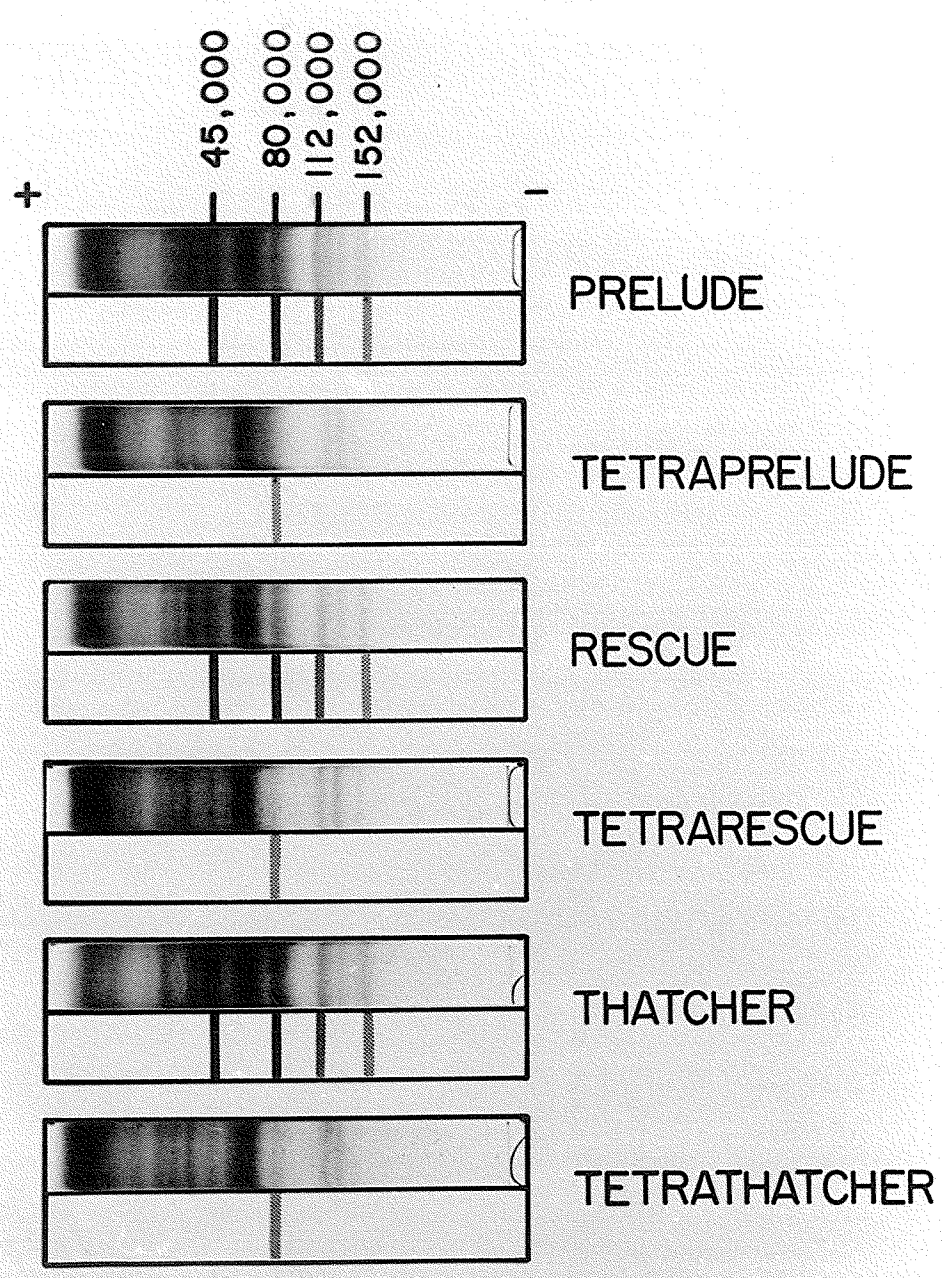
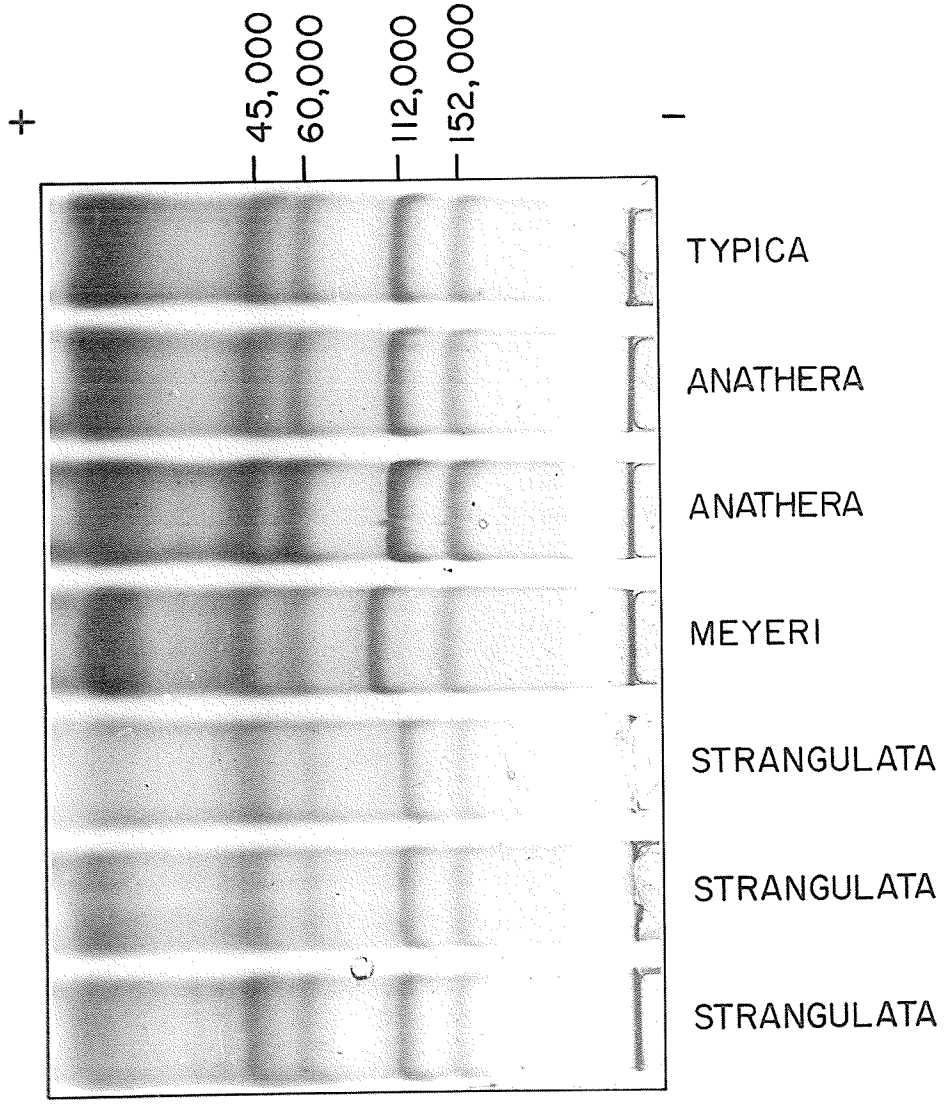

45,000
80,000
112,000
152,000
+
−
PRELUDE
TETRAPRELUDE
RESCUE
TETRARESCUE
THATCHER
TETRATHATCHER

Fig. 3. SDS-PAGE patterns of reduced glutenin of seven accessions of Ae. squarrosa.


+
45,000
60,000
112,000
152,000
−
TYPICA
ANATHERA
ANATHERA
MEYERI
STRANGULATA
STRANGULATA
STRANGULATA

strangulata, meyeri, anathera, and typica. There is a marked similarity of all the SDS-PAGE patterns, but minor differences in the mobility and number of visible bands are evident.

The three accessions of strangulata each have four major glutenin subunits with molecular weights of 152,000; 112,000; 60,000 and 45,000. Minor bands at 39,000 and 30,000 and a broad band of mobility corresponding to a molecular weight of approximately 14,000 are all common to the three strangulata samples. A faint band at 80,000 is also evident.

Anathera and typica patterns were similar to those of strangulata. Subunits that are common to all three varieties have molecular weights of 152,000; 112,000; 60,000; 45,000 and 14,000 (broad band). Some minor differences among these varieties were observed. Both samples of anathera had bands of molecular weights of 80,000 and 39,000 but these subunits appear absent in the typica studied.

The pattern of the variety meyeri was significantly different from the patterns of the other three varieties studied, particularly in the region of the slowest moving subunits. The two largest subunits of meyeri were distinctly of lower molecular weight than the analogous subunits of the other three varieties. In the lower molecular weight region (higher mobility) the pattern of meyeri was essentially identical to that of the the other varieties, with major bands at mobilities corresponding to molecular weights of 60,000; 45,000; 39,000 and 14,000 (broad band).

The similarity of subunit composition of glutenin of the D-genome donors examined makes it impractical to use this criterion in the identi-

fication of the variety of Ae. squarrosa involved in the natural evolution of hexaploid bread wheats. The high molecular weight subunits absent in AABB tetraploids and present in hexaploids (AABBDD) were present in the glutenin of the varieties anathera, typica and strangulata, making either (or all) of these possible D-genome progenitors of the bread wheats.

Relation of Patterns to Baking Quality. Relevant baking quality data for the wheats used in this study has been reported (Kerber and Tipples, 1969; Kaltsikes et al., 1968). Removal of the D-genome from Canthatch caused a marked decrease in quality (Kerber and Tipples, 1969) and on the basis of the present study is associated with the absence of glutenin subunits at MW's 152,000 and 45,000 and a decrease or deletion, of the 112,000 and 60,000 subunits. Production of the synthetic hexaploid restored most of the lost baking quality and the lost glutenin subunits in the SDS-PAGE pattern.

Removal of the D-genome from Rescue and Thatcher caused a large decrease in baking quality (Kaltsikes et al., 1968). Although Tetraprelude gave a satisfactory loaf volume this appears to be due to its relatively high protein content. When the baking results are expressed as loaf-volume per unit protein, Prelude protein (59 c.c. per percent protein on a 14% moisture basis) is of slightly better quality than that of Tetraprelude (55 c.c./% protein).

The decrease in breadmaking quality of the four extracted tetraploid wheats examined in this study was accompanied by the loss of glutenin subunits of MW's 152,000; 112,000 (except for Canthatch where a dilution occurred) and 45,000 and a dilution in either the band at 80,000 or 60,000. Previous

studies (Bietz and Wall, 1972) showed that natural AABB tetraploids, i.e. durum wheats, lacked some of the high MW subunits present in hexaploid wheats. This report was confirmed in the present study (results not shown). All AABB tetraploid wheats, whether natural or synthetically produced, that have been studied, lack some of the high MW glutenin subunits.

Conclusions

The most significant subunit deletions, resulting from the removal of the D-genome of common wheat, are those at MW's 152,000 and 112,000. Since these subunits have not been found in durum glutenins studied to date (Bietz and Wall, 1972; and present study), their absence can be correlated to the lack of breadmaking quality in durum wheats. It might be speculated that these subunits are an essential part of the high molecular weight glutenin which is the elastic component of gluten. Breadmaking quality requires an optimal amount of this type of glutenin for a proper balance in rheological properties.

The high MW subunits could play a dominant role in baking quality in either of two ways. Firstly, they may merely increase the size of the glutenin by their direct contribution to the MW of the intact glutenin molecule. This possibility seems unlikely since these large subunits represent only a small percentage of the total protein in glutenin, as may be inferred from the low intensity of their bands in the SDS-PAGE patterns (Fig. 1 and 2).

The second possibility is that these subunits occupy a critical position in the structure of glutenin. For example they may be located in regions of the molecule where branch points or other characteristic structural features

occur or they may be a requirement for a particular type of tertiary or quaternary structure. Determination of the actual role of these subunits in breadmaking quality will require delineation of the structure of glutenin.

The presence of these large subunits is not the only factor that controls baking quality since all breadwheats contained them regardless of quality (Bietz and Wall, 1972; Orth and Bushuk, 1973b). Obviously bread-making quality requires more than merely their presence but they appear to be necessary.

Acknowledgments

The authors are grateful to D. H. Simmonds, CSIRO Wheat Research Unit, Sydney, Australia for suggestions in the preparation of the manuscript. Tetracanthatch and the synthetic hexaploid wheats were originally produced by E. R. Kerber of the C.D.A. Research Station, Winnipeg. Extracted tetra-ploids of Prelude, Rescue and Thatcher were produced by P. J. Kaltsikes of the University of Manitoba.

Studies of Glutenin. VI. Chromosomal Location of Genes Coding for Subunits of Glutenin of Common Wheat[1]

Abstract

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the reduced glutenins of nullisomic - tetrasomic lines of Chinese Spring showed that four glutenin subunits are coded by genes on chromosome 1D. Analogous studies on ditelocentric lines showed that these genes are located on the long arm of chromosome 1D. This conclusion substantiated the fact that four subunits were absent in the durum wheat, LD 222, and present in its 1D-1B substitution line. Repression of the synthesis of subunits coded by genes on chromosomes of the A or B genomes was observed in lines tetrasomic for chromosomes 2B, 3B, and 6B.

Introduction

Hexaploid (bread) wheats have the genomic formula AABBDD, the A, B, and D genomes each contributing seven pairs of chromosomes. These wheats have 42 chromosomes or 21 pairs. Aneuploid lines, those that have an abnormal number or composition of chromosomes, and substitution lines, those with chromosomes of one genome substituted by homoeologous chromosomes from another, can be used to determine the chromosomal location of genes responsible for coding the synthesis of specific flour proteins.

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[1] Submitted for publication in Cereal Chemistry under the co-authorship of R. A. Orth and W. Bushuk.

Particularly noteworthy in this regard are the two series of aneuploid lines of the common wheat variety Chinese Spring produced by Sears (1954, 1966). The first series is the nullisomic-tetrasomic (nulli-tetra) lines which lack one pair of chromosomes and have a double complement of a homoeologous pair. The second series is the ditelocentrics (ditelos) in which two chromosomes of a pair lack one arm.

Shepherd (1968) determined the chromosomal location of the genes that code for nine of the seventeen protein bands resolved by starch-gel electrophoresis of 2M urea extracts on single seeds of the nulli-tetras of Chinese Spring. The majority of the bands (mainly gliadins) were coded by the homoeologous chromosomes of group 1 and group 6.

Wrigley and Shepherd (1973) employed a two dimensional technique to resolve wheat gliadins into over 40 components. The chromosomal location of genes responsible for the synthesis of more than 30 of these proteins was determined. All genes identified were located on homoeologous chromosomes of group 1 and group 6, in agreement with the findings of Shepherd (1968).

Recent studies by Russian workers (Konarev et al., 1972) showed that cytogenetic removal of the D-genome from hexaploid (common) wheat, to produce extracted tetraploid wheat, resulted in a loss of two slow-moving gliadins that can be identified by gel electrophoresis. Furthermore it was shown that these gliadins were coded by genes on chromosome 1D.

Until the present work, there have been no reports of the chromosomal location of genes that control the synthesis of glutenin (or its subunits).

In a previous paper of this series (Orth and Bushuk, 1973c) we reported that removal of the D genome from four hexaploids resulted in the deletion of a number of glutenin subunits. Two of these subunits, identified by molecular weights of 152,000 and 112,000, are characteristic of bread wheats and are absent in all durum and extracted (AABB) tetraploid wheats studied to date. The presence of these subunits appears to be a necessary, but not sufficient, condition for breadmaking quality (Orth and Bushuk, 1973c; Huebner et al., 1973). Accordingly the chromosomal location of the genes that code their synthesis is of technological interest.

This article presents results on the location of genes for glutenin subunit synthesis, obtained with the two series of aneuploid lines produced by Sears (1954, 1966) and discussed above.

Materials and Methods

Wheat Samples. The grain of seven compensating nullisomic D-tetrasomic B (nulli D-tetra B) and the seven nulli D-tetra A lines of Chinese Spring was generously provided by Dr. E. R. Sears[1]. Each line has one pair of D chromosomes replaced by a homoeologous pair from the B or A genome. For example, nulli 1D-tetra 1B (N1DT1B) is the line in which the 1D pair of chromosomes is replaced by an additional 1B pair. These aneuploid lines retain the normal chromosome number of 42, but the composition

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is abnormal.

Seven ditelocentric lines of Chinese Spring were also provided by Dr. Sears. They are 1DL, 2Dα, 3Dα, 4DL, 5DL, 6Dα, and 7DS. Ditelocentric 1DL (ditelo 1DL) lacks the short arm of chromosome pair 1D, 2Dα lacks one of the arms (as yet not identified) of chromosome pair 2D, 7DS lacks the long arm on chromosome pair 7D.

A durum wheat (AABB), LD 222, and its 1D-1B substitution line, at the tetraploid level, were supplied by Dr. T. Mello-Sampayo[2]. The substitution line was produced by crossing Chinese Spring nulli 1B-tetra 1D to LD 222 and backcrossing to LD 222 three times with appropriate selection and selfing at each step.

Glutenin Preparation. All samples were obtained as whole grain. Approximately 0.5 g of each line was ground with a mortar and pestle, then dispersed in the solvent AUC (0.1M acetic acid, 3M urea and 0.01M hexadecyltrimethylammonium bromide) by homogenization for 5 minutes. Glutenins were then prepared by the pH precipitation technique described previously (Orth and Bushuk, 1973a).

SDS-PAGE. Electrophoresis was performed in a 5% polyacrylamide gel at pH 7.3, according to the method described in an earlier paper in this series (Orth and Bushuk, 1973b). The gels were stained with Coomassie Brilliant Blue (C.B.B.) and were gradually destained by agitation in solutions of acetic acid-isopropanol-water (1:1:8) containing progressively

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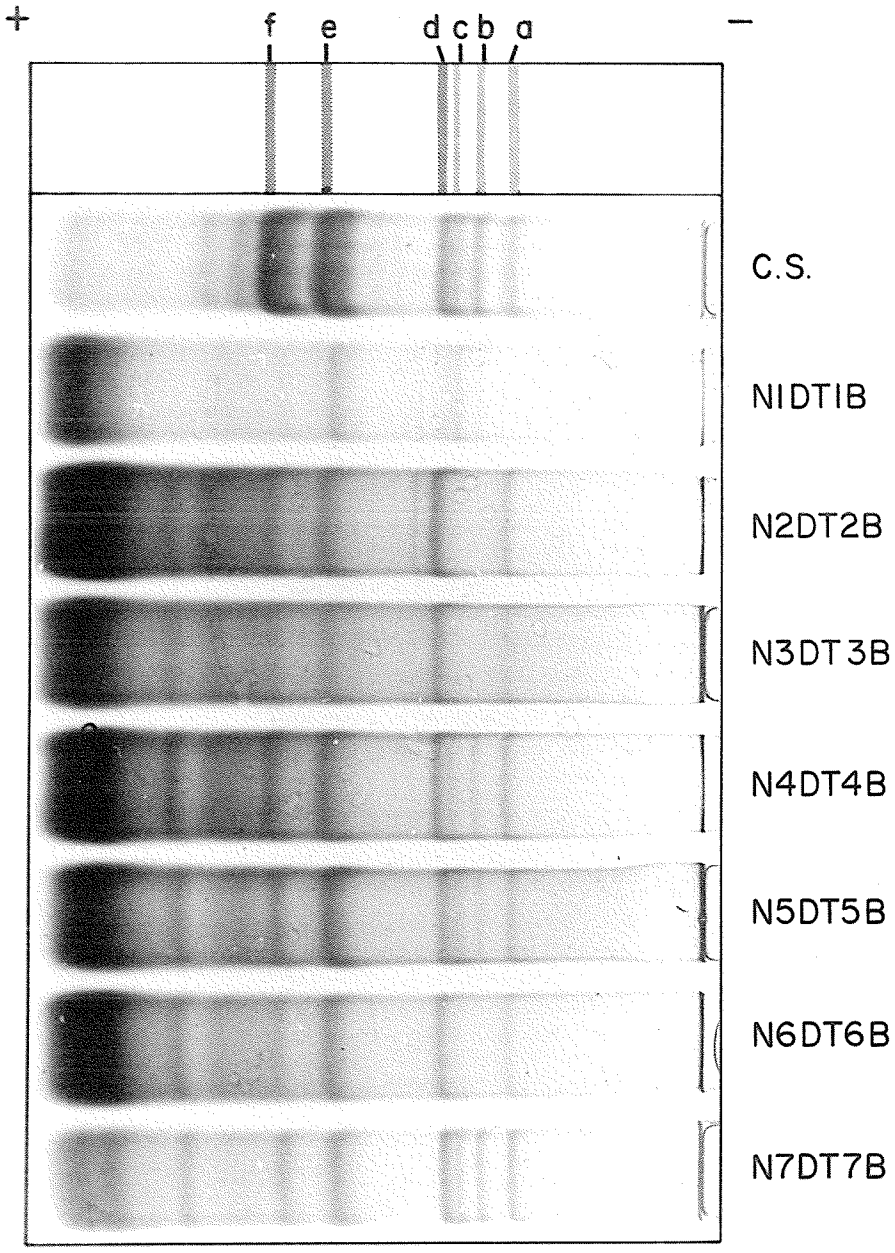
decreasing amounts of C.B.B. The destained gels were stored in 10% acetic acid for subsequent examination and photography.

Results and Discussion

Figure 1 shows the SDS-PAGE patterns of the reduced glutenins of Chinese Spring (C.S.) and its seven nulli D-tetra B lines. The glutenin subunits a to f are identified for reference in discussion that follows. At this point, it is of special interest to note that four of the six identified subunits, a, d, e, and f, have the same electrophoretic mobilities as the subunits deleted on removal of the D genome from hexaploid bread wheats (Orth and Bushuk, 1973c). Figure 1 also shows that they are all present in the parent variety, Chinese Spring. The molecular weights of these four subunits are 152,000, 112,000, 60,000, and 45,000 respectively.

Three of the four subunits, a, d, and f, are absent from the pattern of the nulli 1D-tetra 1B line. On the basis of these results, it is concluded that these three subunits are coded by genes on chromosome 1D of Chinese Spring. It is difficult to determine from the N1DT1B pattern whether the fourth subunit, e, is deleted, or whether it is diluted. If the latter is the case then there may be at least two subunits of the same molecular weight migrating together in the parent and one is deleted. This is probably so since this subunit is not deleted in any of the other nulli D-tetra B lines.

Subunits b and c are deleted in the nulli 2D-tetra 2B, nulli 3D-tetra 3B, and nulli 6D-tetra 6B lines. These deletions are surprising


+
f
e
d
c
b
a
−
C.S.
N1DT1B
N2DT2B
N3DT3B
N4DT4B
N5DT5B
N6DT6B
N7DT7B

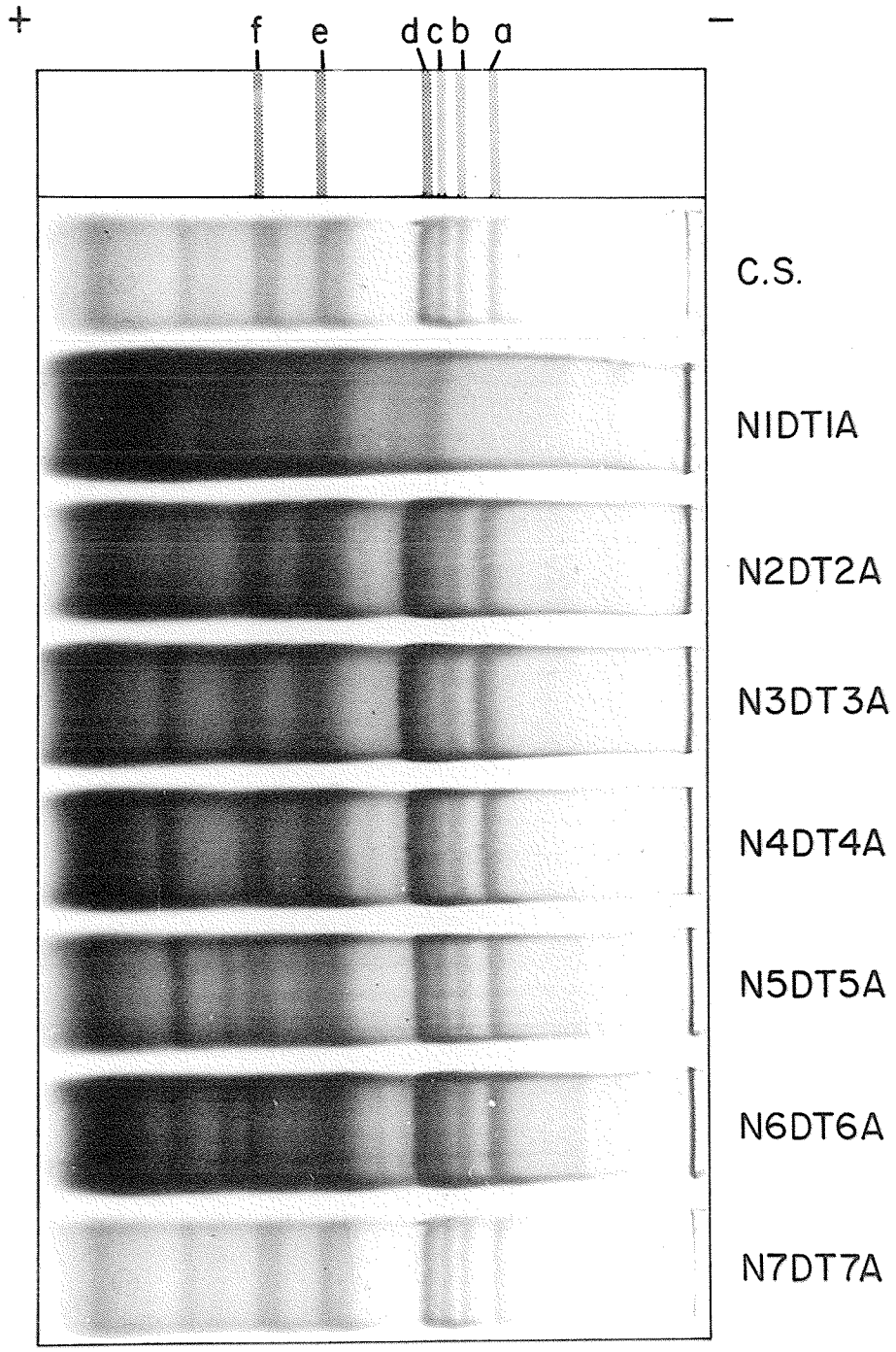
since corresponding subunits were not present in the glutenin from seven accessions of Aegilops squarrosa, genomically DD. Also, these subunits were not deleted in four AABB extracted tetraploids studied (Orth and Bushuk, 1973c).

There are two possible explanations of this apparent anomaly. Firstly, the deletions could be due to removal of D-genome chromosomes coding for these subunits. Secondly, doubling the number of 2B, 3B, or 6B chromosomes, to achieve the tetrasomic condition, may interfere with the normal expression of genes of the A or B genomes.

Results for the nulli D-tetra A lines (Figure 2) offer a means of distinguishing between the two possibilities referred to in the previous paragraph. Once again a schematic representation of the bands under discussion is included in this figure. All lines, except nulli 1D-tetra 1A, exhibit all the bands of the parent Chinese Spring. This observation is consistent with the second of the two explanations - that is, the conversion of chromosomes 2B, 3B, or 6B to the tetrasomic condition has an effect on synthesis of subunits b and c. It is not known whether this repression is at the gene level or at a later stage in the biosynthesis of these polypeptides. An analogous observation was made by Shepherd (1968) in the study of the 2M urea-soluble proteins of Chinese Spring and its nulli-tetra lines. When chromosome 2A was in the tetrasomic condition, the protein forming activity of chromosome 6D was inhibited.

The absence of subunits a, d, and f in nulli 1D-tetra 1A confirms the location of their genes on chromosome 1D. Subunit e is also absent in this

Fig. 2. SDS-PAGE patterns of reduced glutenins of the nulli D-tetra A lines of Chinese Spring.


+
f
e
d
c
b
a
−
C.S.
NIDTIA
N2DT2A
N3DT3A
N4DT4A
N5DT5A
N6DT6A
N7DT7A

line, although this is not very clear from Fig. 2.

On the basis of results of Figs. 1 and 2, it can be stated with some certainty that subunits a, d, e, and f are all coded by genes on chromosome 1D. To confirm this, and to establish that the above subunits are not deleted because of the tetrasomic condition of 1A or 1B, the glutenin subunit compositions of a durum wheat (AABB) and its substitution line produced by substituting chromosome 1D of Chinese Spring for chromosome 1B of the durum, were determined. Figure 3 shows the SDS-PAGE patterns of the reduced glutenins of these two wheats with a schematic of the bands to be discussed.

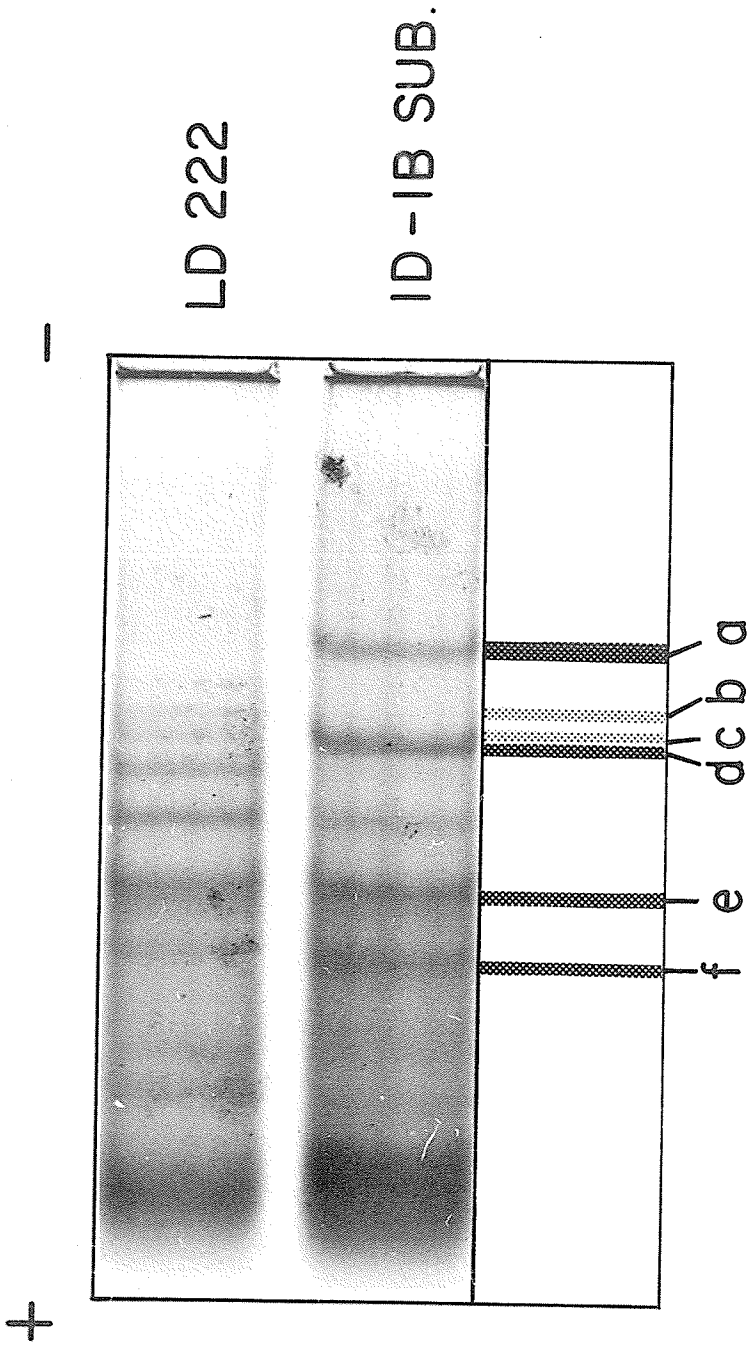
If the genes for subunits a, d, e, and f of Chinese Spring are on chromosome 1D, then we would expect to find these subunits in the pattern of the substitution line. Subunits a and d are obviously present in this line and absent in the durum, confirming the location of their genes on chromosome 1D.

Subunit e is in an area on the gel where an intense band also occurs in the pattern of the durum glutenin. Accordingly, it is not possible to make a definite conclusion regarding the presence of this subunit in the substitution line.

Subunit f, although close in mobility to a slightly higher molecular weight subunit in the durum glutenin, appears as a strong band in the substitution line.

Apart from these interesting additional subunits due to the presence

Fig. 3. SDS-PAGE patterns of reduced glutenins of a durum (LD 222) and its 1D-1B substitution line (1D-1B SUB).


LD 222
ID-IB SUB.
-
+
a
b
c
d
e
f

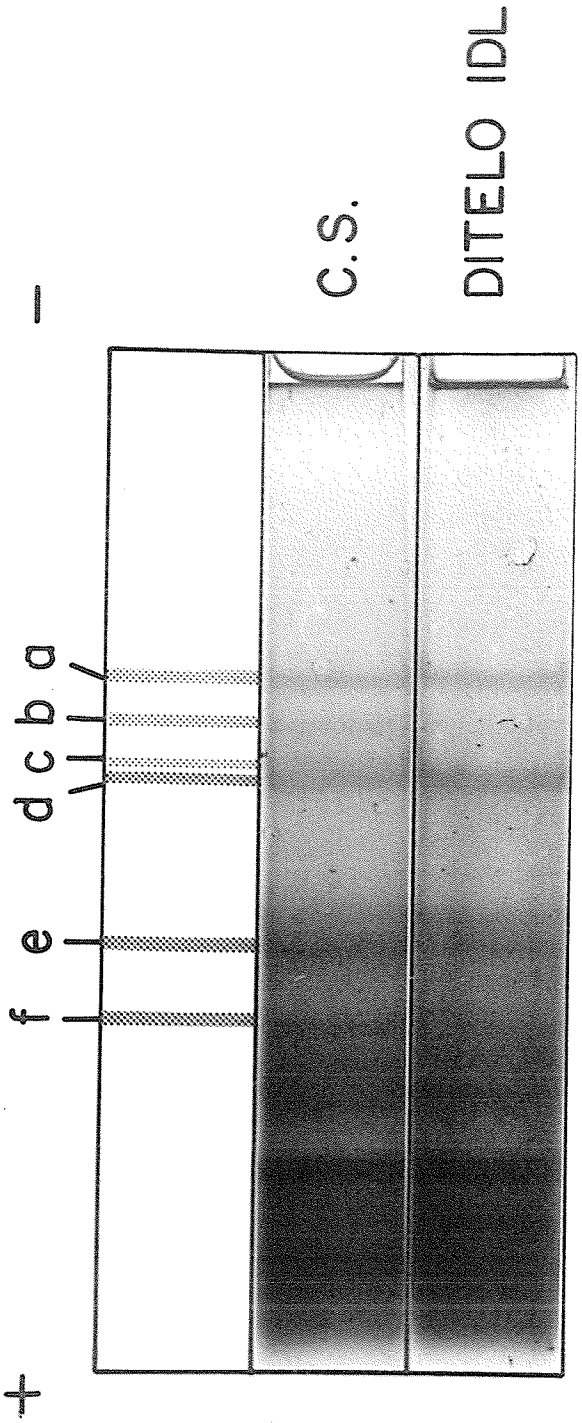
of chromosome 1D, there are also a number of deletions. Subunits b and c are present in the durum but not in the substitution line. This indicates that they are probably coded by chromosome 1B. A similar situation exists for a subunit of slightly lower molecular weight than d (not labeled in Fig. 3). Because there is a possibility of gene repression similar to that observed in some of the nulli-tetra lines (see Fig. 1), it cannot be stated categorically that these subunits are coded by genes on chromosome 1B. There is still the possibility that the addition of chromosome 1D causes repression of the synthesis of subunits b and c. Further work is necessary to clarify this point.

Figure 4 shows the SDS-PAGE patterns of the reduced glutenin of Chinese Spring and its 1DL ditelocentric line (lacks the two short arms of chromosome 1D but has the two long arms). These patterns are qualitatively identical although there appears to be a decrease in intensity of bands b and c in the ditelocentric line. From these results, it can be concluded that subunits a, d, e, and f are coded by genes on the long arm of chromosome 1D of Chinese Spring.

General Discussion

The key role of glutenin (including residue protein) in the bread-making quality of flour is now established (Orth and Bushuk, 1972). Its contribution of elasticity to the rheological properties of dough is consistent with the molecular structure postulated by Huebner et al. (1973) on the basis of amino acid composition of a major subunit, and with the ultrastructure of glutenin as determined by scanning electron microscopy

Fig. 4. SDS-PAGE patterns of reduced glutenins of Chinese Spring (C. S.) and its 1DL ditelocentric line (DITELO 1DL).


C.S.
DITELO IDL
−
+
f
e
d
c
b
a

(Orth et al., 1973a). Information on the structure (all levels, primary, secondary, tertiary and quaternary) of glutenin is basic to the understanding of breadmaking quality at the molecular level.

A major advance in the research on the structure of glutenin has resulted from the application of SDS-PAGE to determine subunit composition (after reduction of disulfide crosslinkages). Most of the work in this area has been carried out in two laboratories; the U.S.D.A. Northern Regional Laboratory in Peoria, Illinois (Bietz and Wall, 1972; Huebner and Wall, 1973; Huebner et al., 1973), and the Department of Plant Science, University of Manitoba, Winnipeg, Canada (Orth and Bushuk, 1973 a, b, c). Studies of the Peoria group are aimed primarily at the fundamental structure of glutenin, whereas the Winnipeg group has concentrated more on the breadmaking and genetic implications. The present article can be classified in the latter category.

It has now been established that one of the ways in which the D genome of bread wheats affects breadmaking quality is by coding for the synthesis of at least four glutenin subunits. One of these is the largest and another the fourth largest that have so far been identified. It appears that these subunits play a key role in the structure of glutenin that is essential for breadmaking quality. They are absent in durum wheats and consequently this is one of the reasons these wheats do not have breadmaking quality (Orth and Bushuk, 1973c). It should be noted that these subunits are not the total answer to breadmaking quality (Orth and Bushuk, 1973 b, c); there are many other factors, some dependent on flour proteins

and some on other constituents (lipids, pentosans, starch).

The mechanism by which these subunits affect the structure of glutenin (and thereby gluten and dough) has not been established. They could occupy a central position in the linking of other subunits into the large glutenin molecule. The extended conformation of the highest molecular weight subunit, based on its molecular weight and amino acid composition (Huebner et al., 1973) indicates it is well suited to a central structural role. Covalent bonds (SS) or secondary interactions between this subunit and other glutenin subunits, would result in long, high molecular weight glutenin molecules. These would be capable of high levels of interchain entanglement and so produce a strong rheological unit or molecular aggregate. Perhaps the observed relationship between breadmaking quality and the proportion of insoluble flour protein (Orth and Bushuk, 1972) can be explained on the basis of the relative stability of glutenin aggregates of wheats of differing quality.

The present work establishes the location of the genes for some of the glutenin subunits identified with breadmaking quality as the long arm of chromosome 1D in Chinese Spring. If this location is general for breadwheats, or the alternate location can be determined, it would be extremely useful to cytogeneticists. In developing synthetic cereal species such as triticale, or in attempting to add (cytogenetically) breadmaking quality to durum (tetraploid) wheats, cytogeneticists should pay special attention to the inclusion in their progeny of the D-genome chromosome(s) responsible for the synthesis of these important glutenin subunits.

Acknowledgments

The authors wish to thank Dr. E. R. Sears and Dr. T. Mello-Sampayo for kindly making available the genetic lines used in this study.

Studies of Glutenin. IV. Microscopic Structure and its Relation to Breadmaking Quality[1]

Abstract

Glutenins of two varieties of HRS wheat, Canthatch and Manitou, one variety of durum wheat, Stewart 63, a synthetic hexaploid (AABBDD) wheat and its tetraploid (AABB) and diploid (DD) parents, and one variety of spring rye, Prolific, were analyzed by scanning electron microscopy. The glutenins of Canthatch, Manitou and the synthetic hexaploid were fibrous in structure while those of Stewart 63 and Tetracanthatch were characterized by ribbon-like and film structures. Rye glutenin showed characteristic rod-like structures. Squarrosa glutenin appeared fibrous like the glutenin of the bread wheats and the synthetic hexaploid. The observed differences in microscopic structure can be related to glutenin elasticity. Glutenin of Manitou was extremely fibrous and of uniform structure. On reduction of its disulfide bonds, this glutenin completely lost its fibrous structure.

Introduction

Although the electron microscope has been used in studies of the wheat kernel, flour, dough and starch (Buttrose, 1963; Seckinger and Wolf,

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[1] Submitted for publication in Cereal Chemistry under the co-authorship of R. A. Orth, B. L. Dronzek, and W. Bushuk.

1970; Simmonds, 1972a,b; Hall and Sayre, 1970; Aranyi and Hawrylewicz, 1969) there is only one report (Seckinger and Wolf, 1970) in the literature of a study of glutenin from one sample of wheat. Because of the extremely important role of this protein in the breadmaking quality of bread wheats, it was felt that its microscopic structure might show a recognizable relationship to its ability to contribute to the formation of the gluten complex that is required for baking quality.

Some results on the structure of gliadin and glutenin were published by Seckinger and Wolf (1970). In films cast from dispersions of these proteins, gliadin showed film-like structures whereas glutenin formed strands.

This article presents scanning electron microscope results on glutenin from a number of related cereal grains and discusses the possible relationship of the microscopic structure to breadmaking quality.

Materials and Methods

Grain Samples. The grain samples used are listed below. All were grown on experimental plots at the University of Manitoba.

Variety, etc.	Type or Class	Chromosome Number	Genomic Constitution
Canthatch	hard red spring wheat	2n=6x=42	AABBDD
Manitou	hard red spring wheat	2n=6x=42	AABBDD
Synthetic Hexaploid	unclassified	2n=6x=42	AABBDD
Tetracanthatch	tetraploid derived from Canthatch	2n=4x=28	AABB
Stewart 63	amber durum wheat	2n=4x=28	AABB
Prolific	spring rye	2n=2x=14	RR
Strangulata	*Ae. squarrosa*	2n=2x=14	DD

Glutenin Preparation. Glutenins were prepared by pH precipitation after extraction of the protein with AUC (0.1M acetic acid, 3M urea and 0.01M hexadecyltrimethylammonium bromide) (Orth and Bushuk, 1973a). AUC extracts were prepared from ground grain of Canthatch, Tetracanthatch, squarrosa, synthetic hexaploid, Prolific (rye) and Stewart 63 (durum) and from washed out gluten of Manitou.

Reduction of Glutenin. Manitou glutenin (50 mg.) was dispersed in 10 ml. of 0.1M phosphate buffer of pH 8.0 containing 1% (v/v) β-mercaptoethanol and 6M urea. The dispersion was shaken overnight at 40°, dialyzed against deionized water for six days and then freeze dried. A control sample was subjected to the same treatment except the β-mercaptoethanol was omitted.

Scanning Electron Microscopy (SEM). Freeze-dried glutenin material was attached to circular stubs with double-sided tape and coated with gold to a thickness of 20-25 nm. The mounted specimens were examined in a Cambridge "Stereoscan" MK IIa scanning electron microscope at an accelerating potential of 10 kV. and photographed on Panatomic X film.

Results and Discussion

Glutenin of Manitou (Fig. 1) showed a characteristic fibrous structure with many thick strands (10μ diameter) intertwined with thin strands (1μ). The strands were generally aligned giving the impression of a unidirectional organization of the fibres. None of the micrographs of this glutenin showed any starch granules which would be readily identifiable because of their

Fig. 1. SEM micrograph of purified freeze-dried glutenin of the bread wheat variety Manitou (X625).

characteristic size, shape and structure. In cross section, the fibres appear circular and there is considerable evidence of filmy material between the strands especially at points of contact.

Reduction of the Manitou glutenin produced a dramatic change in its structure (Fig. 2). All of the original fibrous structure was lost. The reduced glutenin had no distinguishing or regular features. The structural change observed is a conversion of a regular fibrous structure to one that appears completely amorphous. Presumably this structural change would lead to a marked change in the rheological properties of the hydrated glutenin. Fundamentally, it would be expected that the characteristic elasticity of bread wheat glutenins would be completely lost when the structure of Fig. 1 is converted to that of Fig. 2.

The fact that the reduced glutenin lacked the fibrous structure of the control indicates that this feature is not due to sample preparation alone but is dependent on the nature of the glutenin. This conclusion is supported by results obtained in another study (Orth et al., 1973b) in which proteins from Manitou flour, fractionated on Sephadex G-100 in the solvent AUC, showed microscopic structures dependent on their molecular weight. The structure of the low molecular weight proteins was very different to those of high molecular weight.

Glutenin of Canthatch (Fig. 3) appeared as stringy intertwined fibres. In addition it contained some flat filmy material. In structure, this glutenin appeared slightly different from the Manitou glutenin. It is presumed that this alteration of structure is due to the minor difference

Fig. 2. SEM micrograph of reduced glutenin of the bread wheat variety Manitou (X125).

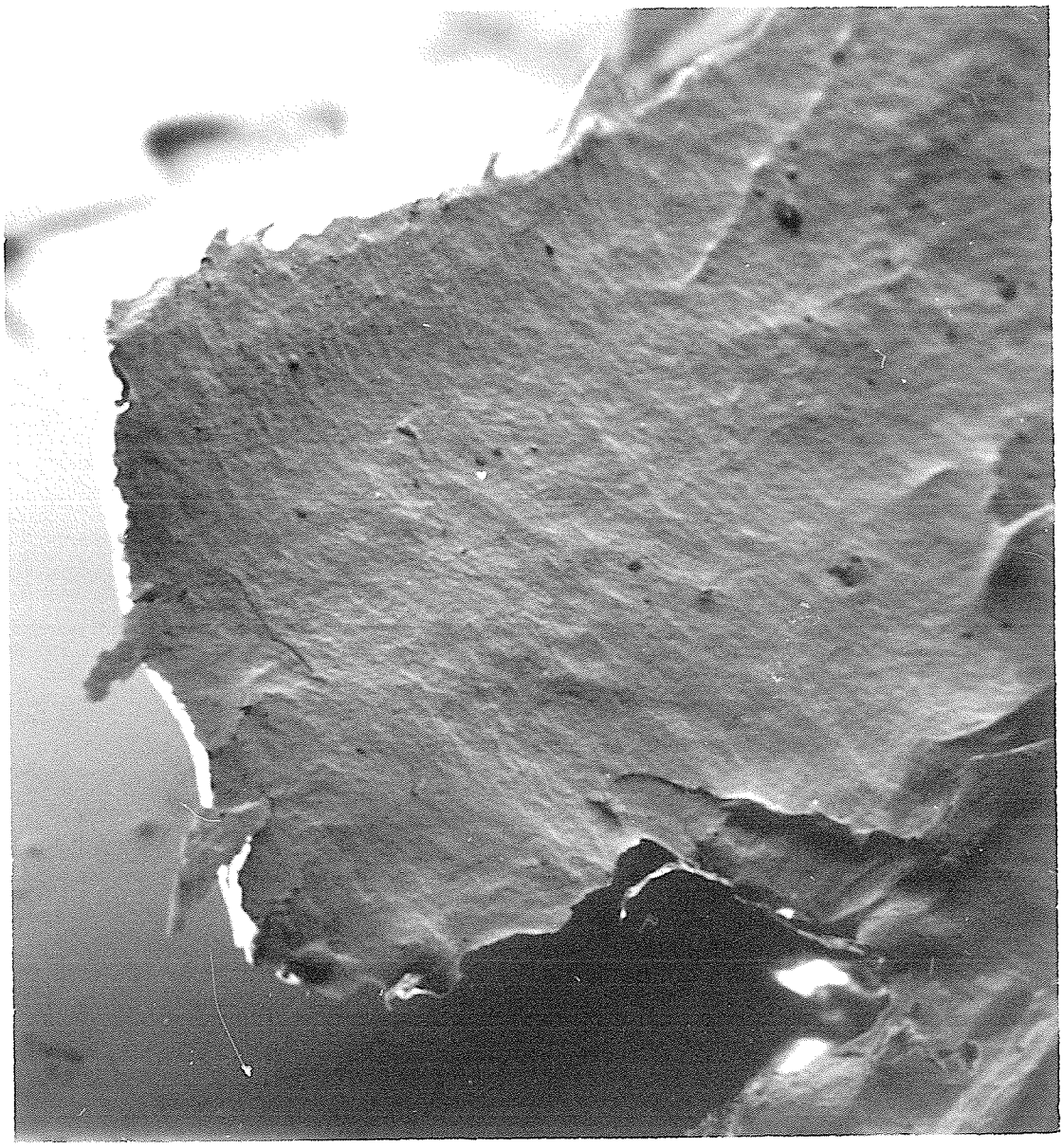
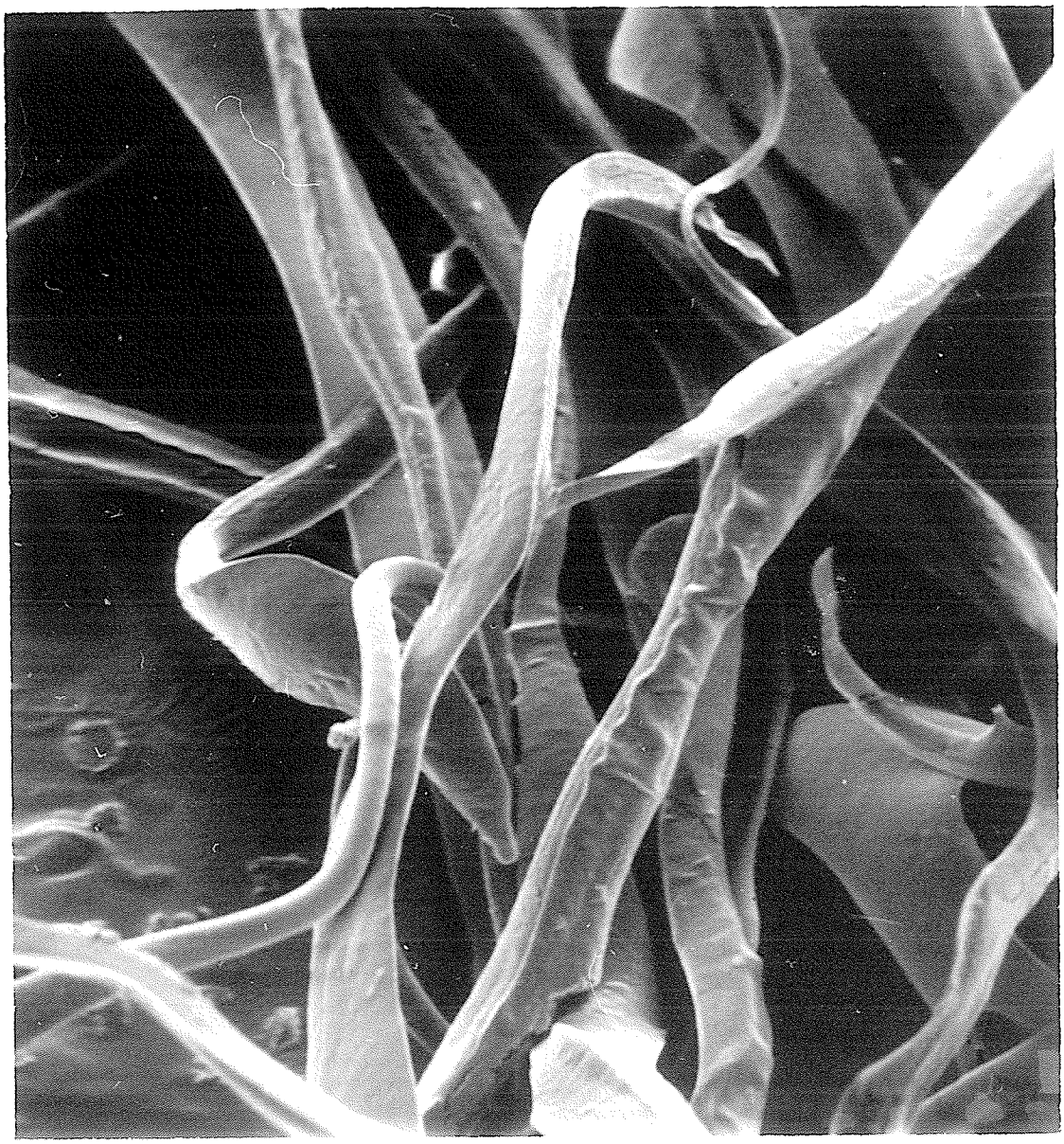


Fig. 3. SEM micrograph of purified glutenin of the bread wheat variety Canthatch (X1250).



in the preparative methods used. However, the typical fibrous structure of bread wheat glutenin is still quite evident.

Figure 4 is a micrograph of the glutenin of the durum wheat, Stewart 63. In contrast to the bread wheat glutenin, the durum glutenin is characterized by flat ribbon-like structures and contains a large proportion of filmy material. When these results are considered relative to functional properties, the durum glutenin would be considerably less elastic than the glutenins of the bread wheats shown in Figs. 1 and 3. The microscopic structural features are generally consistent with the well known rheological properties of bread and durum wheat doughs.

The results presented above suggested a possible relationship between the observed microscopic structure of glutenin and the functional properties of doughs derived from the same wheat. Accordingly, a SEM study of the glutenins of a synthetic hexaploid bread wheat and of the tetraploid and diploid parents was carried out to obtain further information on this relationship.

Figure 5 is a micrograph of the glutenin of Tetracanthatch. This glutenin had very little fibrous structure and consists largely of layered ribbon-like structures. In this respect, Tetracanthatch glutenin was similar to the glutenin from the natural durum wheat (tetraploid), Stewart 63. Also evident in the micrograph of Fig. 5 are discrete disc-shaped particles approximately 5 - 10μ in diameter. These are probably small starch granules.

Removal of the D-genome from Canthatch produced a dramatic change in the microscopic structure of the glutenin. It completely lost its fibrous

Fig. 4. SEM micrograph of purified glutenin of the durum wheat variety Stewart 63 (X1250).

Fig. 5. SEM micrograph of purified glutenin of the extracted AABB tetraploid wheat variety Tetracanthatch (X625).

appearance and became quite amorphous. In breadmaking quality, Tetracanthatch was inferior to Canthatch (Kerber and Tipples, 1969). The loss of baking quality parallels the change in structure of the glutenin.

Addition of the D-genome from Ae. squarrosa var. strangulata to Tetracanthatch to produce a synthetic AABBDD hexaploid wheat gave a noticeable improvement in breadmaking quality (Kerber and Tipples, 1969). The glutenin of the synthetic hexaploid was considerably more fibrous than that of Tetracanthatch with strands of similar diameter and shape as those of Canthatch glutenin (Fig. 6).

The micrograph of the glutenin of the strangulata variety of Ae. squarrosa (progenitor of the D genome of bread wheats), (Fig. 7) had the characteristic fibrous structure typical of bread wheats. Small amounts of sheet-like material and small starch granules were also evident in the Ae. squarrosa glutenin preparation examined. The change of the ribbon-like glutenin of Tetracanthatch to the fibrous glutenin of the synthetic hexaploid can be attributed directly to the D-genome that was added. It appears that this feature of glutenin structure of bread wheat is simply inherited from the tetraploid and diploid parents. The glutenin subunits, as determined by SDS-polyacrylamide gel electrophoresis, also appear to be simply inherited (Orth and Bushuk, 1973c).

Glutenin of the one variety of rye that was examined (Fig. 8) showed characteristic short rod-like structures but essentially no thin fibres. There was very little entanglement of the rod-like particles of this glutenin. Rye glutenin had a structure that was quite distinct from the struc-

Fig. 6. SEM micrograph of purified glutenin of the synthetic hexaploid wheat Tetracanthatch x Ae. squarrosa variety strangulata (X1250).

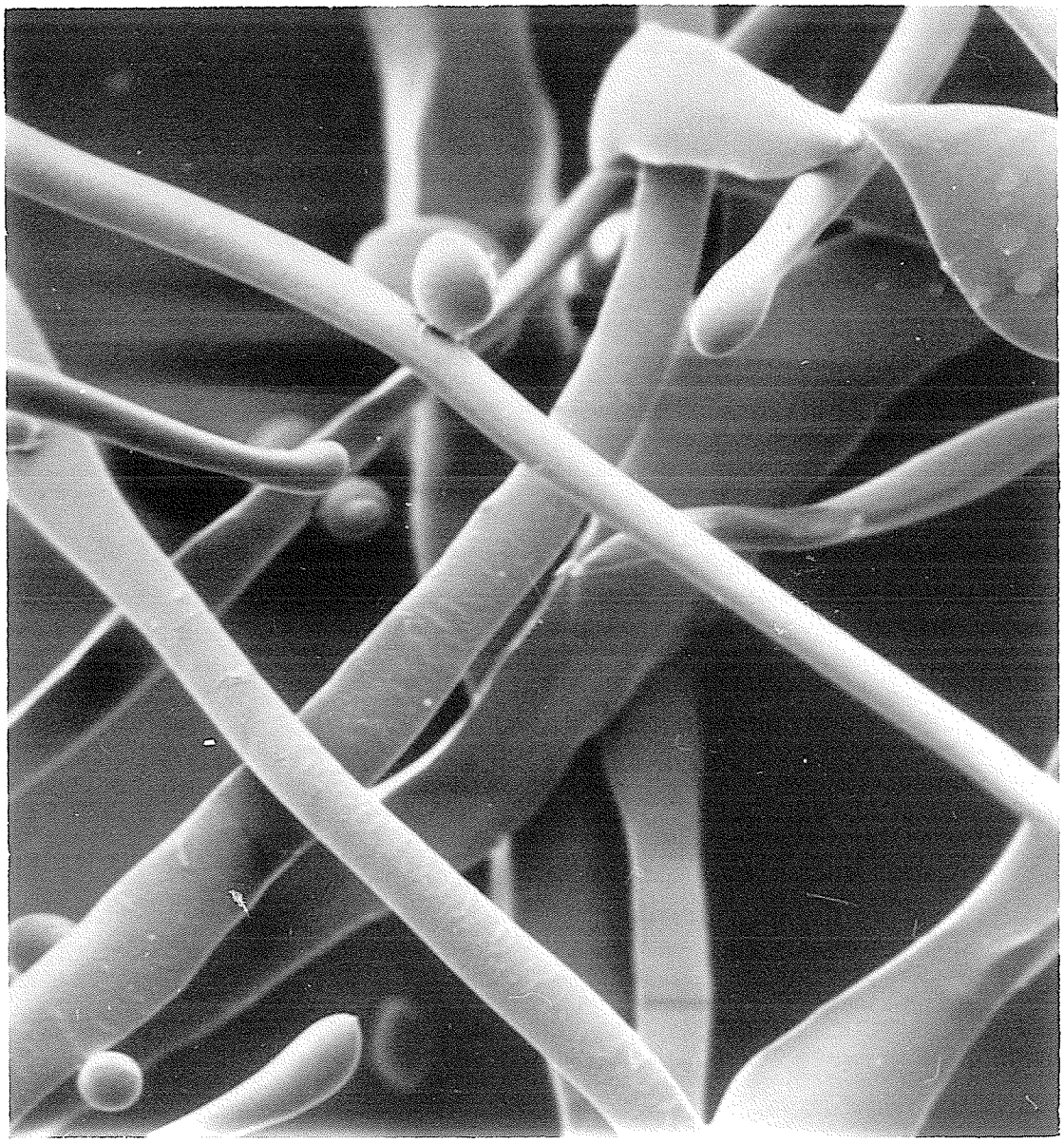
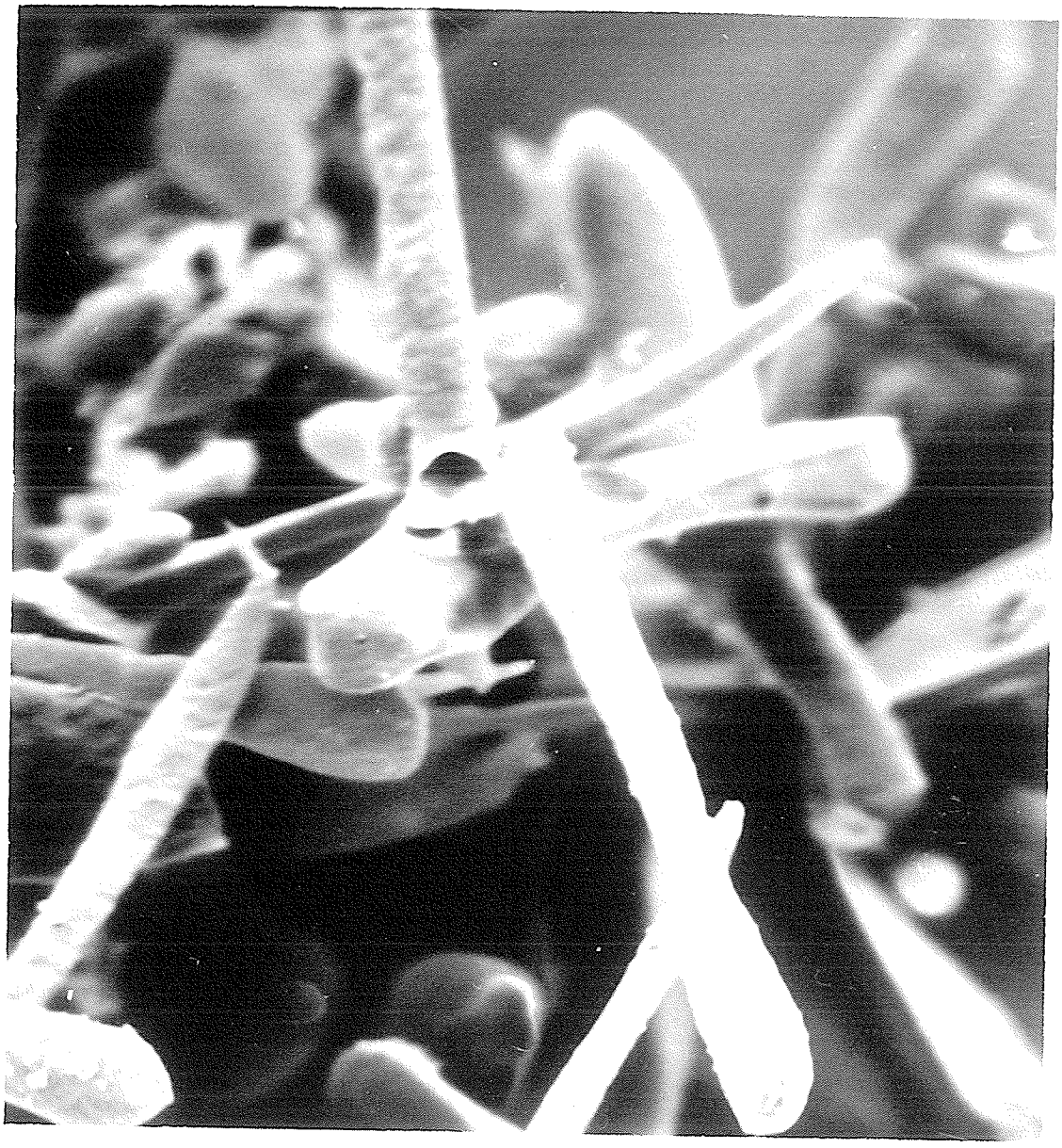


Fig. 7. SEM micrograph of purified glutenin of Ae. squarrosa variety strangulata (D genome, diploid). (X625).

Fig. 8. SEM micrograph of purified glutenin of the spring rye variety Prolific (X3000).



tures of the other glutenins examined in this study. In relation to functional properties, it would be expected that the elasticity of a structure such as shown in Fig. 8 would be considerably less than that of the structures in Figs. 1, 3, 6 and 7.

Results presented in this article showed that the SEM can be used to advantage in the determination of the microscopic structure of glutenin from various grain species. Furthermore the structure of glutenin from bread wheats can be readily distinguished from that of other wheats and grains that do not have the functional properties that are required for breadmaking quality.

Acknowledgment

The grain of Canthatch, Tetracanthatch, the synthetic hexaploid and Ae. squarrosa was provided by Dr. Kerber of the C.D.A. Research Station, Winnipeg.

GENERAL DISCUSSION

Bread wheat glutenin(s) comprises at least fourteen polypeptide subunits, with molecular weights in the range 10,000 to 150,000, linked together by disulfide bonds. Both the acetic acid-soluble glutenin and the insoluble glutenin (residue protein) gave the same SDS-PAGE patterns after reduction. Furthermore, glutenins prepared (from the bread wheat Thatcher) by pH precipitation from both a 0.01M acetic acid solution and the solvent AUC had the same subunit composition as the two fractions (glutenin and residue protein) obtained by solubility fractionation of wheat protein.

These results suggest that the residue fraction is a polymeric (or aggregated) form of acetic acid-soluble glutenin. The crosslinkages involved in this polymerization are as yet unknown; they may be covalent disulfide bonds or secondary bonds, or both. Further research is necessary to determine their nature. It is known however, that these crosslinkages are quite specific and stable and are an essential element of breadmaking quality (Orth and Bushuk, 1972; Tanaka, 1972).

The amino acid composition of the glutenin prepared by pH precipitation, indicates that it is highly pure compared to that prepared by other methods. Its relatively high glycine content, 9.27 mole percent, and low proline content, 12.46 mole percent, can be cited in support of this conclusion. Accordingly glutenin prepared by this method is consid-

ered suitable for studies of subunit composition by SDS-PAGE.

Qualitative differences in the SDS-PAGE patterns of the reduced glutenin of 26 bread wheats were not related to breadmaking quality. The subunit composition of these glutenins was genetically controlled and not affected by location of growth. These results are expected by comparison with analogous results on the other flour proteins. Strong genetic control and no environmental effect has been found for all flour proteins studied to date.

The subunit composition of synthetic and natural tetraploids differs markedly from that of hexaploid bread wheats. Absence of two of the highest molecular weight subunits is the most obvious characteristic of glutenin of the AABB tetraploid wheats. Since these wheats are generally low in breadmaking quality, the involvement of these two subunits in this quality is suggested. However, their presence is not a sufficient condition since all bread wheats, including those of very poor quality, contain them.

A possible way in which these subunits could affect the rheological and baking properties of bread wheats is by a specific involvement in the formation of the insoluble or residue protein (insoluble glutenin). Breadmaking quality, as measured by loaf volume, is directly related to the proportion of residue protein in the flour (Orth and Bushuk, 1972). These high-molecular weight subunits must occupy key positions in the glutenin molecule, allowing for strong inter- or intramolecular interactions. The nature of these interactions remains to be investigated.

Other evidence supporting the hypothesis of a central structural role for these subunits was reported by Huebner et al. (1973). Amino acid analysis of the highest molecular weight subunit, of a breadwheat glutenin, showed that it was relatively high in glutamic acid plus glutamine, glycine, and proline. By analogy with more detailed data on collagen, this composition would allow the formation of extensive helical regions in the subunit. Furthermore, the calculated maximum of two intramolecular disulfide bonds would permit the formation of an extended rather than a globular structure. A polypeptide of this nature would be capable of more extensive interaction with other subunits of glutenin than a globular protein.

The above hypothesis is supported by scanning electron micrographs of glutenins from various wheats. Glutenin from the bread wheat Manitou was very stringy, and comprised groups of parallel fibrils. Durum wheat glutenin, and that of the extracted tetraploid Tetracanthatch, had ribbon-like ultrastructures with very little parallel orientation of the ribbons.

The similarity of the ultrastructure of bread wheat glutenin and that of Aegilops squarrosa variety strangulata confirms the hypothesis that breadmaking quality was originally inherited from the D-genome progenitor of bread wheats.

Rye glutenin has short rod-like fibrils that would not be physically capable of the same degree of entanglement as that attained by the fibrils of bread wheat (Manitou) glutenin.

The ultrastructures of the glutenins are consistent with the known rheological properties of the doughs prepared from bread and durum wheat flours and rye flour. According to the micrographs, doughs from bread wheat flours should be considerably more elastic than doughs from either durum or rye flours. This is generally the case.

Finding that the long arm of chromosome 1D contains the genes that code for four of the glutenin subunits of Chinese Spring has a number of potential uses. The methodology developed can now be applied to nulli-tetra lines of other wheats to determine the location of genes that code the synthesis of subunits implicated in breadmaking quality. When these chromosomes are identified they can be synthetically introduced into cereal species of poor baking quality in the hope of improving them in this utilization property.

In addition, cytogeneticists could now use the presence of the four subunits as evidence of the presence of chromosome 1D of Chinese Spring, where it was suspected in genetic material. Similar "chromosome markers" are needed for other wheat chromosomes.

Although this study does not prove that all bread wheats have their high-molecular weight glutenin subunits coded by chromosome 1D, the study of four different extracted tetraploids indicated that they are coded by chromosomes of the D genome. Furthermore, the glutenins of six samples of Aegilops squarrosa all contained these subunits. A seventh Ae. squarrosa variety, meyeri, contained glutenin subunits of distinctly lower molecular weight than those found in bread wheats, and in the other varieties of Ae.

squarrosa. This indicates that any one of the three varieties, anathera, typica, or strangulata, could be, and that the variety meyeri is not, the D-genome progenitor of bread wheats.

Studies of the nullisomic-tetrasomic lines disclosed an extremely interesting phenomenon. When chromosomes 2B, 3B, and 6B were in the tetrasomic condition, glutenin subunits coded by A- or B- genome chromosomes were absent in the SDS-PAGE patterns of the reduced glutenins of these lines. The mechanism of this repression is unknown and will be difficult to determine since it could be operative at any one of a number of levels in the protein biosynthesis. Unexpected effects of this type illustrate the necessity of careful interpretation of results obtained with synthetically produced genetic material.

This thesis represents a significant contribution to studies of glutenin. In 1971, when this work was commenced, knowledge of the structure and genetics of glutenin was very sparse. Because of research projects at the U.S.D.A. Research Laboratories in Peoria, Illinois, and in our laboratory, this functionally important protein is now as well characterized as the other flour proteins.

CONTRIBUTIONS TO KNOWLEDGE

1. Three preparative methods for glutenin were evaluated by disc-gel electrophoresis and amino acid analysis. A pH precipitation procedure using the dissociating solvent AUC proved to yield a purer glutenin than either the Osborne fractionation or the gel-filtration techniques.

2. Low-molecular weight impurities in glutenin can be removed by overnite stirring of the glutenin with the ion exchanger SE-C50 in the solvent AUC. The impurities are complexed with the ion exchanger and then separated by centrifutition.

3. The subunit composition of glutenin of 26 wheats of diverse baking qualities was examined by SDS-PAGE. Although significant qualitative differences were evident between varieties, no relation with breadmaking quality could be established.

4. Location of growth had no observable effect on the SDS-PAGE patterns of the glutenin subunits of four varieties, each grown at four locations; genetically related varieties had very similar patterns, regardless of baking quality.

5. Using four hexaploid extracted tetraploid pairs, five glutenin subunits that are coded by the D genome were identified. These had molecular weights of 152,000, 112,000, 80,000, 60,000 and 45,000.

6. The glutenins of a synthetic hexaploid (AABBDD), and its extracted tetraploid (AABB) and diploid (DD) parents, were reduced and subjected to SDS-PAGE. The subunit composition of the synthetic hexaploid was simply inherited from its tetraploid and diploid parents. No new bands were evident.

7. The presence of high-molecular weight glutenin subunits is characteristic of bread wheats and, in particular, of the D genome. Accordingly these subunits may be a necessary condition for baking quality. These subunits are absent in durum wheat glutenin. This difference can be used to differentiate bread and durum wheats.

8. Glutenins of seven accessions of Ae. squarrosa were reduced to their subunits and studied by SDS-PAGE. Four varieties were represented by these accessions, meyeri, strangulata, anathera, and typica. The first variety, meyeri, contained two glutenin subunits of high-molecular weight that were distinctly of lower molecular weight than the equivalent subunits of bread wheats studied. The other three varieties had subunits of the same molecular weight (by SDS-PAGE) as those in bread wheats. On the basis of this evidence, any one of the three could be the progenitor of the D genome of bread wheats.

9. Scanning electron microscopy of glutenins of two varieties of bread wheat, one variety of durum wheat, a synthetic hexaploid (AABBDD) wheat, and its tetraploid (AABB) and diploid parents, and one variety of spring rye indicated marked differences in their ultrastructure. The bread wheat glutenins were fibrous and stringy whereas those of the tetra-

ploids were composed of ribbon-like strands Squarrosa (DD diploid) glutenin appeared fibrous like that of the bread wheats glutenin indicating that the latter inherited its fibrous character from the D-genome progenitor. Rye glutenin ultrastructure was characterized by very short rods. The observed differences in structure can be related to the rheological properties of doughs prepared from the flours investigated

10. Intact glutenin from one variety of bread wheat (Manitou) had a highly ordered fibrous ultrastructure as determined by scanning electron microscopy. Reduction with β-mercaptoethanol transformed this structure into an amorphous mass with no ordered structure.

11. Using aneuploid lines of Chinese Spring, chromosome 1D was identified as the location of the genes that code the synthesis of four glutenin subunits. These four include the two high-molecular weight subunits that appear to be necessary for breadmaking quality.

12. Experiments with ditelocentric lines, showed that the genes for these subunits are located on the long arm of chromosome 1D in Chinese Spring.

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