NATURE OF THE ENDOSPERM PROTEINS OF THE EXTRACTED TETRAPLOIDS DERIVED FROM THREE COMMON WHEAT VARIETIES

A Thesis

Submitted to

The Faculty of Graduate Studies and Research

The University of Manitoba

by

Bernard Leo Dronzek

In Partial Fulfilment

of the Requirements for the Degree

Doctor of Philosophy

From the Protein Chemistry Laboratory

Department of Plant Science

Under the Supervision of

Professor W. Bushuk

February 1970

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. W. Bushuk for his encouragement and direction during the course of the research and in the preparation of the thesis and to Dr. P. J. Kaltsikes for supplying the grain samples.

Special thanks are also due to Dr. G. N. Irvine for making the facilities of the Grain Research Laboratory available for the amino acid analyses.

The financial support given by the National Research Council of Canada is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Dronzek, Bernard Leo, Ph. D., The University of Manitoba, February 1970. Nature of the Endosperm Proteins of the Extracted Tetraploids Derived from three Common Wheat Varieties.

Major Professor: Dr. W. Bushuk

The protein compositions of AABB tetraploid wheat derived from three common wheat varieties, Prelude, Rescue and Thatcher, were compared with those of the hexaploid counterparts. The proteins were fractionated by solubility in appropriate solvents into albumins, globulins, gliadins, glutenins and insoluble residue proteins. The solubility characteristics showed that the tetraploid wheats of Rescue and Thatcher contained considerably more gliadin, more glutenin and less insoluble protein residue than the corresponding hexaploids. In contrast, Tetraprelude showed similar amounts of gliadin, glutenin and insoluble protein residue compared to its hexaploid.

Disc and starch-gel electrophoresis were used to separate and correlate the proteins in the four soluble fractions from related wheats. The electrophoretic patterns showed significant qualitative differences among the varieties studied, although the patterns of each extracted tetraploid and its hexaploid counterpart were quite similar.

Amino acid compositions of the flours for each extracted tetraploid and its hexaploid parent showed that the composition are essentially the same. The amino acid composition varied significantly among the solubility fractions; however, the differences for any

one fraction among varieties were insignificant.

In addition to the extracted tetraploids and their parents, the proteins of five accessions of Ae. squarrosa, a synthetic hexaploid and its durum parent were studied. Differences in the electrophoretic patterns were found in the slow-moving gliadin proteins among the five accessions of Ae. squarrosa. Similar patterns were obtained for the synthetic hexaploid wheat and its durum wheat parent.

I INTRODUCTION

Common hexaploid wheat with the genomic formula AABBDD is an allopolyploid wheat. It has been established that the original ancestors of the three genomes A, B and D are the three diploid species T. monococcum, Ae. speltoides and Ae. squarrosa respectively (1). It is presumed that common hexaploid wheat originated through two widely separated evolutionary events. First, the AABB amphiploid resulted from a natural hybridization of the diploid species T. monococcum, and Ae. speltoides. Much later, the D genome of Ae. squarrosa was added, also by natural hybridization. Since both the durum (2n=4x=28=AABB) and the diploid wheats (2n=2x=14=DD) lack the breadmaking quality of the hexaploid bread wheats, the final addition of the D genome chromosomes appears to have contributed the genes for this quality.

Most of the wheat produced in the world is of the common hexaploid type, T. aestivum L. em Thell. (2n=6x=42=AABBDD). It is used primarily in the manufacture of flour for leavened baked products. For many centuries this crop has served as the basic food of man. Since wheat has been of such great economic importance to man himself, a great deal of effort has been devoted to research designed to answer the basic question: what properties of flour constituents determine its breadmaking quality?

It is now well established that the most important single constituent of bread wheat flour associated with its baking quality is its protein. Moreover, it has been recognized for some time that the breadmaking quality is determined by the very complex nature of the proteins which interact to form gluten.

In addition to the peculiar quality factors of the bread wheat proteins which give rise to baking quality, a relatively high quantity of protein is required to produce good bread (2).

In recent years, with the advent of various electrophoretic techniques, numerous workers have tried to associate differences in electrophoretic patterns obtained for various wheat varieties to differences in their breadmaking potentialities (3-5). Although marked differences in the patterns were observed, no direct relationship of particular protein bands to quality has been found. Thus, the genetic control of these proteins, and their relationship to the complex characteristic quality are far from being understood.

The extraction of the AABB components from three common wheat varieties Prelude, Rescue and Thatcher was recently accomplished in the Department of Plant Science, University of Manitoba. Breadmaking quality studies on the extracted tetraploid lines have demonstrated the importance of the D genome in the inheritance of breadmaking quality (6). The breadmaking quality of two of the extracted tetraploids, Rescue and Thatcher, was relatively poor, and not too unlike that of normal durum wheats. The tetraploid of Prelude showed better breadmaking quality than the other tetraploid lines, and was comparable to the three hexaploid wheats used as parents.

The genetic materials used in the present study were the three AABB tetraploid wheats derived from common bread wheats hexaploids , Prelude, Rescue and Thatcher. They will be referred

to as Tetraprelude, Tetrarescue and Tetrathatcher respectively. The purpose of the study was to compare the proteins of the three extracted tetraploids and their hexaploid parents: 1) to determine if the removal of the D genome produced a deletion of certain proteins or a shift in the quantitative distribution of the proteins components which could be attributed to the loss of breadmaking quality, and 2) to determine if the proteins of Tetraprelude, "the durum type wheat with the high breadmaking quality," were similar to those of its hexaploid parent. Stewart 63 was included in the study as the representative of the natural tetraploid wheats with AABB genomic constitution.

In addition to the detailed investigation of the proteins of the extracted tetraploids and their parents, the proteins of two aneuploid lines involving chromosomes of the D genome of the common wheat variety, Chinese Spring, and five accessions of the species Ae. squarrosa and a synthetic AABBDD hexaploid were examined by gel electrophoresis. It was presumed that a study of these would provide additional information on the proteins whose synthesis is controlled by the D genome.

II LITERATURE REVIEW

1. Introduction

Wheat proteins have been of great interest to cereal chemists for over sixty years. This constituent of bread wheat flour determines to a large extent the physical properties of the dough, and in particular its breadmaking potential. It is generally accepted today that breadmaking quality is related primarily to the water-insoluble proteins of the flour which are collectively called gluten. In hydrated state, gluten, with its unique viscoelastic properties, forms the supporting three-dimensional network of the dough that is produced in the first step of the breadmaking process.

Berzelius in 1728 (7) was the first to describe the separation of gluten from wheat flour, but it was not until 1907 that Osborne (8) fractionated and classified these proteins on the basis of their solubility. He classified the proteins into four broad groups: 1) water-soluble proteins or albumins; 2) salt-soluble proteins or globulins; 3) alcohol-soluble proteins or gliadins; and 4) proteins soluble in dilute acid or base, the glutenins. Moreover, Osborne reported that gluten comprised about 80% of the proteins in flour. Gluten comprised two main groups of proteins, gliadins and glutenins, present in nearly equal amounts. With the advent of modern techniques of protein chemistry such as the analytical ultracentrifuge and electrophoresis, it was soon shown that each of the four protein fractions suggested by Osborne was a heterogenous mixture of a

large number of molecularly different proteins.

2. Electrophoresis Studies of Wheat Proteins

Electrophoretic separation of proteins was first reported by Tiselius in 1937 (9). However, this technique was not applied to the separation of the wheat proteins until 1944 (10). In recent years, both moving-boundary and zone electrophoresis have been successfully applied to studies of cereal grain proteins. Zone electrophoresis requires the use of various physical support materials such as filter paper or gels on which the protein migrates while in moving boundary electrophoresis, the proteins move freely in a buffer solution.

Filter paper and agar gels were the first support materials used for separation of proteins by zone electrophoresis (11-12). However, these materials have found very limited application in the separation of cereal proteins. A major development in zone electrophoresis occurred in 1955 when Smithies introduced the use of starch gels as support material for the separation of serum proteins (13). The resolution obtained by starch-gel electrophoresis was much superior to that obtained by other types of zone electrophoresis. Elton and Ewart (14) were the first to use this support material, in conjunction with the aluminum lactate buffer system of Jones et al (15), for the separation of flour proteins. In 1961 Woychik et al (16) improved the separation of flour proteins by starch-gel electrophoresis by the addition of urea to the gel

to increase the solubility of the proteins.

Polyacrylamide gels were introduced for zone electrophoresis by Raymond and Wang in 1960 (17) and adapted to wheat proteins by Lee in 1962 (18). In contrast to starch gels, polyacrylamide gels are transparent, thermostable, non ionic, and their pore size can be readily varied over a wide range by simply changing the concentration of acrylamide used to prepare the gels (19). The electrofocusing technique for separating wheat proteins recently developed by Wrigley (20) also employs polyacrylamide gels as the supporting material.

3. Water and Salt-Soluble Proteins

The water-soluble proteins or albumins and the salt-soluble proteins or globulins of wheat are collectively referred to as the soluble proteins. The first comprehensive study of these proteins was that of Osborne (8). Subsequent investigations prior to 1944 have been reviewed by Bailey (21). In recent years, Pence and coworkers (22-24) have extensively studied these proteins. Most of the early findings were subsequently modified and refined by new techniques of protein chemistry.

Finney (25) reported that the water-solubles were necessary for normal baking performance in two of his three reconstituted flours. In 1951, Pence et al (22) found that water-soluble components from flours with widely different baking characteristics were required for maximum performance of flours reconstituted from all glutens, except the one from durum wheat. A crude albumin

component isolated from the water-solubles produced a positive volume response, and reduced the mixing time. In 1962 Pence (26) concluded that albumin proteins are implicated in the baking performance of the flour. Furthermore, he suggested that differences in soluble constituents might account for the variations in baking quality of different flours.

Recently, Hoseney et al (27) reported that the water-soluble fraction of the flour was not involved in breadmaking quality differences among varieties, although it was required to produce a normal loaf of bread. It was found that the water-solubles contributed to gas production and affected the physical properties of the dough. However, the albumins and globulins were not involved in the breadmaking quality since removal of these proteins from the water-soluble fraction did not decrease the loaf volume of the bread baked from reconstituted flours. On the basis of their reconstitution studies, Hoseney et al (27) suggested that soluble pentosans and glycolipids were the water-soluble constituents that were essential for baking performance.

There have been numerous studies of the physicochemical properties of the soluble proteins of wheat. Laws and France (28) reported in 1948 that a water extract from three flours of different breadmaking quality gave similar patterns in moving boundary electrophoresis. In 1953, Pence and Elder (23) separated the albumins by paper electrophoresis into at least six individual components of similar molecular size. The albumins were characterized by a

higher tryptophan content, but a lower amide nitrogen content than the other wheat proteins. The albumins had an apparent molecular weight of about 28,000 in dilute salt solution, but in the presence of urea or sodium salicylate the molecular weight decreased to about 20,000. A subsequent study by Pence (29) indicated that as many as eleven water-soluble protein components could be detected by paper electrophoresis. The approximate isoelectric points of the eleven individual components varied from 4.5 to 8.7. In 1954 Pence et al (30) demonstrated distinct differences between the compositions of the albumins of durum and common wheat flours. These differences in component distribution appeared to be unaffected by differences in growth location and crop year.

In 1954 Pence et al (24) determined the soluble protein content of 32 flours of widely different type and baking quality. The total soluble protein contents varied in the range, from 13 to 22% of the total flour protein. The amounts of albumins were generally slightly higher than the globulins. Both the albumin and globulin contents, as well as the ratio of the two, varied significantly among the flours.

More recently, Holme (31) separated the albumin fraction from cake flour into at least five components by moving boundary electrophoresis. Elton and Ewart (32) examined the albumins and globulins from 8 wheat varieties by starch-gel electrophoresis. No significant differences were observed in their electrophoretic patterns. Woychik et al (16) separated the water-soluble proteins into at

least 9 components on starch gel in the presence of urea. Later, Elton and Ewart (33) improved the resolution of their starch-gel electrophoresis technique and reported some minor differences in the electrophoretic patterns of the water-soluble proteins from several wheat varieties. In 1963 Nimmo et al (34), using polyacrylamide-gel electrophoresis, found at least 15 protein components in a water extract of bread wheat flour.

Silano et al (35) used disc electrophoresis to analyze the albumin and globulin fractions of several varieties of T. aestivum and T. durum. Fourteen albumin and 15 globulin bands were observed. Varietal differences were found, particularly in the fast moving albumin bands.

Recently, Feillet and Nimmo (36) isolated and characterized two albumins (13A and 13B) from bread flour. Albumin 13B had a relatively high valine content, and no histidine or phenylalanine while albumin 13A contained all the common amino acids and had a relatively high content of alanine. No free sulfhydryl groups were found in either protein. Molecular weights of albumin 13A and albumin 13B were 24,800 and 13,950, respectively, determined from sedimentation-equilibrium data.

Relatively little is known about the salt-soluble proteins (globulins) of wheat flour. So far, no functional role has been attributed to these proteins, although Pence (26) suggested that they may be involved in breadmaking performance. Pence (26) was unsuccessful in his attempts to reconstitute flour from which globulins had been removed. It was not possible to ascertain

whether it was the deletion of the globulins that produced the observed detrimental effect or whether the salt used in the extraction of the globulins damaged the protein system of the flour.

In 1949 Danielson (37) observed two distinct globulins, gamma and alpha, in salt solution extracts of flour by means of the analytical ultracentrifuge. According to this investigator, gamma globulin has a molecular weight of about 210,000. Three different globulin components were found by Pence and Elder (23) in preparations from flour and defatted wheat germ. These globulins were characterized by relatively low tryptophan and amide nitrogen contents, and a relatively high arginine content when compared with the total flour protein. Total globulin content of wheats of different types ranged from 5 to 11% of the total flour protein. Elton and Ewart (32) detected two globulin bands which migrated most rapidly in starch-gel electrophoresis of dialyzed salt extracts of flour. Recently, Fisher et al (38) identified the fast-moving globulin doublet found by Elton and Ewart (32) as purothionin.

4. Gluten or Insoluble Proteins

Finney demonstrated in 1944 (25) that wheat gluten is the fraction of flour that is primarily responsible for its breadmaking quality. Since that time, many attempts have been made to detect differences in the gluten fraction that could be related to differences in baking quality of the flour from which the gluten was obtained. The separation and characterization of the gluten

proteins has been hampered by the lack of suitable solvents capable of solubilizing all the proteins comprising gluten. A major advance was made in 1959 when Jones et al (15) discovered that aluminum lactate buffer of pH3.1 could be used in electrophoresis to separate gluten proteins.

Gluten is normally prepared by washing dough in a stream of water or a dilute salt solution until the starch and solubles are removed. Crude gluten contains about 85% protein, 8.3% lipid, 6% starch, and 0.7% ash, (39). The protein component of gluten is mainly gliadin and glutenin present in approximately equal amounts.

The unique elastic and cohesive properties of doughs are usually associated with analogous properties of the glutenin fraction (15). The glutenin group appears to be a mixture of a number of molecular species which contain a wide range of molecular sizes with the molecular weight ranging from 50,000 to over 2 million (40). Reduction of the glutenin disulfide groups decreases its molecular weight to approximately 20,000 (41).

In starch-gel electrophoresis, glutenin does not migrate into the gel due to its large size (16), although, upon reduction it can be separated into approximately 20 components (42). Seven of the components of reduced glutenin migrated at the same rate as components of reduced gliadin suggesting that the gliadins might be linked by S-S bonds to form glutenin. Elton and Ewart (43) reduced the glutenin from four wheats and separated the components on starch gel. The electrophoretic patterns showed that there were

varietal differences among the reduced glutenins. Huebner et al (44) compared the glutenins isolated from five different classes of wheat. Electrophoretic patterns of the reduced-alkylated glutenins showed significant differences among varieties of the same class, but the greatest differences were among different classes of wheat. Reduced-alkylated glutenin from durum wheat contained few or none of the slow moving components present in the bread wheat glutenins that were examined.

Gliadin, the alcohol-soluble proteins of wheat forms an integral part of gluten and plays an important role in determining the breadmaking potentialities of flour (45). It is highly heterogeneous; the actual number of revealed components depends on the analytical technique used for their separation. Its average molecular weight by sedimentation-diffusion was found to be about 45,000 (46) and its isoelectric point at a pH of about 6.5. Gliadin group of proteins is characterized by extremely high glutamine and proline contents (47). It has a much lower intrinsic viscosity than the glutenins probably because of its smaller size and a more compact globular conformation (48). There is considerable evidence from studies of reduced gliadin that its disulfide bonds are intramolecular (49).

Schwert et al (10) found no differences in the electrophoretic patterns of gliadins from a variety of flour prepared by a number of different methods. Laws and France (28) reported that no significant differences could be detected by moving boundary electro-

phoresis of wheat gluten proteins including gliadins derived from different wheats. In 1954 Mills (50) reported that at least four protein components were present in the gliadin group.

Jones et al (15) used aluminum lactate buffer solution in conjunction with moving boundary electrophoresis to separate gluten proteins into four major and one minor components. Further examination of the electropherograms from four bread wheats (two with good and two with poor baking qualities) showed the same protein composition. However, glutens from two durum wheats, examined similarly, showed quite different electropherograms.

In 1961 Cluskey et al (51) separated by moving boundary electrophoresis protein components of gluten and water solubles from hard and soft wheat flours. On the basis of electrophoretic mobility they designated the gluten proteins into five components identified by Greek letters alpha, beta, gamma, omega, and delta. Hard wheat flours contained more alpha, gamma, and omega components but the same amounts of beta and gamma, the fast moving components. They were able to distinguish between hard and soft bread wheats on the basis of protein composition, although the differences were small.

Woychik et al (16) resolved gluten proteins into nine distinct components by starch-gel electrophoresis in the presence of urea. Eight of the nine components were attributed to the gliadin group. In 1962 Elton and Ewart (32) reported significant differences in

the starch-gel electrophoresis patterns for gluten proteins of different bread wheats. They attributed these to real differences in the proteins. It was suggested that the observed differences might be sufficient to account for the differences in breadmaking quality of the wheats examined. Later the same authors (33) showed that both qualitative and quantitative differences existed between starch-gel the patterns or the gliadins from several wheat varieties.

Lee and Wrigley (5) compared the gluten proteins of eight commercial wheat varieties and four tetraploid wheats by ion-exchange column chromatography and by polyacrylamide-gel electrophoresis. Definite differences in protein profiles for the commercial wheats and tetraploid wheats were observed. However, it was not possible to relate a type of chromatographic pattern to baking quality of the eight commercial wheat studied.

In 1963, Graham (52) using an improved apparatus and procedure for starch-gel electrophoresis, showed that similar protein components were extracted with widely different solvents. Similar patterns were obtained for proteins from similar genotypes while different genotypes showed marked differences in the slow moving or gliadin components. Using the same technique, Coulson and Sim (3) examined the proteins from 34 different varieties of T. aestivum. Major differences in protein patterns were found in the gliadin proteins while the patterns for the fast moving albumin and globulin components were similar for all varieties examined.

Furthermore, there was no obvious correlation between the proteins patterns and the physical or baking-quality characteristics of the flour.

Lee and Ronald (53) studied the effect of environment on wheat gliadin in four T. aestivum varieties grown in Australia under a wide range of growing conditions. Varietal differences in the distribution of gliadin components were found to be much more significant that the differences that could be attributed to environmental factors.

More recently Huebner et al (54) compared the compositions of the gliadin group of proteins from a number of varieties of common, durum, and club wheats by column chromatography and by starch-gel electrophoresis. Greatest differences were observed for varieties representing different classes while differences among varieties of the same class were small.

Using starch-gel electrophoresis, Doekes (4) classified 80 varieties and selected lines of wheat into 5 main groups having similar electropherograms. Group I comprised soft winter wheats with poor baking quality while group V included hard red wheat varieties of relatively good breadmaking quality. Furthermore, group I varieties showed a compact gliadin pattern while group V displayed a gliadin pattern in which the components showed a broad range of mobilities. Groups II, III and IV included

varieties with diverse origins, and considerable variation in baking quality. Their gliadin patterns were intermediate between those of group I and V.

5. Amino Acid Composition

Recent development of highly efficient and rapid analytical techniques for determination of amino acid composition of proteins has produced accurate amino acid analyses of wheat proteins. Although, these data have been extremely useful in relation to nutritional quality of wheat proteins, they are of limited value in relation to the breadmaking quality of wheat varieties (2). Amino acid compositions for a wide variety of wheats that differ widely in baking quality were found to be essentially the same (55).

Glutamine is the predominant amino acid present in wheat proteins, and proline is also present in relatively high amounts (56). Together they make up approximately 50% of the amino acids in gluten proteins (55). Isoleucine, leucine, phenylalanine, serine, tyrosine and valine contents are relatively high in gluten compared to other proteins (55). The soluble proteins generally contain more cystine, tryptophan, basic amino acids and glutamic acid than the gluten proteins (57). The albumins contain more cystine and cysteine than the globulins or gluten proteins.

Glutamic acid and proline contents have been shown to increase, while lysine tends to decrease as the protein content of wheat increases (55). Lawrence et al (58) found that lysine increased

significantly as the protein content decreased below 13.5%. However, for wheats with more than 13.5% protein, there was no significant correlation between lysine and protein contents.

In 1961 Woychik et al (59) determined the amino acid compositions of six protein components isolated from wheat gluten. While their compositions were similar, significant differences were observed which indicate the individuality of these proteins.

Ewart (60) compared the amino acid compositions of the glutenins and gliadins from two strong and two weak wheats. The compositions of the glutenins were similar to those of the gliadins. There were minor differences in the amounts of some amino acids. No significant differences in composition were observed for varieties of the same class or for strong and weak wheats.

Recently, Tkachuk (56) determined amino acid composition of six flours which differed widely in their breadmaking quality. The compositions for the six flours were essentially the same.

6. Genetic Inheritance of Protein Components in Bread Wheat and Related Species

In recent years, a large number of reports have appeared in the literature of attempts to relate electrophoretic or chromatographic patterns for the proteins to genetic relationships among wheats and related grain species. For example, Johnson and Hall (61) used polyacrylamide-gel electrophoresis to obtain evidence of the genetic

origin of bread wheat. The electrophoretic patterns for extracts from T. monococcum, T. dicoccum, and T. aestivum showed that the A and B genomes contributed different proteins. Some evolutionary divergence had apparently occurred in the A genome of T. dicoccum since the proteins of this species were only partially homologous with those of T. monococcum.

Hall et al (62) examined the genome relationships in the Triticinae in terms of protein homology based on electrophoretic patterns. Different degrees of homology were found for the A, B, and D genomes. In 1967, Johnson et al (63) compared the disc electrophoretic patterns for protein extracts from 26 hexaploid and 64 tetraploid wheats. Homology was observed for nine albumin bands in the two groups of wheat examined. Three additional albumin bands in the hexaploids were attributed to the D genome. In addition, differences in the patterns for the slow moving protein bands of the gliadin group were found among (the tetraploid species, and also among the hexaploid subspecies studied) varieties within each group.

The identification of wheat chromosomes involved in the control of breadmaking quality has been hampered by the polyploid nature of wheat. Accordingly, aneuploid and substitution lines have been used to determine the chromosomes which control flour properties that are important in baking quality. A number of chromosomes, mostly of the B and D genomes, have been reported to be involved in the genetic control of breadmaking quality (64-66).

Welsh and Hehn (64) examined the quality of the complete set of monosomic lines of the hard red winter wheat, Kharkof MC22. They observed that chromosome 1D resulted in a drastic reduction of the doughball fermentation time (Pelshenke Test) and an extreme weakening of the farinograph curve.

Morris et al (65) and Schmidt et al (66) studied chromosome substitution lines of the hard red winter variety Cheyenne into the variety Chinese Spring. Mixograph and bake tests indicated that chromosomes 1B, 4B, 7B and 5D were primarily responsible for the high baking quality of Cheyenne. Recently, Welsh et al (67) examined the milling and baking qualities of the complete sets of substitution lines of three hard red spring wheat varieties, Thatcher, Hope and Timstein with Chinese Spring as the common background variety. Only chromosome 2A of Thatcher produced a marked change in the baking properties of the flour from the background variety. On the other hand, 11 Hope and 16 Timstein chromosomes produced notable change. Chromosome 2B of Hope and chromosomes 3B and 6B of Timstein had the greatest effects. On the basis of current state of knowledge, it appears that the genes for breadmaking quality are located on a number of different chromosomes.

There have been a number of reports on the effects of additions or removals of genomes, and of specific chromosomes on the proteins of bread wheats and related species. In 1967, Boyd and Lee (68) reported similar patterns were obtained by starch-gel electrophoresis for protein extracts from single seeds of the

hexaploid wheat Canthatch and its extracted AABB tetraploid, Tetracanthatch. In a subsequent paper, Boyd and Wrigley (69) reported that the AABB Tetracanthatch lacked at least 4 protein bands present in its hexaploid parent. It was indicated that in the earlier study, two samples of the hexaploid parent variety instead of the tetraploid and the hexaploid were examined due to a labelling error. It appears, therefore, that removal of the D genome from the hard red spring wheat variety Canthatch decreases the number of protein components.

In a study of the appropriate ditelocentric aneuploid lines of Chinese Spring, Boyd and Lee (68) showed that removal of the 1D chromosome resulted in a loss of two slow moving protein bands in starch-gel electrophoresis patterns. With the same technique Shepherd (70) examined the endosperm proteins of compensating nullisomic-tetrasomic lines of Chinese Spring and found that nulli 1D-tetra 1A and nulli 1D-tetra 1B lacked two similar slow moving bands. These results agreed with the previous suggestion of Boyd and Lee (68) that the genes for the two missing proteins are on chromosome 1D. Of the 33 nullisomic-tetrasomic lines of Chinese Spring studied by Shepherd (70), 9 of the 17 major protein band of Chinese Spring were accounted for by the removal of individual chromosomes. Presumably the synthesis of the remaining eight components is controlled by diploid or triploid factors on more than one chromosome pair.

Further evidence of the importance of the D genome, and specifically the 1D chromosome, of hexaploid bread wheats to bread-

making quality was reported by Kaltsikes et al (6). For this study, the AABB tetraploid components were extracted from three hard red spring wheat varieties, Prelude, Rescue, and Thatcher. The bread-making qualities of the extracted tetraploid of Rescue and Thatcher were relatively poor, and not too unlike that of normal durum wheats. On the other hand, tetraploid Prelude showed much superior bread-making quality than the other two extracted tetraploids, and was comparable in quality to the three hexaploid parent varieties. This higher quality of Tetraprelude was attributed to two factors: 1) a somewhat higher protein content; and 2) a translocation involving the long arm of chromosome 1D, to a chromosome of either the A or the B genome.

Recent studies of Kerber and Tipples (71) provide further evidence of the importance of the D genome in controlling the baking quality of bread wheats. Results of this study were similar to those of Kaltsikes et al (6). Extracted AABB tetraploid of Canthatch had extremely poor baking quality compared with the hexaploid Canthatch. Five synthetic hexaploids produced by combining the extracted AABB tetraploid of Canthatch with different varieties of Ae. squarrosa which supplied the D genome were superior in baking qualities than the extracted tetraploid but still considerably inferior to Canthatch.

7. Proteins of Alien Genome Combinations

Yong and Unrau (72) used starch-gel electrophoresis to compare the proteins of hexaploid AABBRR Triticale (durum wheat x rye) and

its parental species. These authors reported that the synthetic hybrid had four "new" proteins not present in the parents. It was postulated that the "new" proteins resulted from an interaction between the alien parental genomes. Barber et al (73) compared the esterases of one line of Triticale and its two parents, and found one "new" esterase band in the synthetic amphiploid.

The proteins of the same strain of Triticale and its parents as used by Yong and Unrau (72) were recently re-examined by Chen and Bushuk (74) using a more elaborate separation and electrophoretic technique. No "new" proteins were detected. Chen and Bushuk (74) concluded that the proteins in the Triticale studied were directly inherited from its parental species. Similar results were obtained by Johnson and Hall (61) who examined the proteins of an octaploid AABBDDRR Triticale and its two parental species, T. aestivum (AABBDD) and S. cereale (RR). The polyacrylamide-gel electrophoresis pattern for the Triticale was essentially the same as the pattern for a mixture of extracts from the parent species durum wheat and rye. The discrepancy between the results of Yong and Unrau (72) and Barber et al (73) on the other hand, and those of Johnson and Hall (61) and Chen and Bushuk (74) on the other remains to be clarified.

III MATERIALS

Three common bread wheat varieties Prelude, Rescue and Thatcher, their extracted AABB tetraploids Tetraprelude, Tetrarescue, and Tetrathatcher, and a durum variety, Stewart 63, were used in this investigation. The tetraploids were produced by Kaltsikes (75) using the back-crossing technique developed by Kerber (76). Genetic extraction of the tetraploids from hexaploid wheats was accomplished as follows. The variety Prelude will be used to illustrate the technique. First a cross was made between the common wheat variety Prelude and the durum wheat variety Stewart 63. The resulting pentaploid was backcrossed several times to Prelude, which after selfing gave a tetraploid wheat with the AA and BB genomes derived from Prelude. The seven wheats used in this part of the study were grown together in 1967.

The materials used in the second part of this study, were the disomic parent and the aneuploid lines nulli 1D-tetra 1A and nulli 1D-tetra 1B, of the common wheat variety Chinese Spring, a synthetic AABBDD hexaploid wheat[T. durum (Iumillo x *Ae. squarrosa*)] and five species of *Ae. squarrosa*, one of the diploid progenitors of common wheat. The seeds of Chinese Spring and its aneuploids were provided by Dr. Feldman of the Plant Science Department, University of Manitoba, while the *Ae. squarrosa* seeds and the synthetic hexaploid were part of the University of Manitoba collection. Since there was not sufficient material of this group of samples to mill into flour, their proteins were extracted from single seeds.

IV METHODS

1. Preparation of the Flour

The wheat (200 g.) was tempered to 15.5% moisture and milled into flour on a Buhler experimental mill. The flour was stored at 4°C in sealed containers.

2. Extraction of the Proteins from Flour

Flour proteins of the extracted tetraploids and their hexaploid counterparts were fractionated by the classical technique of Osborne (8). By this procedure five broad fractions are obtained: 1) water-soluble proteins or albumins; 2) salt-soluble proteins or globulins; 3) alcohol-soluble proteins or gliadins; 4) acetic acid-soluble proteins or glutenins; and 5) the insoluble-residue proteins. The fractionation procedure is summarized in Fig. 1.

All extractions were in duplicate and were made in a cold room at 4°C to minimize enzymic activity and the possibility of denaturation. The flour-solvent slurry was continuously stirred in 250 ml. centrifuge bottles with a magnetic stirrer throughout the extraction period. At the end of each extraction period, the mixture was centrifuged at 3,000 rpm. (1860xg.) for 30 minutes. The supernatant was removed and the residue was extracted as above with the subsequent extraction solvent.

3. Water-and Salt-Soluble Proteins

Initially, 10g. (dry weight basis) of flour was extracted with 70 ml. of 0.5M sodium chloride (salt) solution for 2 hours,

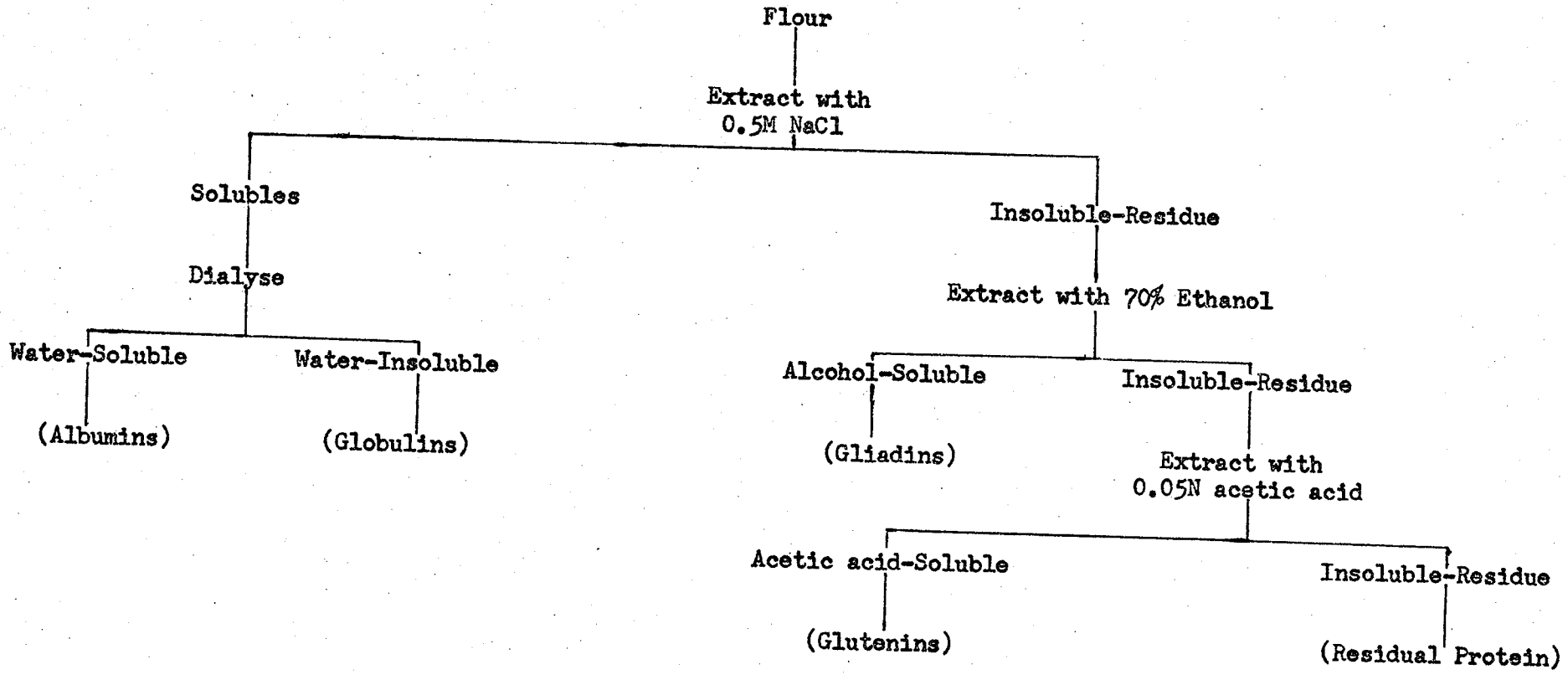


Fig. 1. Solubility fractionation of the flour proteins

followed by a second extraction with 40 ml. of 0.5M salt solution for 1 hour, and a third extraction with 40 ml. of water for 1 hour to remove the residual salt. The three supernatants were combined and dialysed against 4 liters of distilled water for 48 hours. Four changes of fresh distilled water were used in the dialysis. After dialysis, the suspension was centrifuged for 30 minutes to separate the precipitated salt-soluble proteins. The precipitate was washed several times with water, and recentrifuged. The supernatants obtained from all the centrifugations were combined. This contained the water-solubles which will be designated as the water-soluble fraction. The remaining precipitate will be referred to as the salt-soluble fraction.

4. Extraction of Alcohol-and Acetic Acid-Soluble Proteins

The residue obtained from the salt solution and water extractions described above was extracted for 2 hours with 70% ethanol solution, followed by a second 1 hour extraction with 40 ml. of the same ethanol solution. The supernatants obtained were transferred into a 250 ml. round bottom flask. The alcohol was removed from the supernatants under reduced pressure on a rotary evaporator. This fraction will be designated as the alcohol-soluble fraction.

The residue remaining after the ethanol extraction was extracted for 2 hours with 70 ml. of 0.05N acetic acid solution followed by a second extraction for 1 hour with 40 ml. of 0.05N acetic acid solution. The two supernatants were combined to give the acetic acid-soluble fraction. The remaining material will be

referred to as the insoluble-residue fraction.

All five fractions obtained were transferred into weighed trays and frozen. The samples were lyophilized, and the weights of the dried materials were determined for calculation of the yield of various fractions. The dried fractions were transferred into plastic air-tight bottles and stored in a desiccator at -20°C.

5. Determination of Protein Content

Protein contents (N x 5.7) of the flour and the various solubility fractions were determined using the standard macro Kjeldahl and micro Kjeldahl procedures respectively.

6. Starch-gel Electrophoresis

Starch-gel electrophoresis was carried out in the presence of 2M urea in 0.1μ aluminum lactate buffer of pH 3.2. Aluminum lactate was prepared according to Jones and Cluskey (77). To prepare the gel, 65 g. of hydrolyzed starch (Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada) was suspended in 550 ml. of the buffer-urea solution. One ml. of 1% ethylmercurithiosalicylic acid, sodium salt (Thimerosal) was added to the gel mixture to inactivate beta amylase activity during electrophoresis (78). The mixture was heated in a water bath with mild continuous stirring until the temperature reached 75°C. Vigourous stirring was avoided since this produced large air bubbles in the slurry which, on cooling, gave a non-uniform gel. When the temperature reached 75°C. the liquid was poured into a 12 x 30 x 0.6 cm. plexiglass gel tray. The sample slots in the gel were cast using a six-position slot former placed at one end of the tray. The

starch slurry was allowed to cool for 2 hours and then stored in a cold room at 4°C. for 22 hours. The following day, the slot former was carefully lifted from the gel. Finally, the excess gel was removed from the top of the plate by slicing with a cutter made from a thin stainless steel wire.

For the electrophoresis experiment, 0.2 ml. of a 1% protein solution or extract from a single seed was applied to each slot and the slot covered with a glass cover slip. During the experiment, the entire gel was covered with saran wrap to prevent excessive drying out.

The horizontal starch-gel arrangement was used in the entire investigation. The gel was connected to the buffer solutions in the electrode boxes by several sheets of Whatman No. 1 filter paper. Electrophoresis was conducted in a cold room at 4°C. to prevent build up of excessive heat. Each run was for six hours at a potential of 6 volts per cm. At the conclusion of the electrophoresis, the gel was carefully removed from the tray and the top 2mm. of the gel was sliced off with the wire cutter and discarded. The lower part of the gel was stained overnight in 200 ml. of 2% trichloracetic solution containing 10 ml. of 0.7% nigrosin solution. The gel was removed from the staining solution, washed with water, and photographed.

7. Disc Electrophoresis

Disc electrophoresis on polyacrylamide gel was carried out in manner similar to that of Davis (79). The apparatus used was

purchased from Buchler Instrument Inc. In this apparatus, 12 tubes can be run simultaneously. Stock solutions for preparing the gels were made up as shown in Table 1.

The lower gel was prepared by mixing 0.3 ml. of acrylamide solution, 0.6 ml. of buffer solution and 0.3 ml. of catalyst solution per tube. This gave concentration of 7.5% of acrylamide. The gel mixture was covered with a layer of water and photopolymerized for 30 minutes. Excess water was decanted.

The upper gel, comprising 0.1 ml. of buffer solution, 0.05 ml. of acrylamide solution and 0.05 ml. of catalyst solution per tube, was photopolymerized for 15 minutes. After polymerization, the tubes were placed into the upper chamber of the electrophoresis apparatus. The protein solution (25 to 50 μl of a 1% (W/V) in 15% sucrose solution) was then added to each tube. The upper or the anode chamber contained 500 ml. of glycine-acetic acid buffer solution of pH 4.0 while the lower or the cathode chamber was filled with 500 ml. of acetic acid-potassium hydroxide buffer solution of pH 4.3. Electrophoresis was carried out in a water cooled apparatus with a current of 4 ma. per tube applied for approximately 2 hours. Methyl green was used as the visual marker. After completion of the electrophoresis, the gels were removed from the tubes and stained for several hours in 0.5% amido black in 7% acetic acid solution saturated with mercuric chloride. The unabsorbed dye was removed from the gel electrophoretically. The gels were stored in small test-tubes containing 7% acetic acids solution

Table 1. Solutions for Preparation of Gels for Disc Electrophoresis

	Lower gel	Upper gel
Catalyst solution		
Ammonium persulfate	60 mg.	60 mg.
Riboflavin	4 mg.	2 mg.
Water	to 100 ml.	to 100 ml.
Acrylamide solution		
N, N - methylene bisacylamide	0.8 g.	0.8 g.
Acrylamide	30 g.	10 g.
Water	to 100 ml.	to 100 ml.
Buffer solution		
1N potassium hydroxide solution	24 ml.	48 ml.
Acetic acid (glacial)	53.2 ml.	3.65 ml.
Temed*	0.48 ml.	0.2 ml.
Water	to 100 ml.	to 100 ml.

*Temed = N, N, N', N' - tetramethylethylenediamine

during the time required for visual comparison and photographing.

8. Amino Acid Analyses

Amino acid compositions were determined for the flours and the four freeze-dried fractions for each variety or line of grain used in the first part of this study. The analyses were made on a Beckman-Spinco Model 120 Automatic amino acid analyzer using the method of Spackmann et al (80). The amount of each amino acid was determined by comparing the recovery of the particular amino acid with that of the same acid using a standard amino acid mixture.

For preparation of hydrolyzates, the quantity of material containing 20 mg. of protein was mixed with 3 ml. of twice distilled 6N hydrochloric acid in a 17 mm. test tube. The contents of the test tube were frozen and the tubes evacuated and sealed. Hydrolysis time was 18 hours at 110°C. After hydrolysis, the contents of the test tube were frozen and the tube was opened. The frozen hydrolyzates were placed in a desiccator containing solid sodium hydroxide and then evacuated to remove the HCl. Finally, the amino acids were dissolved in a known amount of citrate buffer and an aliquot was applied to the ion-exchange column of the amino acid analyzer.

9. Extraction of Proteins from Single Seeds

For the analysis of proteins in individual seeds used in the second part of this study, the protein solutions were prepared by

extracting ground endosperm with 2M urea or 4M dimethylformamide solution in 0.1N acetic acid solution for 24 hours at 4°C. in 15 ml. centrifuge tubes. The suspensions were centrifuged, and proper aliquots of the supernatant examined by starch-gel or disc electrophoresis.

10. Reduction of Disulfide Bonds of Flour Proteins

Reduction of the disulfide bonds was carried out at room temperature using 1% protein solution in 0.1M phosphate buffer of pH 8.0 containing 6M urea by treatment with 2-mercaptoethanol at a concentration approximately 100 times the disulfide content. After 30 minutes, the liberated sulfhydryl groups were alkylated for 15 minutes by adding a five-fold excess of acrylonitrile. The solution containing the reduced alkylated proteins was adjusted to pH 4.0 with acetic acid, dialysed against 0.1N acetic acid for 48 hours and then freezed-dried.

V RESULTS AND DISCUSSION

1. Breadmaking Quality

The extracted tetraploids and their hexaploid parents used in this study had been previously subjected to a large number of tests to evaluate their baking quality by Kaltsikes et al (6). Since the materials used in the present investigation were from the original seed stocks produced by Kaltsikes, it was felt that the original quality data might be of interest to cereal technologists and plant breeders. Accordingly, the data originally obtained by Kaltsikes et al (6) is included in this thesis as Appendix I.

The results of Kaltsikes et al (6) can be summarized as follows:

(1) Each extracted tetraploid had a higher protein content and gave a lower flour yield than its hexaploid parent.

(2) The dough mixing curves for Tetrarescue and Tetrathatcher were weaker than those of their corresponding hexaploids. Tetraprelude had a stronger mixing curve than the other tetraploids but it was somewhat weaker than that for the hexaploid Prelude.

(3) Tetrarescue and Tetrathatcher had inferior breadmaking quality (considerably lower loaf volume) than their hexaploid counterparts. Tetraprelude, on the other hand, gave a higher loaf volume than the other tetraploids and was comparable in this characteristic to the hexaploid Prelude and the other hexaploid wheats.

Table 2. Protein and Moisture Contents, and Flour Yields for the Three Extracted AABB Tetraploid Wheats, Their AABBDD Hexaploid Parents and Stewart 63 (a Durum Wheat).

	Protein* %	Moisture %	Extraction %
Tetraprelude	15.9	12.6	63.0
Prelude	14.8	12.9	80.0
Tetrarescue	16.4	12.6	50.0
Rescue	13.1	13.1	77.5
Tetrathatcher	14.5	12.7	50.0
Thatcher	14.3	13.2	74.0
Stewart 63	13.3	13.4	70.0

*Protein contents are on a dry basis.

2. Milling of Flour

Protein and moisture contents of flour milled from 200 g. of wheat of the three extracted tetraploids, their parents and a durum wheat variety Stewart 63 are given in Table 2. These results are essentially the same as those published by Kaltsikes et al (6) for the same wheat samples. As noted previously (6), Tetraprelude and Tetrarescue had a higher protein content than their hexaploid parents while Thatcher and Tetrathatcher had similar protein contents. The hexaploid wheat gave a higher flour yield than their extracted tetraploids.

3. Solubility Fractionation

At the outset of the present study it was decided to separate and characterize the proteins of the three extracted tetraploids and their hexaploid parents according to the Osborne (8) classification of cereal proteins. Extraction conditions were carefully controlled throughout to ensure that quantitative and qualitative differences between fractions for a particular wheat sample and between wheats for a particular fraction would not be masked by variations in the fractionation procedure.

4. Solubility Distribution on a Weight Basis

The total weights of extractable material expressed in percent of the total material recovered, are given in Table 3. Recoveries of the total weight of the original flour were between 92.6 and 97.6%. The incomplete recovery could be attributed to losses during dialysis of low molecular weight compounds that are extracted in the salt fraction.

Table 3. Solubility Distributions of Flours from Extracted Tetraploid Wheats and Their Hexaploid Counterparts

	Tetra-thatcher	Thatcher	Tetra-prelude	Prelude	Tetra-rescue	Rescue	Stewart 63
	%	%	%	%	%	%	%
Water-soluble	4.5	3.2	3.4	3.2	5.0	3.6	3.6
Salt-soluble	1.3	1.3	1.6	1.6	1.7	1.5	1.4
Alcohol-soluble	6.9	5.6	6.3	5.6	7.6	5.2	6.7
Acetic acid-soluble	4.6	3.4	3.7	3.1	6.1	3.4	2.6
Residue	82.7	86.5	85.0	86.5	79.7	86.4	85.7
Recovery	94.9	97.4	97.4	97.5	92.6	97.6	96.0

It can be seen from Table 3 that the solubility distributions for the extracted tetraploids are quite different from those for the hexaploid parents. All except one of the soluble fractions from the three extracted tetraploids contained more material than the analogous fraction from the hexaploids. The exception was the amount of the salt-soluble fractions which was essentially the same for all the wheats studied. Of the four soluble fractions the alcohol-soluble fraction comprised the highest percentage of the original flour. Of the three varieties, the distribution for Tetraprelude was closest to that of its hexaploid parent. This seems to parallel the observation that this extracted tetraploid was similar to its hexaploid counterpart in breadmaking quality.

5. Protein Content of the Solubility Fractions

The protein contents (N x 5.7) of the various solubility fractions are given in Table 4. Nitrogen recoveries for the samples were above 96% and are considered satisfactory. In general, the protein contents of the soluble fractions from the extracted tetraploids were somewhat lower than those of the analogous fractions from the hexaploid parents. There were a number of minor deviations from this generalization. The water-soluble fractions contained from 45 to 58% protein while the alcohol-soluble fractions contained from 70 to 75% protein. The salt-soluble fractions were the highest in protein (76 - 78%) while the insoluble residue had the lowest protein content. The fact that the protein content for all of the soluble fractions was considerably less than 100% indicates that relatively large and variable amounts of non-protein

Table 4. Protein Contents of the Various Solubility Fractions

	Water-Soluble	Salt-Soluble	Alcohol-Soluble	Acid-Soluble	Residue
	%	%	%	%	%
Tetraprelude	51.3	77.0	74.5	70.3	6.8
Prelude	54.2	76.5	75.1	73.1	6.0
Tetrarescue	47.3	76.0	72.1	63.3	5.6
Rescue	57.6	77.9	70.5	69.6	5.3
Tetrathatcher	45.0	77.5	73.2	64.5	5.0
Thatcher	51.9	76.4	75.0	69.2	5.7
Stewart 63	47.9	75.7	69.8	58.8	4.2

material were extracted by the solvent used. Although carbohydrate analyses were not made in this study, a comparison of the data of Table 4 with those of Yong (81) suggests that most of the non-protein material is probably carbohydrate.

6. Distribution of Proteins in the Solubility Fractions

The distribution of proteins in the five fractions are given in Figs. 2 and 3. Figure 2 compares the results for the extracted tetraploid from Thatcher and Rescue with those for its hexaploid counterparts while Fig. 3 shows analogous results for Prelude. Both figures include results for Stewart 63, the durum wheat, for comparison purposes.

All seven species contained relatively small quantities of water (about 12%) and salt (about 8%) soluble proteins. Together these proteins made up approximately 20% of the flour proteins. There were no significant differences in the amounts of water-soluble and salt-soluble proteins among the seven wheats used in this investigation.

On the other hand, Tetrarescue and Tetrathatcher contained considerably more alcohol-soluble proteins, or "gliadin", and acetic acid-scluble protein, or "glutenin" and less insoluble protein than their hexaploid counterparts. In this respect, the extracted tetraploids of these two varieties were similar to the durum wheat Stewart 63. In contrast Tetraprelude and Prelude contained essentially the same amounts of protein in these three fractions.

It appears that removal of the D genome from the hexaploid to produce the extracted tetraploid wheat markedly affected the

Fig. 2. Distribution of proteins in the five solubility fractions for Tetrathatcher, Tetrarescue, Thatcher, Rescue and Stewart 63.

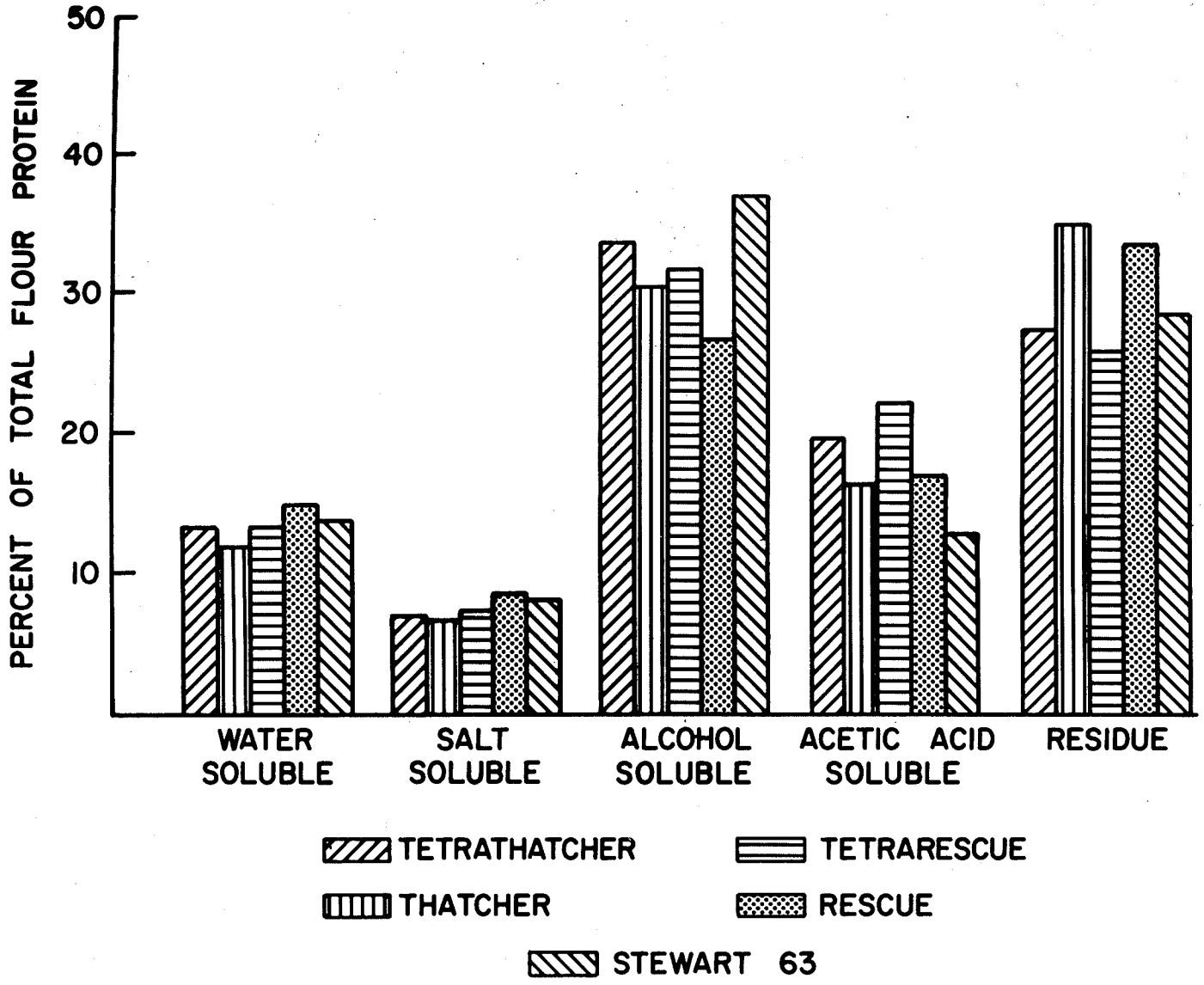
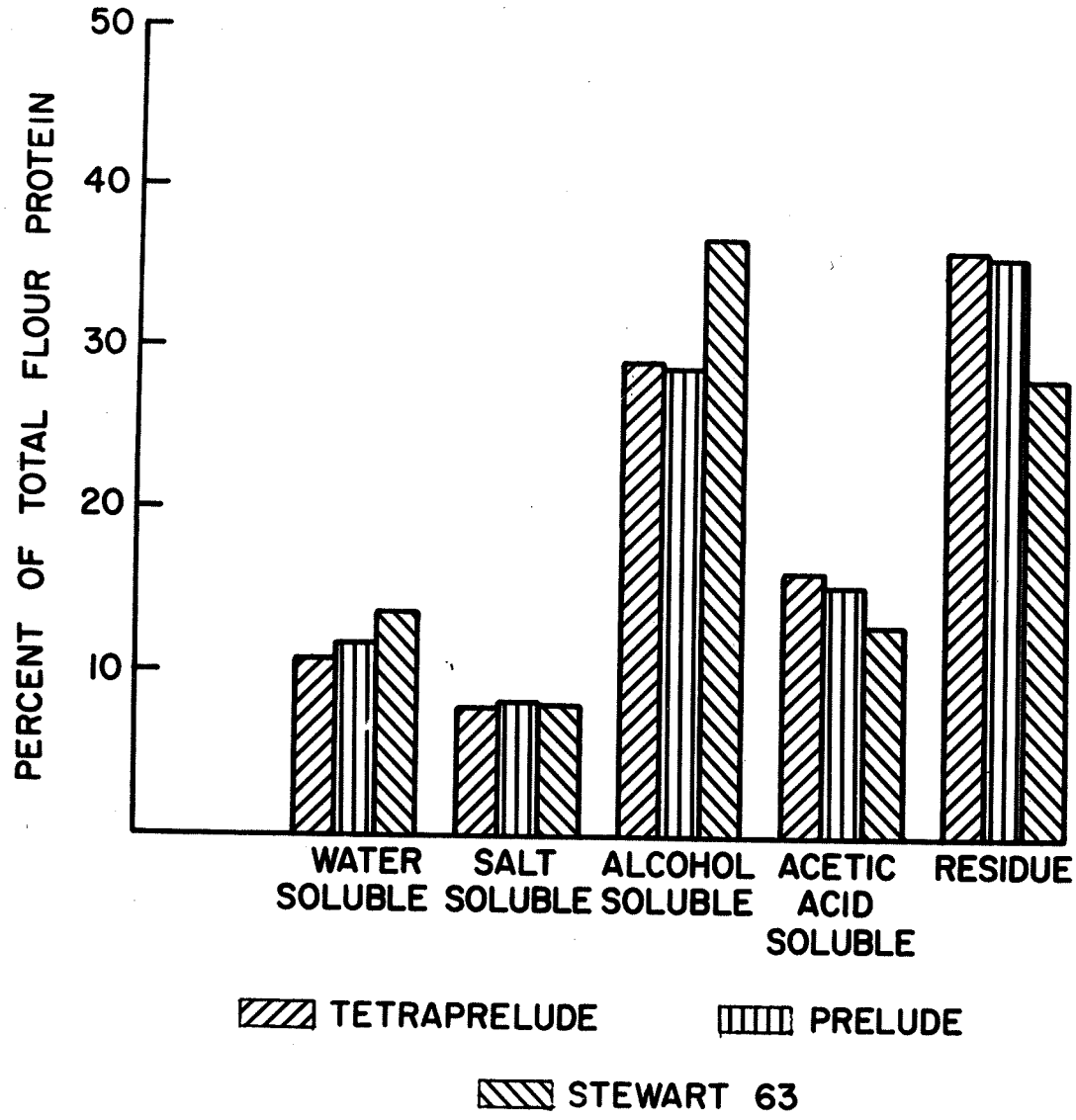

PERCENT OF TOTAL FLOUR PROTEIN
50
40
30
20
10
WATER SOLUBLE
SALT SOLUBLE
ALCOHOL SOLUBLE
ACETIC ACID SOLUBLE
RESIDUE
TETRATHATCHER
TETRARESCUE
THATCHER
RESCUE
STEWART 63

Fig. 3. Distribution of proteins in the five solubility fractions for Tetraprelude, Prelude and Stewart 63.


PERCENT OF TOTAL FLOUR PROTEIN
50
40
30
20
10
WATER SOLUBLE
SALT SOLUBLE
ALCOHOL SOLUBLE
ACETIC ACID SOLUBLE
RESIDUE
TETRAPRELUDE
PRELUDE
STEWART 63

quantitative distribution of the proteins among solubility fractions in two of the three varieties. Tetraprelude, "the durum-type wheat with the high breadmaking quality" had essentially the same quantitative protein distribution as its hexaploid parent. These results suggest that breadmaking quality of wheats is directly related to the solubility composition of its proteins. However, since solubility of protein depends on chemical composition and structure, the present findings do not preclude the possibility that there might be qualitative differences within a particular solubility fraction that can affect the breadmaking quality. In the sections that follow, the proteins of various fractions will be examined to determine if there are any qualitative differences among them.

7. Amino Acid Composition

The amino acid compositions of the flour and of the various solubility groups were determined on a Beckman-Spinco Model 120 amino acid analyzer. In all analyses, nitrogen recoveries following chromatography of the hydrolyzates was over 95%. No corrections were applied for the loss of threonine or serine during hydrolysis. Cysteine, cystine, and tryptophan contents were not determined. The amino acid contents will be expressed in g. of amino acid per 100 g. protein.

Amino acid compositions of the flours from the three extracted tetraploids, their parents and the durum wheat variety Stewart 63 are given in Table 5. Comparison of the data for each extracted tetraploid and its hexaploid counterpart, shows that the compositions

are essentially the same. No significant differences in amino acid composition were found in all the seven flours analyzed. These results are in general agreement with the findings of Tkachuk (56) who reported that hard red spring wheat flours of good and poor baking quality as well as flours from four different classes of Canadian wheat showed little variation in their amino acid compositions.

The amino acid compositions of the various solubility fractions obtained from the seven flours are given in Tables 6, 7, 8 and 9. The compositions of the four protein fractions for a single variety or species are quite different. However, the compositions of any one fraction, from different varieties and species were essentially the same. There was no significant differences in amino acid composition in any of the soluble protein fractions from wheats of good and poor baking quality. Although, the tetraploids of Rescue and Thatcher had significantly different solubility distribution patterns than their corresponding hexaploids (Fig. 2), this difference was not reflected by the amino acid composition data. Presumably, the differences in protein composition were not large enough to show significant differences in amino acid composition of the total endosperm.

Particular solubility fractions were characterized by relatively high contents of certain amino acids. The water and salt-soluble fractions were relatively rich in basic amino acids and poor in glutamic acid (Tables 6 and 7). The alcohol-soluble fraction was characterized by high glutamic acid and proline contents

Table 5. Amino Acid Compositions of the Flours

(g. of amino acid per 100 g. of protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	2.5	2.3	2.7	2.7	2.4	2.6	2.4
Histidine	2.5	2.3	2.5	2.5	2.3	2.7	2.2
Ammonia	4.4	4.6	4.1	4.2	4.1	4.5	3.7
Arginine	4.7	4.8	4.5	4.8	4.4	4.7	4.3
Aspartic	5.8	5.4	4.9	5.2	5.7	5.5	5.0
Threonine	3.2	3.1	3.1	3.2	3.1	3.1	2.9
Serine	5.1	5.4	4.9	5.3	5.8	5.5	4.8
Glutamic	43.6	46.5	42.8	44.4	43.8	45.5	48.8
Proline	13.9	13.6	13.0	13.5	13.6	14.1	13.2
Glycine	4.2	4.4	4.2	4.6	4.2	4.4	4.1
Alanine	3.5	3.7	3.5	3.7	3.8	3.9	3.5
Valine	5.1	5.1	4.6	4.8	5.1	5.1	4.7
Methionine	1.4	1.4	1.3	1.4	1.5	1.4	1.4
Isoleucine	3.9	4.3	4.0	4.0	4.6	4.2	4.5
Leucine	8.5	8.4	7.8	8.0	8.7	8.3	8.8
Tyrosine	3.0	3.0	3.0	3.4	2.9	2.9	3.1
Phenylalanine	6.3	6.5	5.7	6.0	6.0	6.0	6.5

Table 6. Amino Acid Compositions of Water-Soluble Proteins

(g. amino acid per 100 g. of protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	4.8	4.7	5.1	5.1	5.2	4.6	4.7
Histidine	2.6	2.3	2.7	2.7	2.8	2.4	2.3
Ammonia	2.9	2.8	2.4	2.7	2.7	2.3	2.4
Arginine	5.0	5.2	6.2	6.4	6.2	6.0	5.6
Aspartic	8.2	7.8	8.8	8.3	8.2	8.3	8.9
Threonine	4.2	3.9	4.3	4.1	4.6	4.2	4.4
Serine	5.3	4.9	5.5	5.2	5.3	5.1	5.1
Glutamic	30.2	27.2	27.2	28.2	29.7	26.7	26.8
Proline	12.3	11.4	10.7	11.8	11.3	11.0	10.4
Glycine	5.0	4.9	5.5	5.1	5.5	5.0	5.3
Alanine	5.6	5.4	6.3	6.3	6.3	6.0	5.9
Valine	5.8	5.8	6.4	5.8	6.0	5.8	6.1
Methionine	2.1	1.9	2.3	2.6	2.7	2.3	2.5
Isoleucine	4.1	3.8	4.2	4.3	4.1	4.0	4.2
Leucine	8.3	8.5	8.7	9.4	9.0	8.5	8.7
Tyrosine	3.9	3.9	3.9	4.1	3.7	3.6	4.0
Phenylalanine	5.9	6.0	5.2	5.6	5.4	5.0	5.0

Table 7. Amino Acid Compositions of Salt-Soluble Proteins
(g. of amino acid per 100 g. of protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	7.3	6.3	6.2	6.4	6.1	6.7	5.5
Histidine	3.6	3.1	3.3	3.3	3.1	3.1	3.2
Ammonia	1.9	1.7	1.7	1.7	1.7	1.7	1.8
Arginine	8.6	8.2	10.3	9.4	9.5	9.8	9.6
Aspartic	7.4	9.3	7.5	7.9	8.4	7.4	6.9
Threonine	4.0	4.6	3.6	3.9	4.1	3.9	3.6
Serine	5.2	5.6	4.7	4.7	5.3	4.8	5.0
Glutamic	17.4	20.1	17.9	17.8	19.3	16.9	19.9
Proline	4.8	5.7	5.0	5.2	5.5	5.0	5.3
Glycine	6.7	5.9	6.2	5.5	6.2	5.6	5.1
Alanine	7.0	6.0	5.6	5.5	5.9	5.3	4.8
Valine	6.6	6.4	6.2	6.0	6.1	6.2	4.9
Methionine	1.3	1.5	2.0	2.0	2.2	2.3	1.4
Isoleucine	4.3	4.6	4.0	4.1	4.5	4.6	4.0
Leucine	8.4	8.6	7.5	8.0	8.5	8.2	7.3
Tyrosine	3.2	3.2	3.7	3.6	3.1	3.5	3.1
Phenylalanine	4.6	4.8	5.0	4.5	4.7	5.2	4.6

Table 8. Amino Acid Compositions of Acid-Soluble Proteins

(g. of amino acid per 100 g. protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	1.7	1.7	1.5	1.4	1.5	1.4	1.6
Histidine	2.1	2.3	2.3	2.1	2.1	2.3	2.3
Ammonia	4.3	4.6	5.2	4.9	4.4	5.0	5.4
Arginine	3.5	3.3	3.8	3.5	3.2	3.3	3.5
Aspartic	3.0	2.9	3.2	2.9	3.0	2.9	3.1
Threonine	2.3	2.7	3.1	2.7	2.9	2.9	2.7
Serine	5.4	5.1	5.6	5.0	5.1	5.6	5.4
Glutamic	50.2	55.1	58.8	55.1	49.6	54.5	58.5
Proline	14.4	15.6	15.9	15.0	12.9	15.0	16.0
Glycine	5.4	4.5	5.3	4.7	4.6	4.3	4.4
Alanine	2.8	2.4	2.7	2.3	2.6	2.5	2.7
Valine	3.7	3.8	4.1	4.6	3.5	4.1	4.4
Methionine	1.5	1.3	1.7	1.5	1.2	1.5	1.2
Isoleucine	3.6	4.1	4.4	3.8	3.9	4.7	4.9
Leucine	7.4	7.9	8.4	7.2	7.7	8.3	8.5
Tyrosine	4.5	4.3	5.0	4.4	3.6	3.3	4.5
Phenylalanine	6.1	7.3	6.7	7.1	5.8	7.7	8.4

Table 9. Amino Acid Compositions of Alcohol-Soluble Proteins

(g. of amino acid per 100 g. of protein)

	Tetrathatcher	Thatcher	Tetraprelude	Prelude	Tetrarescue	Rescue	Stewart 63
Lysine	0.7	0.6	0.6	0.6	0.6	0.6	0.7
Histidine	2.0	2.1	2.3	2.2	2.1	2.3	2.0
Ammonia	4.3	4.6	4.5	4.9	4.3	5.1	5.0
Arginine	2.4	2.4	2.7	2.4	2.5	2.7	2.8
Aspartic	2.7	2.8	2.9	3.0	2.9	3.0	3.3
Threonine	2.0	2.0	2.0	2.0	2.1	2.3	1.8
Serine	4.4	4.8	4.7	4.2	4.4	5.1	4.2
Glutamic	54.1	57.3	55.8	55.8	54.0	56.4	59.6
Proline	17.9	19.4	17.2	18.0	16.8	17.5	18.9
Glycine	1.5	1.6	1.6	1.7	1.6	1.8	1.9
Alanine	2.1	2.1	2.3	2.3	2.3	2.4	2.4
Valine	3.8	4.1	4.1	4.4	4.0	4.6	4.7
Methionine	1.1	1.1	1.2	1.3	1.4	1.3	1.8
Isoleucine	4.6	4.6	4.7	4.6	4.5	4.7	5.3
Leucine	8.1	8.2	8.2	8.3	7.9	7.8	8.7
Tyrosine	2.5	2.9	2.8	3.3	2.4	2.8	3.8
Phenylalanine	7.4	7.6	6.7	7.0	7.3	7.2	8.3

and low lysine content (Table 8).

The composition of the alcohol-soluble fractions was, in general, quite similar to that of the acid-soluble fractions. Similar results were obtained by Ewart (60). Some minor differences do exist between these two fractions. The alcohol-soluble protein fraction is richer in proline than the acid-soluble fraction. On the other hand, the acetic acid-soluble fraction had much higher glycine, lysine, and tyrosine contents.

8. Starch-Gel and Disc Electrophoresis Results

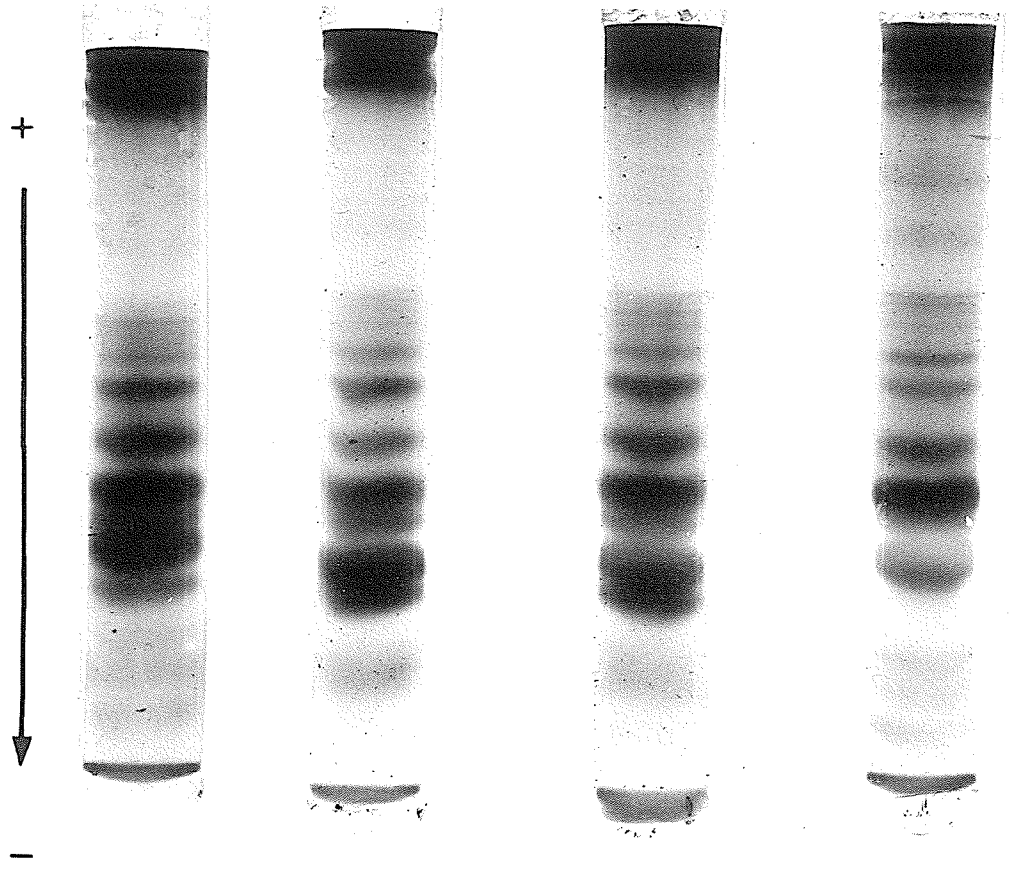
(i) Introduction - Both disc and starch-gel electrophoresis were used to compare the proteins of the various solubility groups, as well as the protein extracts obtained from single seeds of the other cereal species studied. Disc electrophoresis has greater resolving power than the starch-gel electrophoresis technique. Another advantage of disc electrophoresis is its ability to yield rapid and reproducible separations of the proteins studied. The main difficulties encountered with starch-gel electrophoresis were the lack of reproducibility and the overall inconvenience of the technique. Accordingly, disc electrophoresis was used for most of this study. However, starch-gel electrophoresis results were included in some cases for comparison.

The proteins of the extracted tetraploids, their parents as well as the other cereal species studied were compared on the basis of their patterns after electrophoresis. Relative mobility of the protein components identified as individual bands was used as the

criterion of identity or homology of the proteins in the same fraction or extract from different varieties or species. This procedure is similar to that employed by Johnson et al (63) in the analysis of genome and species relationships in wheat. Proteins which had the same electrophoretic mobility were considered to be identical or very similar in physical and chemical properties. To confirm the electrophoretic identity of the protein components of each extracted tetraploid and its parent, protein mixtures made up of equal parts of the same fractions from the two related species were examined by electrophoresis under identical conditions. Identical proteins from the two species in the mixture will appear as a single band.

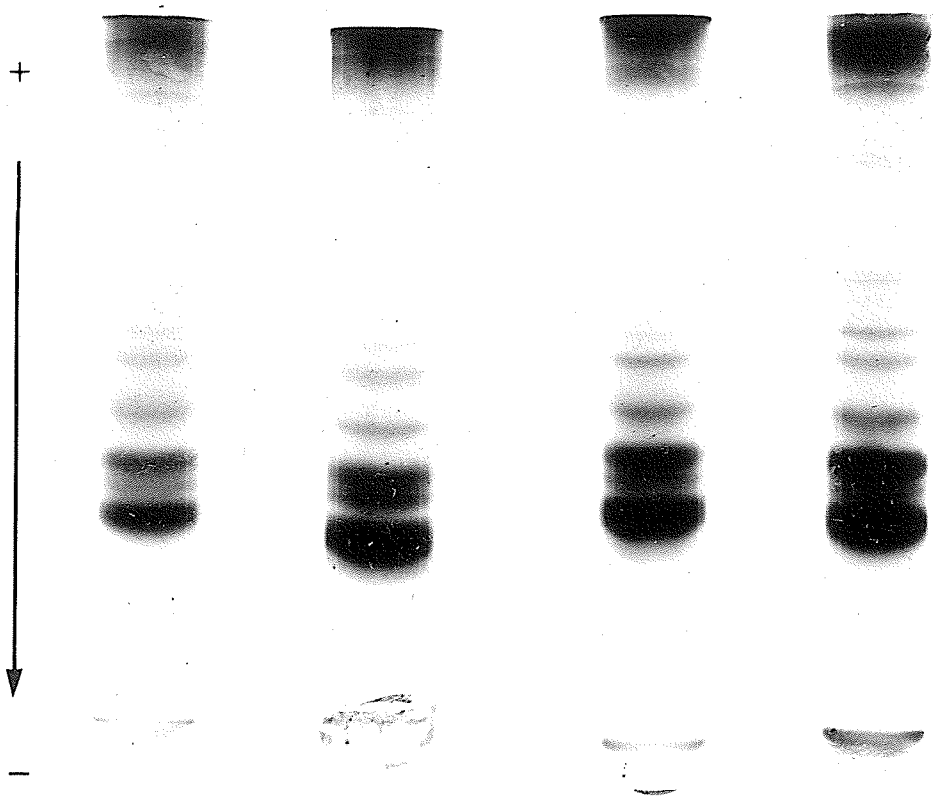
(ii) Water-Soluble Proteins - Figures 4 - 6 show the disc electrophoresis patterns of the water-soluble proteins or the "albumins" of the three extracted tetraploids, their parents, Stewart 63 and mixtures of the fractions from each tetraploid and its parent. The water-soluble proteins are characterized by intermediate and fast moving protein bands with relative mobilities (R_f) from 0.35 to 0.90. The patterns for each extracted tetraploid was essentially the same as that for its hexaploid counterpart. This was confirmed by comparing patterns for the tetraploid, its parent and their mixture. Detailed examination of the patterns showed that the water-soluble proteins from hexaploid Rescue might contain one very minor protein band (R_f of 0.75) that is not present in the pattern for the fraction of its extracted tetraploid. On the basis of these results it appears that the removal of the

Fig. 4. Disc electrophoretic patterns for the albumins from Tetraprelude (4xP), Prelude (6xP), Stewart 63 (ST63) and a mixture (M) of the albumins from Tetraprelude and Prelude.



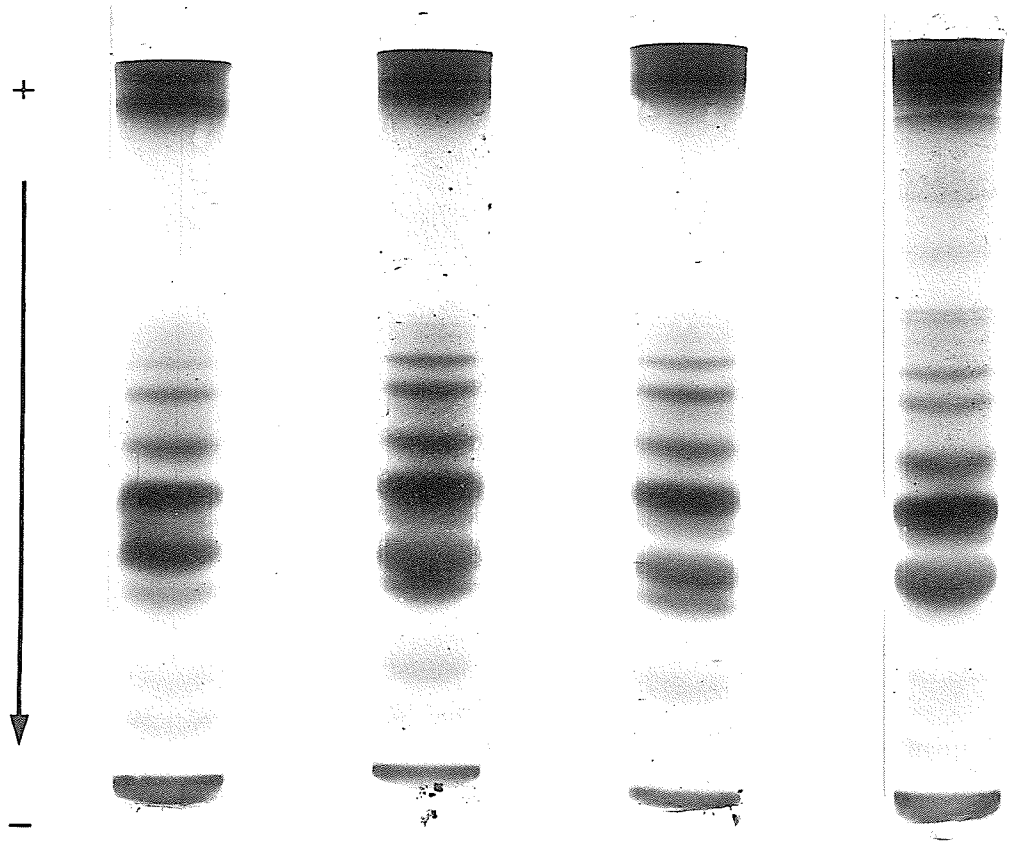
ALBUMINS

Fig. 5. Disc electrophoretic patterns for the albumins from Tetrarescue (4xR), Rescue (6xR), Stewart 63 (ST63) and a mixture (M) of albumins from Tetrarescue and Rescue.



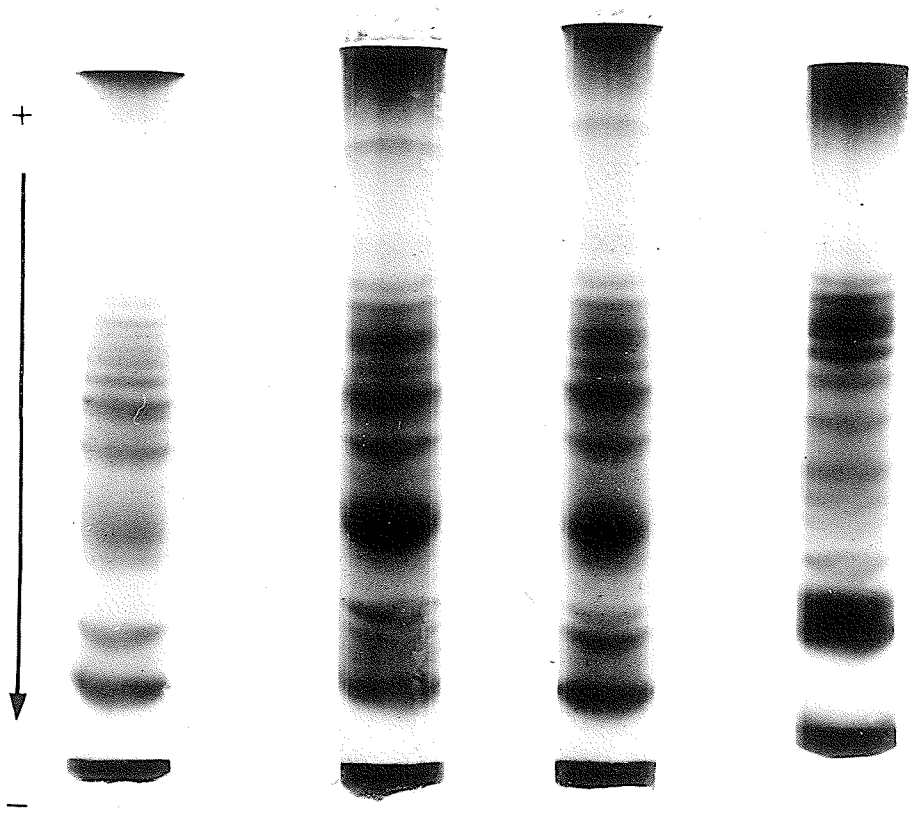
ALBUMINS

Fig. 6. Disc electrophoretic patterns for the albumins from Tetrathatcher (4xT), Thatcher (6xT), Stewart 63 (ST63) and a mixture (M) of albumins from Tetrathatcher and Thatcher.



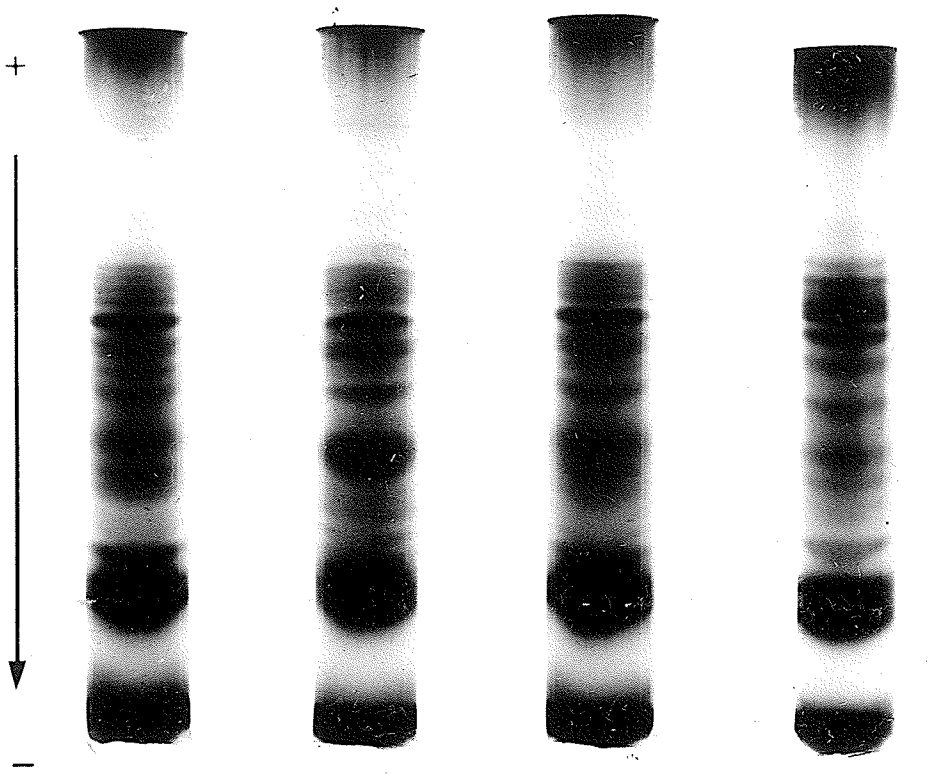
ALBUMINS

Fig. 7. Disc electrophoretic patterns for the globulins from Tetraprelude (4xP), Prelude (6xP), Stewart 63 (ST63) and a mixture (M) of globulins from Tetraprelude and Prelude.



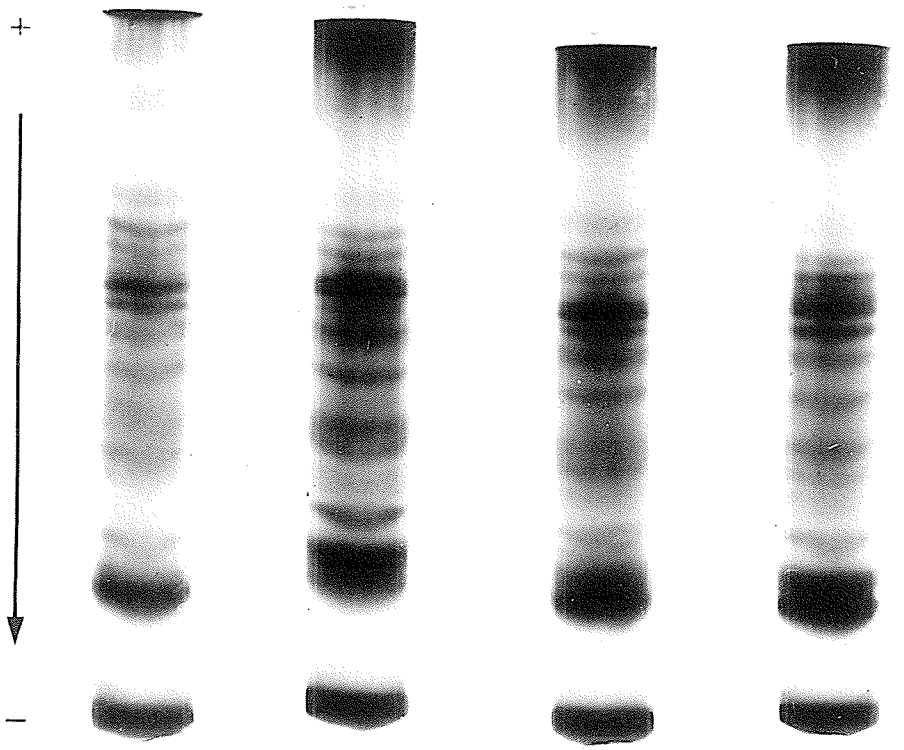
GLOBULINS

Fig. 8. Disc electrophoretic patterns for the globulins from Tetrarescue (4xR), Rescue (6xR), Stewart 63 (ST63) and a mixture (M) of globulins from Tetrarescue and Rescue.



GLOBULINS

Fig. 9. Disc electrophoretic patterns for the globulins from Tetrathatcher (4xT), Thatcher (6xT), Stewart 63 (ST63) and a mixture (M) of globulins from Tetrathatcher and Thatcher.



GLOBULINS

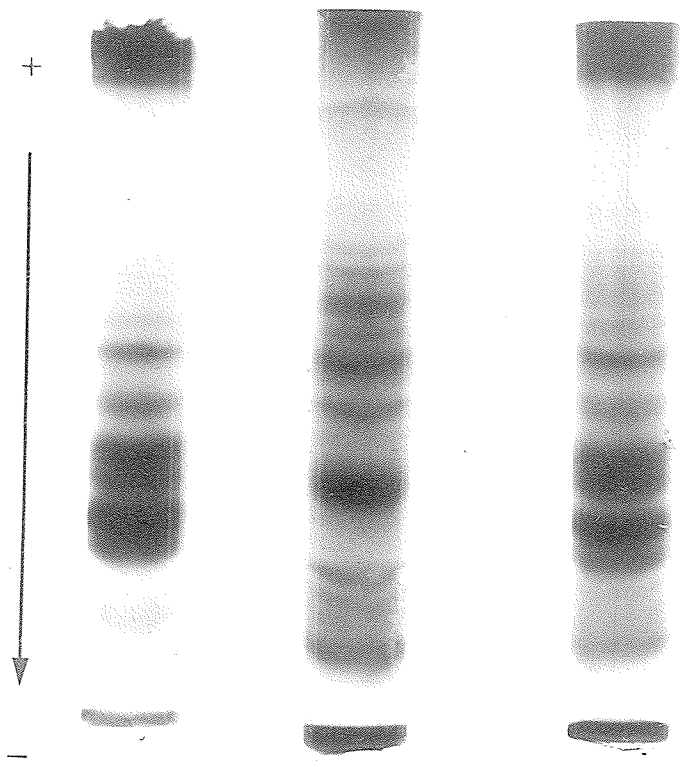
D genome from the three hexaploid wheats did not alter the qualitative composition of the water-soluble proteins, except perhaps the deletion of one minor protein band in the variety Rescue.

The three hexaploid wheats studied were very homogenous with respect to the water-soluble proteins. Their protein patterns comprised at least 10 detectible bands. Four major protein bands appeared in the R_f region from 0.56 to 0.70. It was not possible to ascertain by the electrophoretic technique used whether any of these 4 major bands contained more than one protein. A dark band at the origin and a few light bands in the R_f region from 0 to 0.35 were observed for all the wheats examined. These appear to be due to contamination with the alcohol and acetic acid-soluble proteins extracted in the water-soluble fraction.

Comparison of patterns for the water-soluble proteins of the durum and the three bread wheat varieties showed that all of the water-soluble proteins of the durum wheat were present in the three hexaploid wheats. However, the hexaploid wheats had one minor additional fast moving protein band at R_f 0.75 which was not present in the durum wheat. Two of the three extracted tetraploids (Tetraprelude and Tetrathatcher) retained this minor, fast moving band. Accordingly, it appears that in the variety Rescue this band was contributed by the D genome, while in Thatcher and Prelude, the genes that control its synthesis are also present in the A and B genomes.

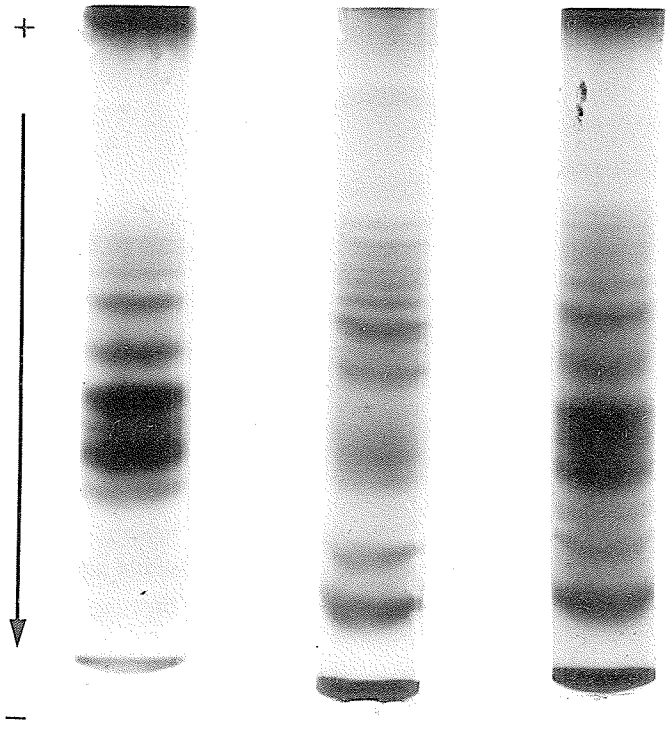
(iii) Salt-Soluble Proteins - The disc electrophoresis patterns for the salt-soluble proteins or "globulins" of the three

Fig. 10. Disc electrophoretic patterns for the albumins (Alb), the globulins (Glo) and their mixture (M) from Prelude.



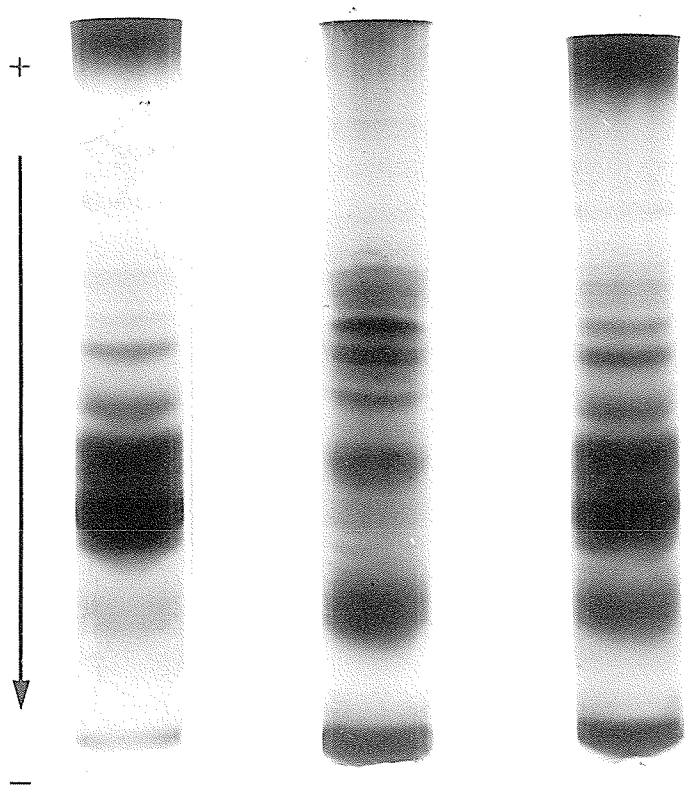
PRELUDE

Fig. 11. Disc electrophoretic patterns for the albumins (Alb), the globulins (Glo) and their mixture (M) from Tetra-prelude.



TETRAPRELUDE

Fig. 12. Disc electrophoretic patterns for the albumins (Alb), the globulins (Glo) and their mixture (M) from Rescue.



RESCUE

Fig. 13. Disc electrophoretic patterns for the albumins (Alb), the globulins (Glo) and their mixture (M) from Tetra-rescue.