SOME CHEMICAL AND PHYSICAL CHANGES IN WHEAT DURING SPROUTING

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ABSTRACT

Hwang, Paulina Miaw Jin, Ph.D., The University of Manitoba, February, 1972. Some chemical and physical changes in wheat during sprouting. Major Professor: Dr. W. Bushuk

Effects of sprouting of wheat on milling and baking qualities and on some chemical components of flours were investigated for one variety of hard red spring wheat. Sprouting was detrimental to both milling and baking qualities; the extent of deterioration of quality increased with increasing degree of sprouting. α-Amylase and proteolytic activities of the flour increased 27,000 and 17 fold respectively during 8 days of sprouting. Starch molecules were degraded, presumably by the action of amylases, to sugars. This chemical degradation produced two types of erosion on the surface of the starch granules. This erosion, named enzymic damage, was studied by scanning electron microscopy. The extent of enzymic damage increased with sprouting. The amount of damage and the free sugar content increased with increasing α-amylase activity of the flours. Quantitative and qualitative changes in the endosperm protein occurred during sprouting. Solubility fractionation showed a marked decrease in the amount of insoluble or residue protein. This change would be particularly detrimental to breadmaking quality. The decrease in the amount of the high molecular weight protein component and the increase in the number of amino groups were directly related to the increase in proteolytic activity of the flours. The increase in the number of amino groups during sprouting was taken as evidence of proteo-

lytic cleavage of peptide bonds. Analyses of SH and S-S groups showed a 30% decrease in the former and a 10% increase in the latter when wheat was sprouted for 8 days. These groups do not appear to be involved in the observed changes in the proteins.

INTRODUCTION

The annual economic loss to the Canadian grain industry resulting from unwanted sprouting of wheat has been estimated at over a million dollars. In a year when the harvest period is unusually wet, the losses are many times greater. The damaging effects of sprouting are multiple but are mostly cumulative. Firstly, sprouting decreases the efficiency of recovery of the grain during threshing. This decreases the yield per acre. Secondly, an additional economic loss occurs when the grain is degraded on account of sprouting when the farmer sells it to the grain merchant. A further loss results during the milling of the wheat into flour. Here the main effect is a decrease in the yield of flour. A portion of the solid matter of the endosperm is converted to rootlets and coleoptiles in sprouting; these are removed in preparing the wheat for milling. Excessive sprouting of wheat generally leads to a lowering of the grade (color) of the flour that is milled from the wheat. Finally, sprouting produces a detrimental effect on the functional or end use properties of the wheat. It is with this last effect that the present study is concerned. Furthermore, the study is restricted to one variety of bread wheat. Accordingly, the pertinent properties that were investigated are those that are related to the breadmaking quality of the flour.

As will be cited later, the detrimental effect of sprouting on breadmaking quality has been generally attributed to the abnormally high α-amylase activity that develops during premature germination. However, there are many other biochemical changes that occur in the

endosperm when the wheat sprouts. On the whole, these have not been investigated in detail and their functional significance has not been delineated. Specifically, the present study deals with some changes in the endosperm proteins, starch and proteolytic enzymes, in addition to α-amylase activity.

REVIEW OF THE LITERATURE

Technological Implications of Sprouting in Wheat

Economic Magnitude of Sprout Damage in Canada and Other Parts of the World

In 1932, Harrington (1932) wrote, "on the plains of Western Canada the average annual loss from the effects of weather on the unthreshed grain runs into millions of dollars. A large part of this loss is caused by the sprouting of grain in the stook and windrow.... Sprouted grain has distinctly less value for seed purposes or for milling than undamaged grain, consequently resistance to after-harvest sprouting is an important advantage in a cereal variety...This is particularly true with respect to wheat varieties since the wheat of Western Canada is grown largely for export trade and must be of high uniform quality if it is to command a satisfactory price". Eight years later, Harrington and Knowels (1940) reported that it was economically desirable to have varieties which remained dormant for several weeks following maturity. Marquis, which at that time had been for many years the most extensively grown wheat variety in Western Canada, had a relatively long dormancy period and was popularly considered as being highly resistant to after-harvest sprouting. There were, however, other licenced varieties grown in Canada, e.g. Garnet, which did not possess such high sprouting resistance.

The damage caused by sprouting in North-West Europe has been greater than in Canada. Belderok (1961) pointed out that in the Netherlands the farmer suffers economically, since sprouting adversely

affects crop yields and grain quality. He estimated that annual losses varied from a few per cent to as high as 20% of the total value of the crop. Extensive economic losses due to sprouting occur annually in the United Kingdom and New Zealand.

Effects on Grade

Soundness is one of the factors considered in grading cereal grains in Canada. The maximum amounts of sprouted wheat permitted in grades from No. 1 Manitoba Northern to No. 6 wheat are 0, 1½, 5, 8, 8 and 12% respectively. Accordingly, a sample of wheat can be degraded if its content of sprouted kernels exceeds the maximum for the grade. For example, No. 1 Manitoba Northern should be free from sprouted material; it will be degraded to No. 2 Manitoba Northern if it contains up to 1½% sprouted wheat, and so on.

Prior to and during sprouting, wheat usually is subjected to wetting and drying processes. Bushuk and Hlynka (1960) showed that this can produce a decrease in grain density and a concomitant reduction of weight per bushel. If this decrease is extensive, it could lead to a further lowering of the grade, since bushel weight is one of the prime grading factors for wheat in Canada and the United States.

Effect on Milling Quality

Studies of Kneen et al. (1942) and Baker and Golumbic (1970) showed that sprouting reduced the dry weight of the kernel and consequently lowered the yield of flour milled from the wheat. The loss of dry weight during germination was 28% for an unspecified

hard red winter wheat (Kneen et al., 1942) and 23.5% for Cheyenne, a hard red winter wheat (Kneen, 1944). Geddes et al. (1941) studied the effect of wheat type, protein content, and malting (sprouting) conditions on the properties of malted wheat flour. They found that malting did not affect the ash content of the flour, although the protein content decreased slightly. The flour milled from the malted wheat was considerably darker than that from sound wheat. Accordingly, sprouting damages milling quality by decreasing flour yield and color.

Effect on Breadmaking Quality

The breadmaking quality of flour milled from wheat samples containing sprouted kernels has been studied extensively (Read and Haas, 1936; Hildebrand and Burkert, 1942; Kneen, 1944; Bushuk et al., 1966; Farrand, 1969). An analogous series of investigations dealt with the effects of additions of malted flour (flour from wheat in which all kernels are sprouted). In either case, the observed effects of sprouting have been generally attributed, directly or indirectly, to the excessively high α-amylase activity of the flour milled from sprouted wheat.

Alpha-amylase, together with the β-amylase present in normal flour, is directly involved in the production of fermentable sugars and thereby increases the rate of carbon dioxide production in a bread dough during fermentation. Accordingly, a definite optimum level of α-amylase activity is essential for proper gassing of the dough. In baking technology, this level of activity is usually controlled by the addition of malted flour (wheat or barley).

However, the level of α-amylase activity of flour milled from wheat samples that contain sprouted kernels is usually much higher than the optimum, and it is this excessively high activity that leads to the deterioration of baking quality.

The detrimental effects of α-amylase activity on breadmaking quality are reasonably well delineated (Jones, 1940; Bushuk et al., 1966; Farrand, 1969; Williams, 1969). At extremely high levels of activity the detrimental effects are apparent quite early in the breadmaking process, actually at the dough stage. The dough becomes extremely sticky to the point where it cannot be handled in the normal manner by dough make-up equipment. A partial solution to this problem is to cut back on the amount of water used to form the dough. This, however, is undesirable since it reduces the yield of bread. At intermediate levels of α-amylase activity, the detrimental effect is usually not observed until the loaf is removed from the oven. First of all, there is a deterioration in the crumb grain due to the appearance of large holes resulting from a combination of excessive gassing and weak crumb. Under some baking conditions, the resulting bread will also have an undesirably sticky crumb. Again, these harmful effects can be minimized by decreasing the amount of water used to mix the dough.

The introduction of mechanical dough development to baking technology has accentuated the detrimental effects of α-amylase activity on bread quality. With this new baking technique, a high water absorption can be maintained in a dough from a cheaper flour of lower protein content by increasing the level of starch damage produced

by milling (Tipples and Kilborn, 1968). Since damaged starch is readily attacked by α-amylase (Farrand, 1969; and Williams, 1969), the level of damaged starch that can be tolerated depends inversely on the α-amylase activity of the flour. The actual level of activity is extremely critical for proper utilization of flours with high starch damage. The fraction of sprouted wheat that can be tolerated in a grist for milling flours with abnormally high starch damage is extremely low.

Biochemical Changes in the Endosperm

Alpha-amylase

The literature contains many articles on studies of the development of amylase activity in wheat during laboratory germination (or malting). Most of these studies concerned α-amylase because of its significance in breadmaking, although in some cases β-amylase was investigated also. In some of the older references, these two activities were referred to as dextrinogenic and saccharogenic activities respectively. Geddes et al. (1941), Kneen et al.(1942), Dickson and Shands (1942), Dickson et al. (1947) and Fleming et al. (1960) demonstrated that during laboratory germination, α-amylase activity increased with increasing steeping moisture, time and temperature. In addition, Geddes et al. (1941) studied the effect of protein content on the properties of malted wheat flour. They found high-protein hard red spring wheats had the highest α-amylase activity.

The effect of variety and class of wheat on α-amylase activity of malted wheat in relation to production of malted flour was studied

by Dickson and Geddes (1949), Geddes et al. (1941) and Fleming et al. (1960). On the basis of rapid development of α-amylase activity, they rated soft white wheat as the best for malting followed by soft winter, hard red winter and hard red spring.

Yomo (cited by Bonner and Varner, 1965) and Paleg (1965) showed independently that during germination in the presence of gibberellic acid α-amylase was synthesized de novo in the aleurone layer of the kernel in barley and wheat respectively. On the other hand, the concomitant increase in β-amylase activity was due to activation of latent β-amylase and not a result of new synthesis.

Daussant and Abbott (1969) also showed that the increase in α-amylase activity of wheat during germination and early growth resulted from synthesis of new enzyme rather than from an activation of a zymogen. Recently, Olered and Jonsson (1970) studied the pattern of α-amylase in maturing and germinated wheat by agar gel electrophoresis. They found that there were two α-amylase systems which act during different stages of the development of the grain. The two α-amylase systems were designated as "green" α-amylase, found in immature wheat, and "malt" α-amylase synthesized during germination. The activation of "green" α-amylase was stimulated by an increase in moisture whereas the activation of "malt" α-amylase appeared to be a result of DNA-controlled synthesis during germination -- a process in which the gibberellins have a control function. It was concluded that the "malt" α-amylase activity increased approximately a thousandfold during sprouting by an irreversible process. On the other hand, the activity of the "green" α-amylase remained at a fairly low level throughout the development

and subsequent germination of the grain. Furthermore, it was readily activated or inactivated in ripening grain by environmental conditions such as moisture and temperature.

As far as the writer is aware, there are no reports in the literature dealing with the kinetics of the development of amylase activity in wheat during sprouting in the field. This is probably due to the difficulty in sampling at a particular stage of sprouting which is greatly influenced by the environmental conditions such as rainfall, temperature, etc.

Proteinases

Our knowledge of the proteinase present in wheat flour is considerably less compared with the knowledge on the starch-degrading enzymes. It is generally believed (Hites et al., 1953) that flour from sound wheat has extremely low proteinase activity and that this activity is of no consequence to bread making quality. Johnson and Miller (1949) studied the effect of two types of proteinases in breadmaking using enzyme supplements prepared from malted wheat flour and fungal extracts. A commercial sponge-dough procedure was used in the baking. The observed effects of increasing concentrations of the two enzymes were different. The difference depended on the type of flour used in the bread formula. With hard red spring wheat flour, wheat proteinase produced a marked increase in loaf volume, whereas winter wheat flour did not show any response. Both flours showed a decrease in loaf volume with increasing amounts of fungal proteinase.

The usefulness of certain proteinases in breadmaking was demonstrated by Johnson et al. (1956). They showed that the addition of a

fungal proteinase to the sponge in the sponge and dough baking procedure reduced the mixing requirements at the dough stage. These proteinase treated doughs were more extensible and could be sheeted and molded with greater ease. These effects were particularly beneficial with certain flours that normally produced bucky doughs.

Hanford (1967) investigated the proteolytic enzymes of wheat and flour and their effects on bread quality in the United Kingdom. He suggested the existence in wheat of at least two enzymes with differing action: one which produced gluten softening and the other which produced soluble nitrogen as measured by the modified Ayre-Anderson test. These two enzymes were referred to as alpha- and beta-proteinases respectively, a terminology analogous to that used for amylases. It was suggested that variations in activity of the alpha-proteinase could be responsible for differences in "gluten quality" observed by baking and rheological tests on flours that are essentially the same by analytical tests. The direct involvement of wheat proteinases in gluten softening was confirmed by Redman (1971).

Kaminski and Bushuk (1969) detected four proteolytically active components in endosperm extracts by starch gel electrophoresis. Proteolytic activity was detected in all the fractions (albumins, globulins, gliadins and glutenins) of the endosperm proteins. Recently, a proteolytic enzyme from malted flour of one variety of Canadian hard red spring wheat was isolated by affinity chromatography and partially characterized by Chua (1969) and Chua and Bushuk (1969). The purified enzyme contained three major and one minor protein bands detected by disc electrophoresis on polyacrylamide gel. However, it appeared to be

homogeneous by column chromatography on Sephadex G-150 and by ultrafiltration. The molecular weight of this purified enzyme, determined by gel filtration, was 34,000; its sedimentation coefficient ($S_{20,w}$) was 3.8. The purified enzyme was inactivated by sulfhydryl blocking agents and activated by reducing agents but was not affected by soybean trypsin inhibitor. The optimum pH of this enzyme was 3.8 on hemoglobin substrate and 7.5 on casein substrate. Unfortunately, its effect on gluten and dough was not investigated.

The published work that is most pertinent to the present investigation is that of Beresh (1969). This Soviet worker showed that the proteinase activity, measured by the formation of amino groups, increased six fold during five days of germination.

Other Enzymes

Besides changes in the activity of the amylases and proteinases, changes in the activity of other enzymes have been observed during germination and sprouting of wheat. Most of these, however, are not considered to be related to the baking quality of flour. Bhatia and Nilson (1969) studied electrophoretic properties of 0.01M sodium pyrophosphate buffer-soluble enzymes from dry and sprouted wheat. The enzymes examined were alcohol dehydrogenase (ADH), peroxidase, and nonspecific esterases. Multiple forms (isoenzymes) of all of these enzymes were observed in the dry seed. The ADH pattern did not change during sprouting. Esterase and peroxidase patterns changed both qualitatively and quantitatively. The number of peroxidase bands increased from 0 to 8 during sprouting to the first leaf stage.

Changes in the multiple forms of various enzymes during early growth of wheat were investigated by Macko et al. (1967). Electrophoretic patterns for glucose-6-phosphate dehydrogenase and malate dehydrogenase remained unchanged during germination. However, germination produced a definite increase in the activities of 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, glutamate dehydrogenase, peroxidase and esterase. In some cases, qualitative changes in the electrophoretic patterns were observed. In the case of alcohol dehydrogenase a decrease in activity was observed when dormant wheat was germinated. Drapron et al. (1969) reported that lipolytic activity of wheat grain showed a slight decrease at first and then increased on further germination regardless of temperature or light conditions. All of these enzymes are essential for the metabolic processes of the new plant but they have not been implicated in the functional (breadmaking) properties of the flour.

Proteins

In spite of the extreme importance of the endosperm protein of bread wheats in breadmaking quality, there have been relatively few studies of the changes in this component during sprouting. Coulson and Sim (1965) examined the changes in the endosperm proteins of one variety of common wheat (cv. Als) during the life cycle of the wheat kernel using starch gel electrophoresis. They found extensive degradation of endosperm proteins during the early stages of germination to lower molecular weight materials, but did not observe any qualitative changes in the patterns of the acetic acid-soluble proteins.

After 6 days of germination, however, a definite decrease in the amount of the slow-moving fractions could be inferred from the intensity of the bands. Components of higher electrophoretic mobility appeared to be unaltered throughout germination up to 13 days. A few new components of high mobility were observed for all stages of germination and were presumed to result from the degradation of some slower-moving components. There were no qualitative changes in the free amino acids of the endosperm and the embryo throughout germination.

Macko et al. (1967) compared the patterns of the Tris buffer-soluble proteins extracted from dormant and germinated wheat kernels by polyacrylamide gel electrophoresis. They found that the main difference between the two extracts was in fastest migrating components.

The most extensive studies of the changes in wheat endosperm proteins are those of two Soviet groups. Although these studies were initiated about 10 years ago, the published articles became known to the writer only during the time this thesis was being written.

Shorina and Vakar (1965) and Shorina et al. (1966a, 1966b, 1967) showed that, with increasing sprouting, the endosperm proteins rapidly lose their gluten forming ability. No gluten could be washed out from flour milled from wheat that was sprouted for three days. Viscosimetric measurements led to the conclusion that the structure of the protein complexes of gluten gradually became more open (loose) during sprouting. This loosening was attributed to a reduction of disulfide bonds (presumably by protein disulfide reductase) with a concomitant breakdown of the hydrogen bonding system. Careful examination of the data, however, does not show an unequivocal decrease in the number of

disulfide bonds in the gluten during sprouting. These workers did not detect any breakdown of peptide bonds in the same samples.

On the other hand, another Soviet worker, Beresh (1969), observed the same changes in gluten during sprouting as did Shorina and Vakar (1965) but he also observed a marked (4-6 fold) increase in the number of amino groups. Accordingly, Beresh (1969) attributed the changes in the physical properties of the gluten to proteolytic action. Results of Redman (1971) on flours from grists containing a small percentage of sprouted wheat are in general agreement with those of Beresh (1969).

Starch

So far as the writer is aware, there is no report in the literature dealing with changes that occur in the starch during germination and sprouting of wheat. Williams (1971), using ordinary light microscopy, examined a number of starch samples prepared from sprouted wheat. He observed that some of the granules were partially eroded (damaged) and that the granules that had suffered this type of damage appeared quite different from those damaged by milling or other types of grinding. The implications of the enzymic degradation of the starch during germination and sprouting in breadmaking have not been investigated. However, from knowledge of the role of starch in the water-holding capacity of dough and in the crumb structure of bread, it is presumed that any changes that occur might be quite important.

Genetic Implications of Sprouting

Very little research has been done on the inheritance of sprouting resistance. Several authors have shown that dormancy is a heritable character readily transferred to hybrid populations from the parents (Gfeller and Svejda, 1960). Other investigators (Greer et al., 1948; Wellington, 1956; Wellington and Durham, 1958; Everson and Hart, 1961; and McEwan, 1965-1967) associated dormancy with seed coat color; white wheats were least resistant to sprouting.

Detailed studies of inheritance of post-harvest seed dormancy and kernel color in genetic lines of spring wheat were reported by Gfeller and Svejda (1960). They observed a regular association between red seed coat color and dormancy. However, there were significant differences in the degree of dormancy within the red lines. It was suggested that lines with a greater number of genes controlling seed coat color might be the most dormant.

In breeding wheat varieties resistant to sprouting, McEwan (1965-1967) also studied the nature of the inheritance of the red grain character. He concluded that as many as three genes might be involved in controlling grain color. Furthermore, he suggested that the range of sprouting reaction exhibited by red-grained varieties could be explained on the basis of dosage effect; the slightly resistant varieties would have a single gene for red grain color, the moderately resistant lines two genes, and the highly resistant ones all three. This hypothesis remains to be verified. The same study showed that when the three genes for red grain color in the sprout-resistant variety Hilgendrof 61 were

introduced separately into tester stocks, by backcrossing with the white-grained variety Aotea, each one produced a level of sprouting resistance similar to that of the resistant parent.

Although it is generally believed that red-colored varieties are more dormant than white wheats, there are some red-grained wheat varieties, e.g. Garnet (Harrington, 1932) and Mexican varieties Sonora 64, Inia 66 and Ciano 66, that are quite susceptible to sprouting. On the other hand, there are also a few white wheats that are reasonably resistant to sprouting, e.g. Brevor (Everson and Hart, 1961), and C-518 and K-321.BT.1B.1 (Czarnecki, 1971). Boyd and Gordon (1971) also found that the commonly held view of the direct relationship between dormancy and red pericarp color was not absolute. Their work showed that the white wheats Gabo, Stewart and Camenja have good resistance to germination.

The question of heritability of resistance to sprouting in bread wheats is currently under very active investigation in Canada. This has become particularly important since α-amylase activity has become an extremely important factor in breadmaking quality (see above). In the 1969 Western Cooperative Test, there were a number of experimental lines rejected on account of low amylograph viscosity (Bushuk, 1971).

Environmental factors, mainly moisture and temperature during the final stages of maturing and harvesting, greatly affect the sprouting tendency of wheat. In Austria, Fritz (1933-34) collected data on precipitation, temperature, sprouting-damage and harvesting methods during years when visible sprouting occurred. These data covered a number of widely different wheat varieties. He observed a clear

relationship between weather conditions and sprouting.

Studies of the germination of a sprout-resistant red wheat and a sprout-susceptible white wheat were carried out by Wellington (1956). He observed that the proportion of the red grains that germinated increased with storage time after harvest without any further desiccation, but the increase was greater when the moisture content of the grains was substantially reduced.

The subject of the effects of environment and environment-variety interaction on sprouting has been studied in detail, and reviewed extensively by Belderok (1961). First, he carried out basic studies on the effects of heat treatment at pre-ripe stages on the dormancy or sprouting resistance of a large number of Dutch wheat varieties. Heat treatment (25°C) for 1 or 3 days had only a minor effect on the duration of dormancy. However, treatments of 6 days or more shortened the dormant period quite considerably. Subsequently, he extended these studies to field conditions. On the basis of these investigations, he established a dormancy resistance curve for each variety relating length of dormant period to accumulated temperature during dough ripeness stage in day-deg. C. These studies are extremely significant technologically to wheat production in the Netherlands. Each new variety is tested for dormancy resistance before it is licensed. If its resistance to sprouting is less than 10 days, it is not recommended for commercial production. In addition, these studies have led to a practical sprouting warning system whereby the farmer receives advice on the correct harvesting time for his particular variety depending on prevailing temperatures during the ripening period.

Recently, a study of pre-germination in cereal grains during ripening was initiated at The University of Manitoba by Gordon (1970). In this study, a hard red spring wheat, cv. Manitou, a durum wheat, cv. Stewart 63, and a spring rye, cv. Prolific were examined for premature germination. It was demonstrated that a characteristic early rupture of the pericarp-testa during ripening of the grain led to varying amounts of premature germination and growth of the embryo. Some evidence has already been accumulated which indicates that absence of this early rupture of the pericarp-testa can be used as an indicator of dormancy or sprouting resistance. Accordingly, selection for the absence of this characteristic should prove very useful for identifying dormant varieties at a very early stage in the breeding program.

MATERIALS

One variety of hard red spring wheat (Manitou) was used in this study. The grain was grown in Manitoba during the 1967 crop year which was normal so far as temperature and moisture were concerned. Sprouted samples were produced by germinating wheat that was first soaked in two lots of distilled water at 10°C for 48 hours. Germination was for specified periods of time at a constant temperature (20°C) to ensure even germination. After zero, two, four and eight days of germination, two and half kg.portions of wheat were frozen and then freeze-dried. The rootlets and coleoptiles were removed from the dry sprouted wheat by moderate shaking on a coarse wire sieve. All five (control, soaked, and three germinated) samples were milled into a straight-grade flour on a Buhler experimental mill after tempering to 15.5% moisture. The flours were used as the starting material for the work described in this thesis. Pertinent technological characteristics of the original wheat are listed in Table 1.

TABLE 1. TECHNOLOGICAL CHARACTERISTICS OF THE WHEAT

Bushel weight, lb.	66
1000 kernel weight, g.	32.8
Protein content, % (13.5% m.b.)	14.9
Moisture, %	8.4

Sephadex G-150 used for gel filtration was obtained from Pharmacia (Canada) Ltd. β-Amylase, required for determination of α-amylase activity, was the sweet-potato type from Nutritional Biochemicals Corporation. All other chemicals used were reagent grade.

METHODS

Technological Tests on Wheat and Flour

Bushel Weight

The bushel weight of the wheat was determined by the AACC Approved Method 84-10 (A.A.C.C.,1969).

1000 Kernel Weight

Thousand kernel weight of the wheat was calculated by multiplying the weight of 200 kernels by 5.

Moisture Content

Moisture content of the wheat was determined by the AACC Approved Method 44-11. Moisture content of the flour was determined on the Brabender Rapid Moisture Tester. According to the procedure for this apparatus, the samples were dried for 1 hour at 130°C.

Flour Color

Flour color was determined by the Kent-Jones and Martin Flour Colour Grader using the procedure supplied by the manufacturer of the instrument (Kent-Jones & Amos, 1967).

Ash Content

Ash content of the flour was determined by the AACC Approved Method 08-01.

Protein Content

Protein content of both wheat and flour was determined by the AACC Approved Method 46-12 (macro Kjeldahl, boric acid modification).

Sedimentation Test

Sedimentation value was determined according to the AACC Approved Method 56-60.

Farinograph Test

Farinograph test was carried out as described by the AACC Approved Method 54-21. This is the constant flour weight procedure.

Amylograph Test

Amylograms were obtained by using Brabender VISCO/AMYLO/GRAPH according to the AACC Approved Method 22-10.

Baking Test

The Remix baking test described by Irvine and McMullan (1960) was used to evaluate baking quality.

Analyses on Flour

Amino Acid Composition

Amino acid compositions were determined on a Beckman Model 121 automatic amino acid analyzer. The hydrolyzate was prepared using 6N hydrochloric acid and hydrolysis time of 24 hours under vacuum at 110°C. Data reported in this thesis are the average of two analyses. Replicate results differed by less than 3%. Cystine and cysteine were estimated from the disulfide and sulfhydryl contents determined amperometrically (see below). Tryptophanwas not determined by the standard procedure using acid hydrolyzates.

Sulfhydryl and Disulfide Contents

Sulfhydryl contents of the flours were determined by titrating with silver nitrate according to the procedure of Sokol et al. (1959). To determine disulfide contents, these groups were first reduced to sulfhydryl groups by a pretreatment with sodium sulfite as outlined by Tsen and Anderson (1963). Total sulfhydryl content was then determined by amperometric titration. The disulfide content was calculated by subtraction. Half a gram of flour (0% moisture) was used for the determination of the original sulfhydryl content and 0.1 g. (0% moisture) was used after reduction of the disulfide groups. The concentration of sulfhydryl groups was read off a standard curve obtained by plotting the volume of silver nitrate solution used to titrate increasing amounts of cysteine hydrochloride.

Amino Groups

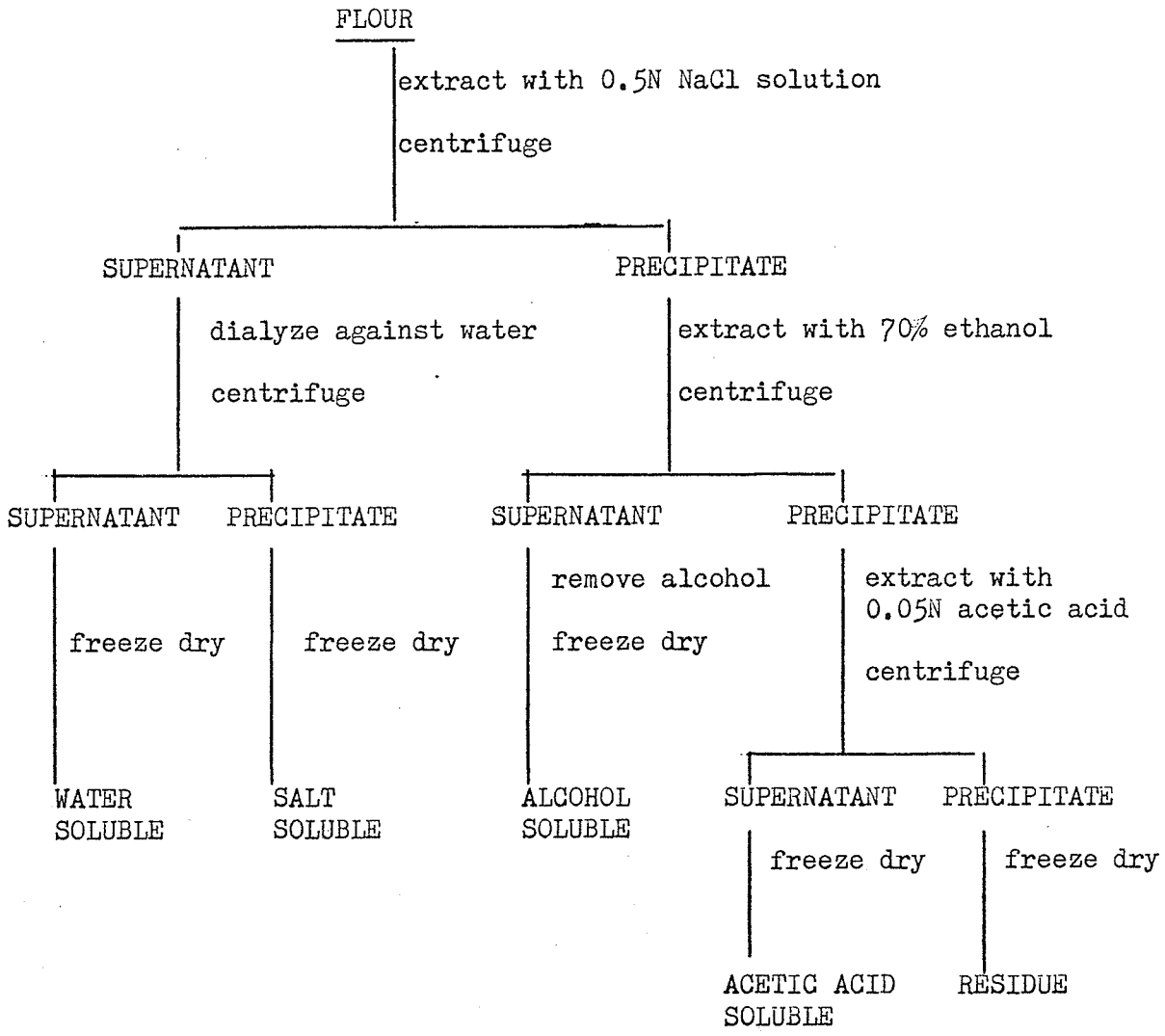
The proteins of the flour were first solubilized in 1% acetic acid solution using a flour to acetic acid ratio of 1 to 10. Extraction was carried out in a cold room (for one hour) to minimize further proteolytic action. The suspension was centrifuged at 20,000 xg for one hour. This extraction procedure solubilized about 80-90% of the total proteins for all five flours. An aliquot (0.1 to 1.0 ml., depending on amino nitrogen content) of the clear supernatant was measured into a test tube and mixed with 1 ml. of 2% ninhydrin reagent. The mixture was heated at 100°C for 30 min. to develop the characteristic purple color. The optical density of the solution at 570 nm. was determined on a Zeiss spectrophotometer using a water blank. The amount of amino nitrogen in the aliquot was read off a standard curve which was constructed using a series of solutions containing a mixture of known amounts of 18 common amino acids.

Solubility Fractionation of Flour Proteins

Flour proteins were fractionated by a modified Osborne procedure (1907). The procedure used is summarized in Fig. 1. Five protein fractions were obtained: (1) water-soluble proteins (albumins); (2) salt-soluble proteins (globulins); (3) alcohol-soluble proteins (gliadins); (4) acetic acid-soluble proteins (glutenins); and (5) insoluble residue. All extractions were made in a cold room (6°C) to minimize the effects of enzymes or thermal denaturation. The extracted fractions were dialyzed as required and freeze-dried, and stored in a

Figure 1.

Summary of the solubility fractionation procedure.


FLOUR
extract with 0.5N NaCl solution
centrifuge
SUPERNATANT
PRECIPITATE
dialyze against water
centrifuge
extract with 70% ethanol
centrifuge
SUPERNATANT
PRECIPITATE
SUPERNATANT
PRECIPITATE
freeze dry
freeze dry
remove alcohol
freeze dry
extract with
0.05N acetic acid
centrifuge
WATER
SOLUBLE
SALT
SOLUBLE
ALCOHOL
SOLUBLE
SUPERNATANT
PRECIPITATE
freeze dry
freeze dry
ACETIC ACID
SOLUBLE
RESIDUE

freezer in closed vials. These protein fractions were later used in the disc gel electrophoresis, gel filtration and proteolytic activity experiments. Protein contents of the five fractions were determined by the Nessler procedure of Williams (1964).

For molecular sieve gel filtration on Sephadex G-150, the flour proteins were extracted by the procedure of Meredith and Wren (1966). This extraction uses a strongly dissociating solvent comprising 0.1M acetic acid, 3M urea and 0.1M cetyltrimethylammonium bromide. This ternary solvent will be referred to as AUC. One and a half g. of flour was extracted in 26 ml. of AUC for one hour by stirring with a magnetic stirrer at room temperature. The mixture was centrifuged for 20 minutes at 20,000 xg and the supernatant was recentrifuged for 30 minutes at 100,000 xg to obtain a clear extract. The extraction was repeated twice and the supernatants were combined. This extraction procedure solubilizes over 98% of the flour protein (Bushuk and Wrigley, 1971).

Fractionation by Gel Filtration

Preparation of Sephadex Columns Using AUC Solvent

The Sephadex G-150 column was packed as described by Bushuk and Wrigley (1971). According to their procedure beads of Sephadex G-150 (4.7 g.) were suspended in AUC (500 ml.) and left overnight at 70°C with moderate stirring or for 72 hours at room temperature without stirring. The Sephadex suspension was deaerated by applying vacuum and then poured into a column tube (2.5 x 34 cm.) containing several inches of AUC at the bottom to prevent trapping of air bubbles. Before any of the solvent was drained, the gel was allowed to settle for 30 to 60 minutes. During packing and use of the column, it is important to keep

the operating pressure (head) below 10 cm. of water to prevent undue compacting of the swollen Sephadex beads. The column was equilibrated, using downward flow, overnight at a flow rate of 20 to 30 ml. per hour. The direction of flow was then reversed carefully to prevent introduction of air bubbles. During a gel-filtration experiment, the effluent passed through an ISCO model UA-2 UV analyzer and then to the siphon of a volume-activated fraction collector. The O.D.* of the UV analyzer was recorded on a strip chart recorder in the usual manner.

Gel Filtration

Protein fractions obtained by solubility fractionation (20 mg. protein) were dissolved in AUC solvent (3 ml.) and clarified by centrifugation, if necessary. The five fractions and the AUC extracts of the flours were examined chromatographically on Sephadex G-150 with AUC solvent as described by Bushuk and Wrigley (1971). Bovine plasma albumin, ovalbumin, aldolase, trypsin, myoglobin and trypsin inhibitor were used to calibrate the column for estimation of molecular weight (see Fig. 2). The peaks of the elution curve were designated as proposed by Meredith and Wren (1966): glutenin, comprised material over 100,000 in molecular weight; gliadin, 25,000 to 100,000; albumin, 10,000 to 25,000; and non-protein material, below 10,000. These four fractions were collected separately and dialyzed against distilled water with frequent changes of water in a cold room for one day. The dialyzed fractions were freeze-dried and examined by electrophoresis.

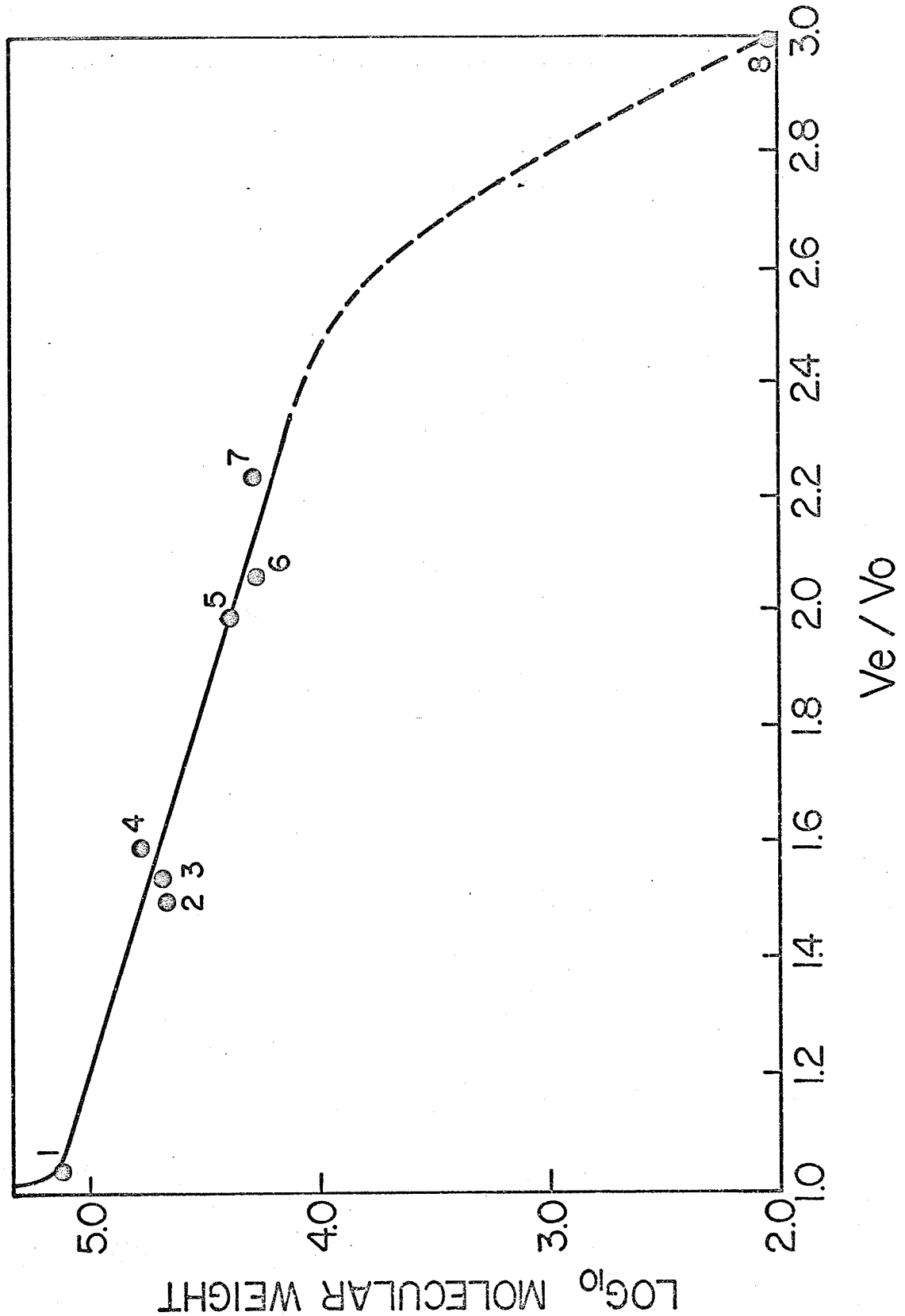
*O.D. = optical density at 280 nm.

Figure 2.

Calibration curve for Sephadex G-150 using AUC solvent.

Calibration Standards Used:

1. bovine plasma albumin (dimer) 129,800 M.W.
2. ovalbumin 45,000 M.W.
3. aldalase (monomer) 52,700 M.W.
4. bovine plasma albumin (monomer) 64,900 M.W.
5. trypsin 23,800 M.W.
6. myoglobin 17,000 M.W.
7. trypsin inhibitor 21,500 M.W.
8. NEMI (N-ethylmaleimide) 125 M.W.


LOG10 MOLECULAR WEIGHT
5.0
4.0
3.0
2.0
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
Ve / Vo
1
2
3
4
5
6
7
8

Polyacrylamide Gel Electrophoresis

The water-, salt- and alcohol-soluble protein fractions and the fractions from the Sephadex fractionation were examined by electrophoresis at pH 3.2 on 7.5% polyacrylamide gels as described by Chen (1969) and Chen and Bushuk (1970). This procedure uses the principles of analytical polyacrylamide disc gel electrophoresis described by Davis (1964).

The gel electrophoresis procedure used to separate and detect α-amylase activity was described by Kruger and Tkachuk (1969). For this purpose, acrylamide gels (7%), containing 0.2% β-limit dextrin were used. The running pH was 8.9. In all experiments, duplicate gels were electrolyzed. One gel was used to determine the location of the protein bands and the other for the location of α-amylase activity. The latter set was incubated in 0.2M acetate buffer (pH 5.5) for $\frac{1}{2}$ hour to 1 hour, depending on the activity. After incubation, the gels were placed in a solution containing 0.2% iodine and 2% potassium iodide. The background color of the gels was reddish-brown. A colorless band appeared where the dextrin had been degraded by α-amylase. The location of protein bands was determined in the usual manner using Amido black stain.

The compositions of solutions for the acrylamide gels for experiments at pH 3.2 and 8.9 are listed in Tables 2 and 3 respectively.

TABLE 2. SOLUTIONS FOR GEL ELECTROPHORESIS AT pH 3.2

Chemicals	Upper gel pH 4.9 per 100 ml.	volume ratio	Lower gel pH 3.2 per 100 ml.	volume ratio
Solution A		1		1
acrylamide	10.0 g		30.0 g	
N,N'-methylene bisacrylamide	0.8 g		0.8 g	
Solution B		1		2
1 N potassium hydroxide	48.0 ml		24.0 ml	
acetic acid	3.6 ml		53.2 ml	
N,N,N',N'-tetra methyl-ethylene diamine	0.2 ml		0.4 ml	
Solution C		2		1
ammonium persulfate	60.0 mg		120.0 mg	
riboflavin	1.0 mg		2.0 mg	

Stock buffer solutions*	Upper buffer(pH 3.7) per liter	Lower buffer(pH 4.1) per liter
glycine	28.1 g	--
acetic acid	3.05 ml	43.0 ml
1 N potassium hydroxide	--	120.0 ml

Other solutions

0.01% methyl green used as electrophoretic front marker.

0.5% amido black in 7% acetic acid solution for staining protein bands.

* dilute to $\frac{1}{2}$ strength for both upper and lower electrolytes

TABLE 3. SOLUTIONS FOR GEL ELECTROPHORESIS AT pH 8.9

Chemicals	Upper gel pH 6.7 per 100 ml.	volume ratio	Lower gel pH 8.9 per 100 ml.	volume ratio
Solution A		1		1
1N HCl	48.0 ml		48.0 ml	
TRIS	5.98 g		36.6 g	
N,N,N',N'-tetra methyl-ethylene diamine	0.46 ml		0.23 ml	
Solution B		2		2
Acrylamide	10.0 g		28.0 g	
N,N',-methylene bisacrylamide	2.5 g		0.735 g	
Solution C		1		4
ammonium persulfate	--		0.14 g	
riboflavin	4.0 mg		--	
Solution D		4		1
Water				

Stock buffer solution for upper and lower electrolytes*

	g/l.
TRIS	6.0
glycine	28.8

* dilute to 1/10 strength for both upper and lower electrolytes.

Other solutions

0.001% bromophenol blue as marker.
0.5% amido black in 7% acetic acid or 0.25% coomassie blue in 20% TCA for staining protein bands.

Determination of Proteolytic Activity

Two slightly different procedures were used to prepare the enzyme extracts for determination of proteolytic activity of the flour samples. The first was the method of Chua (1969). According to this procedure the enzyme in 3 g. flour was extracted with 10% saturated ammonium sulfate solution (12 ml.), containing L-cysteine hydrochloride (0.125 g./100 ml.) and disodium ethylene diamine tetraacetate (EDTA, 0.1 g./100 ml.), by stirring with a magnetic stirrer at 6°C for one hour. The extraction mixture was centrifuged for 30 minutes at 3,000 xg and the supernatant was dialyzed against a number (at least six) of changes of distilled water at 6°C for 2 days. For the second extract preparation, 0.2M acetate buffer (pH 3.8) was used for the initial extraction according to the procedure of Wang and Grant (1969).

Proteolytic activity of the extracts was determined by a modified Ayre-Anderson procedure (Chua, 1969) using hemoglobin as the substrate and the method published by Lowry et al. (1951) to estimate the amount of tyrosine released during incubation. The activity was expressed as μmoles of tyrosine released per minute per g. of flour.

Proteolytic activity of the protein fractions obtained by solubility fractionation was estimated by suspending the freeze-dried fraction (5-10 mg. protein) directly in the 1% hemoglobin substrate solution (5 ml.). Following incubation at 38°C and pH 3.8 for 2 hours, the amount of tyrosine released by the enzyme was again measured by the method of Lowry et al. (1951). The activity was expressed as indicated above.

Determination of α-Amylase Activity

Preparation of the β-Limit Dextrin Substrate

β-Limit dextrin was prepared as recommended by MacGregor (1971). Waxy maize starch (20 g.) was suspended in one l. of 90% aqueous dimethyl sulphoxide and stirred until a homogeneous suspension was obtained (ca. 24 hours). The solution was diluted with five l. of distilled water containing 100 ml. of 0.2M acetate buffer (pH 4.6) and 0.2 ml. of sweet potato β-amylase suspension. This reaction mixture was stirred for 24 hours at room temperature. An additional 0.2 ml. of β-amylase was added and allowed to react for another 24 hours at room temperature. The resulting suspension was dialyzed against frequent changes of distilled water for 4-5 days in a cold room (6°C). The dialysate was boiled for 5 minutes to inactivate the β-amylase, cooled, and freeze-dried. The resulting β-limit dextrin was stored in the refrigerator until used.

Activity Assay

The enzyme was extracted from the appropriate flour sample (0.2 g.) with 50 ml. of 0.2M acetate buffer solution of pH 5.5 by magnetic stirring at 6°C for 18 hours (MacGregor *et al.*, 1971). The resulting suspension was filtered through Whatman No. 1 filter paper. The filtrate (2 ml.), containing the α-amylase, was added to 2 ml. of 0.05% β-limit dextrin solution in a test tube and incubated at 35°C for 10, 20 and 30 minutes. The reaction was stopped at the end of each incubation period by adding 10 ml. of 0.005% iodine solution. The mixtures were then left at room temperature for 30 minutes to allow the iodine to

react with the remaining dextrin. The dextrin-iodine complex gave a reddish-brown color. The optical density of this solution was measured at 540 nm. against the iodine blank. The decrease of intensity of the dextrin-iodine color, expressed in O.D., was recorded.

A standard curve was constructed as recommended by MacGregor et al. (1971). This curve is plotted on a semi-log paper using the vertical axis (logarithmic) for O.D. units and the horizontal axis for "relative time" units. For the standard line 0.6 O.D. units was used for 0 "relative time" and 0.4 O.D. units for 100 "relative time". The experimental values of O.D. were normalized to "corrected" O.D. units (C.O.D.) by multiplying each one by 0.6 divided by the O.D. of the substrate blank. The C.O.D. value was then used to read off the relative time from the standard curve. This graphically derived relative time was divided by the actual time of incubation to give an index of relative activity.

Microscopic and Chemical Studies of the Starch

Examination by Ordinary Microscope

Starch granules, washed-out by hand from the five flour samples used in this study, were examined by ordinary light microscopy using the iodine staining technique described by Williams (1969). In each case, the same field was also examined by ordinary microscopy using polarized light. As required, the image was photographed using a 35 mm. camera and Kodachrome II film.

Scanning Electron Microscopy (SEM)

Three types of specimens were prepared for viewing by SEM: 1) flour, 2) starch (washed from flour samples), and 3) cracked seeds. The starch and flour were sprinkled onto double-backed Scotch tape attached to the specimen stubs. The whole seeds were cracked in half in order to expose the endosperm cells and attached to the specimen stubs by a spot of silver dag. All the samples were coated in a vacuum evaporator first with carbon and then by approximately 200-250 Å of gold. The samples were rotated and tilted during evaporation in order to ensure good coverage. The coated specimens were viewed in a Cambridge Stereoscan MKIIa scanning electron microscope at 5 kv accelerating potential and photographed on 35 mm. Kodak Tri X film.

Determination of Starch Damage

Two methods were used to determine the magnitude of the so-called starch damage. The first was the procedure of Farrand (1964). This method is based on the known fact that only damaged starch is readily attacked by cereal α-amylase in aqueous suspension. In the actual assay, the flour is incubated with a relatively large amount of wheat α-amylase extract. The amount of maltose released is a direct measure of the starch damage which is expressed in arbitrary Farrand units. The second procedure was described by Williams and Fegol (1969). This procedure is based on the color that develops when iodine is added to an extract of the flour obtained with a strong solution of sodium sulfate containing 15% formamide and 0.2% sulfosalicylic acid. The results are recorded as absorbance at 555 nm. and starch damage is read off a calibration

curve obtained by analyzing flours of known starch damage (determined by some other more elaborate procedure) such as that of Farrand (1964).

Determination of Free Sugars

The amount of free sugars in the flours was determined as follows. First, the sugars were extracted using the procedure described by Shannon (1968). The sugar content of the extract was then determined by the phenol-sulfuric acid method. Glucose was used as the standard in the determination and the actual amount of free sugars was expressed in mg. glucose per g. of flour.

RESULTS AND DISCUSSION

Effects of Sprouting on Properties Related to Milling and Baking Quality

Technological data related to milling and baking quality of the five flour samples are summarized in Table 4. Standard farinograms are shown in Fig. 3 and simple correlations between parameters are listed in Table 5.

Effects on Milling Quality

Flour yield of wheat decreased considerably upon germination and sprouting (Table 4). The ash content of flours milled from sprouted wheats was definitely lower than that of the control. The lowering of endosperm ash content during soaking and sprouting can be attributed to leaching and metabolic utilization of inorganic ions. The color of the flour from the 4- and 8-day sprouted samples was much darker (higher color units) than that of the control. The protein content decreased slightly throughout sprouting. In conclusion, sprouting damages milling quality by decreasing flour yield and color. The results are in general agreement with the more limited data of Geddes et al. (1941). They found that flour milled from malted wheat was considerably darker than that from sound wheat.

Effects on Baking Quality

Table 4 also shows a gradual decrease of flour protein during sprouting. This observation is in agreement with those of McCalla (1934) and Coulson and Sim (1965). These workers observed a significant loss of endosperm protein during sprouting. Coulson and Sim (1965) attributed

TABLE 4. EFFECTS OF SPROUTING ON MILLING AND BAKING QUALITY OF A HARD RED SPRING WHEAT

		Germinated			
	Control	Soaked	2 Days	4 Days	8 Days
Yield, %	73.5	75.1	71.9	71.9	63.8
Ash, % (14% m.b.)	0.40	0.36	0.25	0.29	0.27
Color, units	0.6	0.1	0.6	1.4	2.7
Protein (14% m.b.)	13.4	13.5	12.6	12.3	12.0
Sedimentation value	58.5	69.0	44.5	42.5	30.0
Specific sedimentation value	4.4	5.1	3.5	3.4	2.5
Amylograph viscosity	500.0	50.0	50.0	50.0	50.0
Starch damage (Farrand units)					
Farrand (1964)	22.5	0.0	14.4	34.8	44.4
Williams and Fegol (1969)	25.7	5.9	20.3	30.3	59.7
Farinogram					
absorption, %	67.1	58.8	58.9	56.4	54.3
development time, min.	3.5	3.0	2.0	1.5	1.0
M.T.I., B.U.	25.0	65.0	120.0	260.0	350.0
Baking absorption, %	63.1	54.8	51.9	--	--
Loaf volume, cc. (remix)	925.0	805.0	660.0	--	--
No malt loaf volume, cc. (remix)	800.0	870.0	610.0	--	--

Figure 3.

Farinograms of flours milled from control, soaked, 2-, 4- and 8-day germinated wheat.

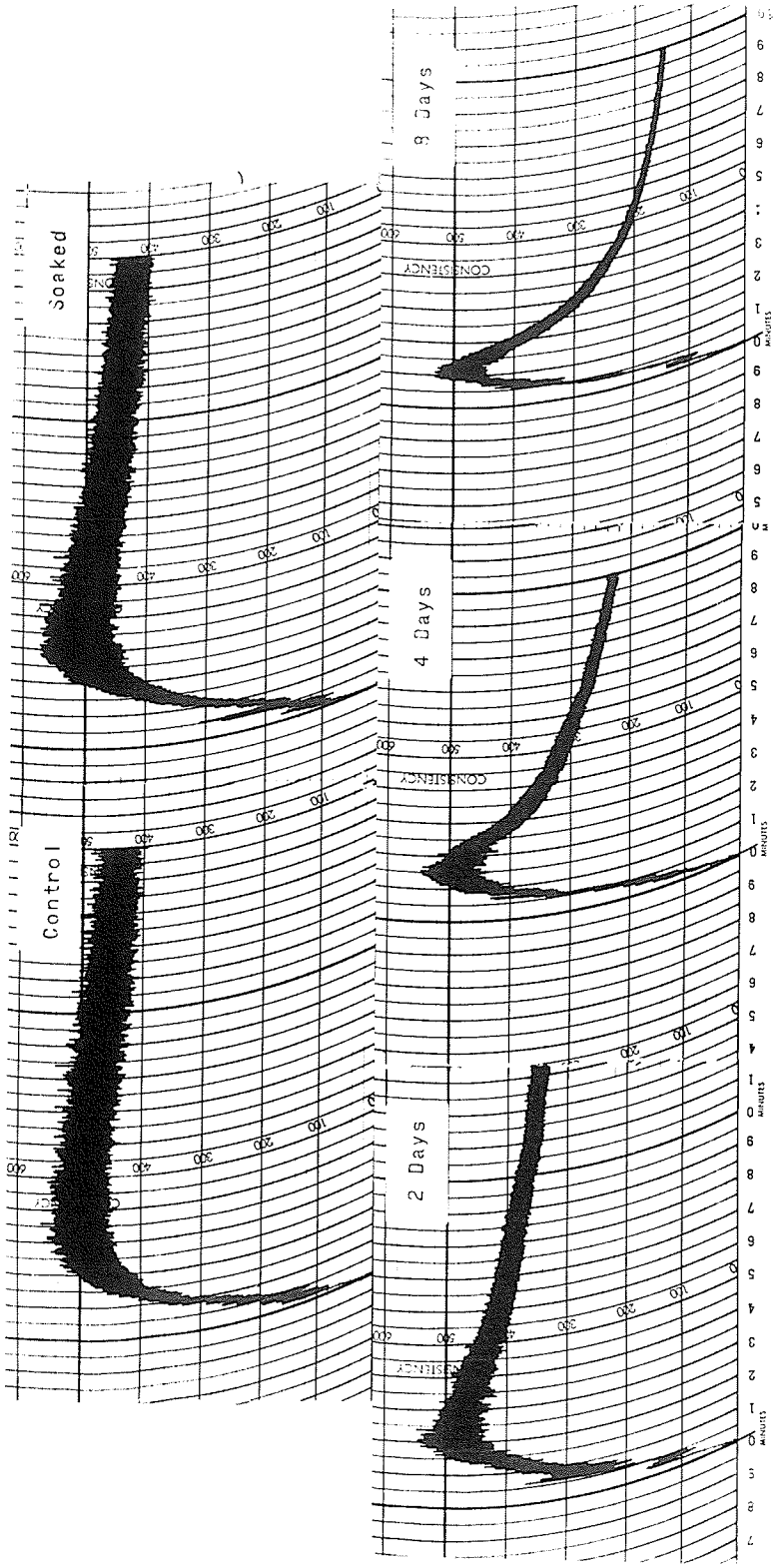

Control
Soaked
2 Days
4 Days
8 Days
CONSISTENCY
MINUTES

TABLE 5. CORRELATIONS BETWEEN SOME QUALITY PARAMETERS

Variables	r value	n
Farinograph absorption versus:		
flour protein	+0.76 *	5
Starch damage (enzymic procedure)	-0,95 **	4
α-amylase activity	-0.99 **	4
Sedimentation value versus:		
flour protein	+0.97 **	5
starch damage (enzymic procedure)	-0.91 *	4
Starch damage (enzymic procedure) versus:		
α-amylase activity	+0.98 **	4
free sugars	+0.96 **	4
α-Amylase activity versus:		
free sugars	+0.99 **	4

** significant at the 1% level

* significant at the 5% level

this loss to degradation and utilization of the proteins during sprouting.

The sedimentation value decreased considerably with increasing sprouting. On the other hand, the flour from the soaked sample had a much higher value than the flour from sound (control) wheat. If the sedimentation value is divided by percentage of protein in the flour, a "specific sedimentation value" is obtained. This value can be used as an index of protein quality as suggested by Zeleny (1947). For the flours used in this study, the specific sedimentation value decreased gradually with increasing sprouting. The soaked sample again gave a higher value than the control. This decrease in specific sedimentation value indicated a rapid deterioration of baking quality (as measured by the sedimentation value). The fact that the sedimentation value can be increased by soaking underlines one of the main shortcomings of this test as a measure of breadmaking quality.

The amylograph viscosity of all flours, except the control, was below 50 B.U. These very low values indicate that α-amylase activity of these samples was extremely high.

The starch damage values with both the Farrand (1964) and the Williams and Fegol (1969) procedures showed similar trends. Both methods gave extremely low values for the soaked sample. As the wheat sprouted the starch damage increased rapidly. The values for the 4- and 8-day samples were considerably higher than for the control. The nature of starch damage in the sprouted samples will be discussed later.

The gradual deterioration of baking quality with sprouting is also indicated in the properties measured on the Farinograph (see Table 4 and also Fig. 3). There was a large decrease in absorption, a decrease

in dough development time and a marked increase in mixing tolerance index with increasing sprouting. The rapid weakening of the farinograph mixing is particularly evident from the farinograms in Fig. 3.

Table 4 also gives the results of the baking tests. Because of the high α-amylase activity of some of the flours, all five flours were also baked without any malt in the formula. Of the flours from sprouted wheat, only the 2-day sample could be baked into bread. The 4- and 8-day samples were too sticky and could not be handled in the usual manner after mixing. For the remix baking test a suitable baking absorption is usually four percentage units lower than the farinograph absorption. For the 2-day sample, an additional decrease of three units was made to produce a dough that could be handled. This marked decrease in absorption is in general agreement with the observation of Bushuk et al. (1966) who found that the absorption dropped from 66% to 63% when a small amount of malt was added to decrease the Brabender amylograph viscosity from 900 to 300 B.U.

The loaf volume, by the normal remix procedure, was higher for the control. The soaked sample had a satisfactory volume but the volume of the 2-day sample was completely unacceptable. When the malt was omitted from the formula, the soaked sample had the highest volume, indicating that its excessive α-amylase activity was probably the reason it performed poorly in the regular baking test. These baking results confirm published reports and common knowledge of the extreme importance of α-amylase activity in baking quality.

To obtain further information on the interrelationships between baking quality tests, simple statistical correlations were calculated

for a number of pairs of tests. In spite of the very low number of samples, it was felt that this exercise would provide useful information. Correlations that were significant at the 1 and 5% levels are listed in Table 5.

As anticipated, baking absorption was positively correlated with protein content and negatively with α-amylase activity. Normally, absorption increases with increasing starch damage. For example, Bushuk et al. (1966) showed that for the remix test the optimum absorptions for the same flour with starch damage values of 23.3 and 30.5% were 66 and 68% respectively. The anomalous results obtained in the present study suggest that an increase in damaged starch produced by amylase action does not have the same effect on water absorption as does physically damaged starch. The data obtained could also result from the overriding effect of proteins on absorption. As sprouting proceeds, the proteins become degraded (to be discussed later) and thereby lose their ability to bind water. Concomitantly, the amount of enzymic starch damage increases.

Sedimentation value was positively correlated with protein content at the 1% level of significance. It is common knowledge that, for flour samples from sound wheats of similar type, the sedimentation value increases directly with protein content over the normal range of contents. The significant negative correlation between the sedimentation value and the starch damage of the flours used in the present study probably arises from the same factors that gave rise to the negative correlation between absorption and starch damage (see above).

Correlations involving α-amylase activity and free sugar content will be discussed later after data on these analyses are presented.

Analyses on Flour

Amino Acid Composition

It was of interest to determine the amino acid composition of flours used in the present study because a change in composition would indicate a change in the proteins which could subsequently be reflected as a change in baking quality.

The amino acid compositions for the five flour samples are given in Table 6. Sixteen amino acids were determined on the automatic analyzer and cysteine and cystine were determined by amperometric titration. The compositions showed only minor changes during sprouting. Arginine decreased during sprouting. The three sprouted samples were significantly lower than the control and the soaked sample. Cysteine also showed a small but significant decrease. Of the other amino acids, proline showed a gradual decrease while cystine increased slightly with soaking and germination. The change in cystine for any flour relative to the value for the control was not equivalent to the change in cysteine.

Sulfhydryl and Disulfide Contents

Sulfhydryl (SH) and disulfide (S-S) contents were determined to check the results of Shorina et al. (1967). These workers obtained some indication, although not too clear cut, that the number of S-S groups in gluten decreased while the SH content increased during sprouting.

The results obtained for the five flour samples used in the present study are given in Table 7. Each value is an average of three analyses.

Flour from soaked wheat had a slightly higher SH content than the control. The content decreased by 20-30% during sprouting. There was

TABLE 6. AMINO ACID COMPOSITIONS FOR THE FIVE FLOURS *

Sample	Control	Soaked	2-day	4-day	8-day
% Protein, 0% m.b.	15.4	15.5	14.5	14.1	13.9
Lysine	2.27	2.14	2.21	2.18	2.03
Histidine	3.49	3.45	3.48	3.54	3.52
Ammonia	21.51	22.06	21.30	21.20	21.07
Arginine	6.92	6.83	6.15	5.99	6.14
Cysteine	0.06	0.06	0.04	0.04	0.04
Aspartic Acid	2.45	2.53	2.51	2.54	2.62
Threonine	1.83	1.82	1.82	1.74	1.76
Serine	3.66	3.62	3.68	3.51	3.50
Glutamic Acid	22.33	22.28	22.40	21.65	21.00
Proline	10.10	9.65	9.96	9.61	9.57
Glycine	4.05	3.97	4.00	3.91	3.89
Alanine	2.68	2.65	2.67	2.64	2.66
Cystine	1.14	1.30	1.60	1.33	1.41
Valine	2.78	2.83	2.75	2.77	2.72
Methionine	0.88	0.84	0.93	0.80	0.89
Isoleucine	2.28	2.31	2.25	2.30	2.24
Leucine	4.56	4.63	4.58	4.53	4.42
Tyrosine	1.43	1.39	1.37	1.29	1.36
Phenylalanine	2.69	2.82	2.67	2.79	2.64
N % Recovery	97.11	97.18	97.44	94.36	93.48

* g. of amino acid per 100 g. of protein

TABLE 7. SULFHYDRYL AND DISULFIDE CONTENTS OF THE FLOURS

	-SH (μeq./g.flour)	S-S (μeq./g.flour)
Control	1.06	12.0
Soaked	1.14	13.7
Germinated		
2 days	0.78	15.0
4 days	0.70	12.5
8 days	0.72	13.0

essentially no difference in the values for the 2-, 4-, and 8-day samples.

The disulfide contents of the flour from the soaked wheat and the 2- and 8-day samples were significantly higher than that of the control. Other than the normal sampling error which is quite high for wheat, the reason for this difference is not obvious. The experimental error in the disulfide determination is about 5%. Disulfide content of the 4-day sample was essentially the same as that of the control flour.

The results obtained in the present study on flour apparently disagree with those obtained on washed-out gluten of Shorina et al. (1967). It is quite possible that the decrease in disulfide content of gluten during sprouting that these workers obtained resulted indirectly from a partial fractionation of the gluten during washing. For example, the gluten from normal wheat could be quite different than the gluten from sprouted wheat owing to the degradation of the proteins that occurs (to be discussed later). Also it must be kept in mind that the wheat used by the Soviet workers was different from the one used in the present study.

Amino Groups

The flours were analyzed for amino groups in order to examine the possibility of peptide bond cleavage during sprouting. Results obtained are tabulated in Table 8.

The number of amino groups increased rapidly during sprouting, indicating a highly significant hydrolysis of peptide bonds. In general, these results agree with those of Beresh (1969) who showed that flour from wheat sprouted for four days had about eight times as many free

TABLE 8. AMINO GROUPS OF THE FIVE FLOURS

	μmoles/g. N_2
Control	1.75
Soaked	2.55
Germinated	
2 days	4.18
4 days	7.17
8 days	10.18

amino groups as the flour from dormant wheat. Beyond four days of germination he observed a decrease in the number of amino groups. A similar decrease was not observed in the present study. It would be expected however that as the new wheat plant continues to grow, eventually the endosperm should show a decrease in amino groups due to the depletion of the amino acids by the metabolism of the young plant. The time when the amino content would be at a maximum would probably depend on the wheat variety and on conditions (temperature, moisture, etc.) of sprouting.

Solubility Fractionation of Flour Proteins

The distribution of protein (Kjeldahl nitrogen x 5.7) among the five solubility fractions obtained by the modified Osborne fractionation technique is given in Table 9.

A considerable increase in the proportion of the water-soluble proteins was obtained when the wheat was soaked. With sprouting, there was a slight decrease in this fraction compared with that of the control. Results from gel chromatographic fractionation (to be discussed later) indicate that decreases in three of the solubility fractions (water-soluble, salt-soluble and residue) are paralleled by increases in the amount of low molecular weight components. Presumably in the solubility fractionation, these are removed in the initial salt-solution extract. Subsequently most of this material would be lost in the dialysis step used to separate the water- and salt-soluble components. The nature of the dialyzable components was not investigated.

TABLE 9. PROTEIN SOLUBILITY DISTRIBUTION FOR THE FIVE FLOUR SAMPLES

Protein fractions	Control	Soaked	Germinated			S.D.*
			2 Days	4 Days	8 Days	
	Percent of Flour Protein					
Water-soluble	7.9	11.9	6.5	6.7	5.1	0.5
Salt-soluble	4.1	3.0	1.5	2.5	1.2	0.3
Ethanol-soluble	43.7	38.3	37.4	42.2	38.7	3.6
Acetic acid-soluble	11.6	12.6	18.3	24.2	28.5	2.8
Residue	26.7	29.0	25.3	11.1	7.2	1.0
Acetic acid-soluble + residue	38.3	41.6	43.6	35.3	35.7	-
Recovery	94.0	94.8	89.0	86.7	80.7	-

* Standard deviation based on five fractionations

The amount of the salt-soluble fraction decreased during soaking and germination and sprouting. On the other hand, alcohol-soluble proteins remained rather constant for all five flours. The most notable change observed was the gradual increase with sprouting in the acetic acid-soluble fraction and a parallel decrease in the residue proteins. The sum of the latter two fractions showed only a small gradual decrease with sprouting. These results suggest that part of the residue protein gradually becomes soluble in acetic acid solution during sprouting.

The solubility fractionation procedure was also applied to the five wheats ground on an analytical micro mill, instead of flour. All but one solubility fraction (see Appendix I) followed similar trends as those for flours shown in Table 9. The salt-soluble fraction of ground wheats increased during sprouting whereas the same fraction from flour showed a decreasing trend. The sum of water- and salt-soluble fractions of whole wheats was higher than the sum of the same fractions of the flours. This is likely due to the fact that wheat bran has higher percentages of water- and salt-soluble proteins than the endosperm (Teller and Teller, 1932). On the other hand, gluten, which includes the alcohol-soluble, acetic acid-soluble and residue proteins, was lower for ground whole wheats than for the flours obtained from the same wheats.

Sephadex Chromatography Using AUC Solvent

Using Sephadex G-150 as the support material and AUC as the eluting solvent, Meredith and Wren (1966) suggested that wheat flour proteins can be readily fractionated into four fractions of decreasing molecular weight:

Fractions	Molecular weight
I	> 100,000
II	25,000-100,000
III	10,000-25,000
IV	< 10,000

In addition, Meredith and Wren (1966) referred to fractions I, II and III as glutenin, gliadin and albumin respectively. The use of these terms is perhaps unfortunate since, as will be seen later, these fractions are not equivalent to the same fractions obtained by the Osborne solubility fractionation technique. Fraction IV obtained by Sephadex chromatography is considered to be low molecular weight peptides and free amino acids.

AUC Extracts

The amount of protein extracted out by AUC solvent was approximately 95% which is similar to that reported by others who have used this solvent (Meredith and Wren, 1966, and Bushuk and Wrigley, 1971).

The gel elution profiles of the extracts of ground wheats are shown in Fig. 4. The distributions of the four fractions obtained by the gel filtration technique are given in Table 10.

The results (Fig. 4 and Table 10) show a marked decrease during sprouting in the amount of fraction I and II proteins. The amount of fraction III proteins changed very little. A definite increase in fraction IV was observed. The increase in fraction IV agrees with the results of Beresh (1969) who found a marked increase in non-protein nitrogen and decrease in protein nitrogen during germination and sprouting.

Figure 4

Elution profiles of AUC extracts of ground wheats.

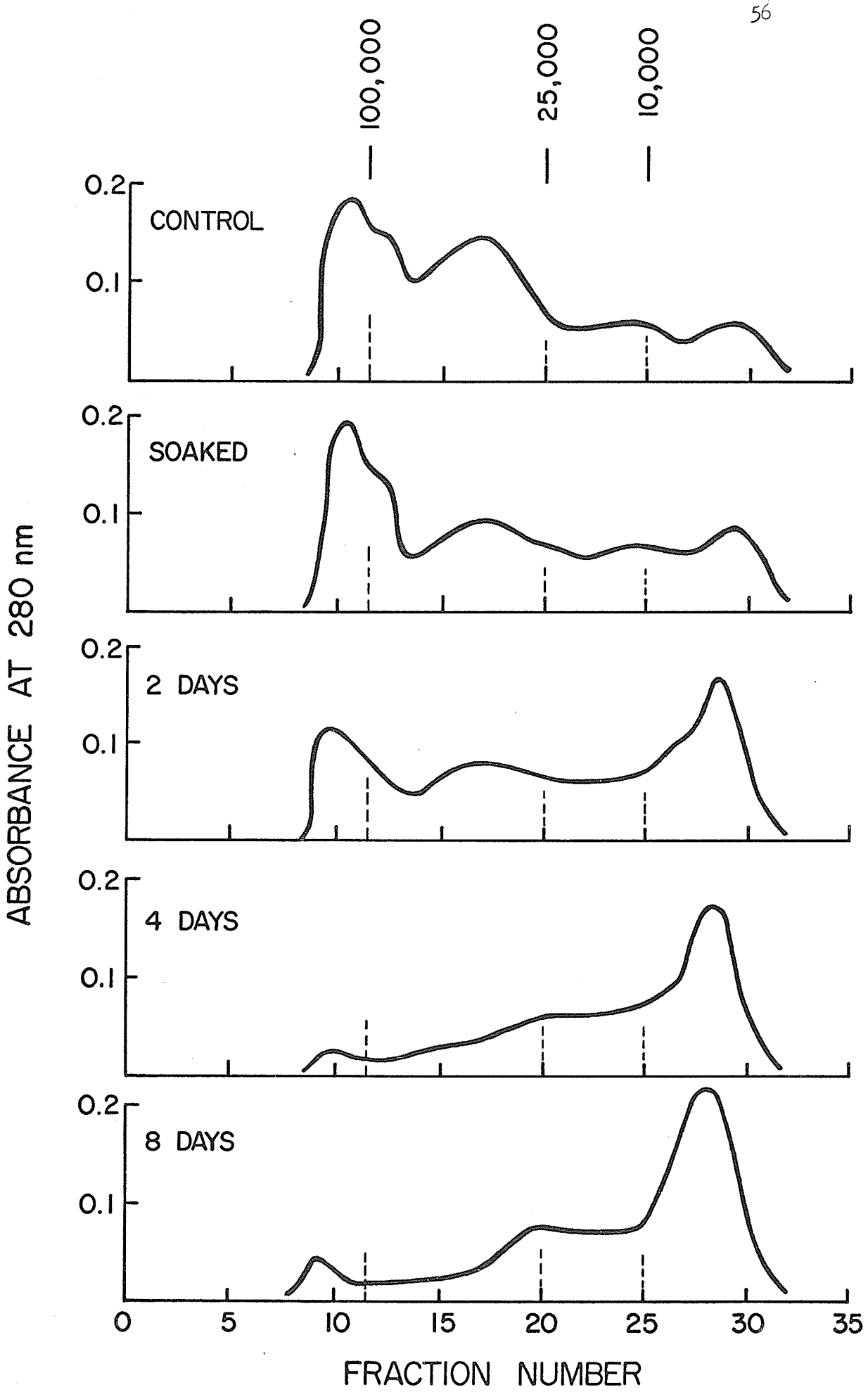

100,000
25,000
10,000
CONTROL
SOAKED
2 DAYS
4 DAYS
8 DAYS
0.2
0.1
ABSORBANCE AT 280 nm
0
5
10
15
20
25
30
35
FRACTION NUMBER

TABLE 10. FRACTIONATION OF AUC EXTRACTS OF GROUND WHOLE WHEATS BY SEPHADEX G-150 CHROMATOGRAPHY

Fraction	I	II	III	IV
Mol. wt.	> 100,000	100,000-25,000	25,000-10,000	< 10,000
	%	%	%	%
Control	33.0	31.6	16.5	18.9
Soaked	23.0	34.5	19.1	23.4
Germinated				
2 days	21.0	27.0	16.0	36.0
4 days	6.3	18.0	25.2	50.5
8 days	6.5	17.6	18.9	57.0

The elution profile of whole wheat extracts changed very little after two days of soaking (Fig. 4). The elution profile for the extract of the 2-day sample showed a considerable change from the control. A tremendous change in the profiles was observed for the 4- and 8-day samples.

The AUC extracts of flours (instead of ground whole wheat) were also fractionated on Sephadex G-150. These results are shown in Fig. 5 and Table 11.

Qualitatively the elution profiles for the flour extracts (Fig. 5) were similar to those for ground wheat (Fig. 4). The main difference in the profiles for the control and soaked samples was that the flours had relatively more of group I components and less of group IV components. These observations agree with the results obtained by the solubility fractionation (see above).

The changes during sprouting shown by the profiles for the flours were not as marked as those obtained for the extracts of ground wheats. Qualitatively, the same changes were observed. With increasing sprouting the proportion of fraction I proteins decreased while that of group IV increased (see also Table 11). The changes in the two intermediate fractions were small and insignificant and showed no definite trend with increasing sprouting.

The results for the control flour agree quantitatively with the results of Meredith and Wren (1966) and Bushuk and Wrigley (1971). Accordingly, it appears that the gel filtration technique might be a good reproducible technique for fractionating flour proteins.

Figure 5

Elution profiles of AUC extracts of the five flours.

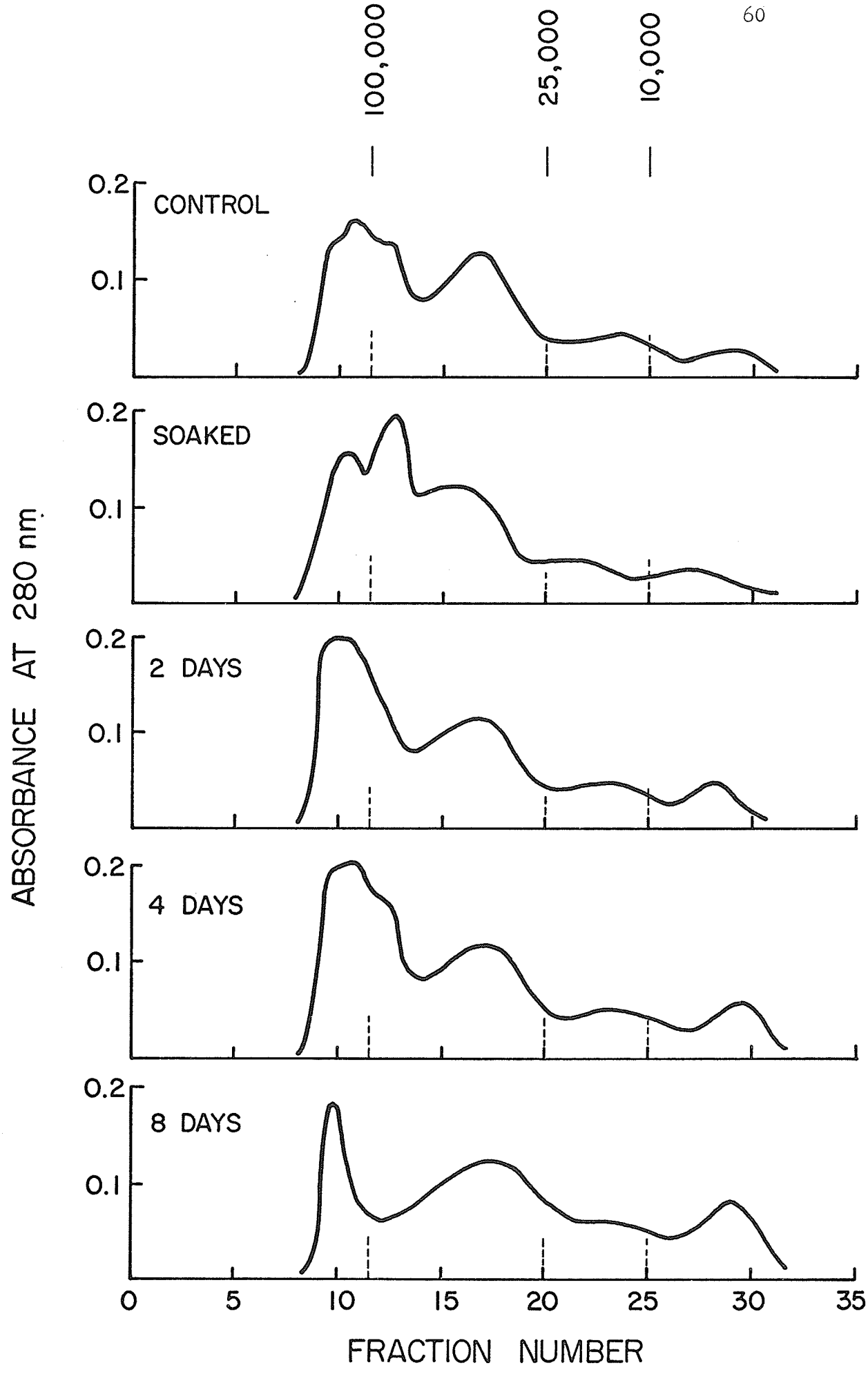

100,000
25,000
10,000
CONTROL
SOAKED
2 DAYS
4 DAYS
8 DAYS
0.2
0.1
ABSORBANCE AT 280 nm
0
5
10
15
20
25
30
35
FRACTION NUMBER

TABLE 11. FRACTIONATION (%) OF AUC EXTRACTS OF FLOURS BY SEPHADEX G-150 CHROMATOGRAPHY

Fraction	I	II	III	IV
Mol. wt.	>100,000	100,000-25,000	25,000-10,000	< 10,000
	%	%	%	%
Control	30.7	45.4	15.4	8.5
Soaked	37.6	45.0	11.8	5.6
Germinated				
2 days	31.7	42.0	13.5	12.8
4 days	25.6	45.5	13.3	15.6
8 days	19.8	40.3	19.1	20.8

Solubility Fractions

Protein fractions obtained by the modified Osborne fractionation procedure were dispersed in AUC solvent and fractionated into four groups according to molecular weight using Sephadex G-150 as the support material and AUC as the eluting solvent. The purpose of this experiment was to compare the equivalent fractions (e.g. glutenins, gliadins and albumins) obtained by the two fractionating techniques. To avoid possible confusion, solubility fractions will be identified by capital letters A to E and the gel filtration fractions by Roman numerals I to IV.

Results for flour from sound control wheat are presented in Table 12 and the elution profiles for the five solubility fractions are shown in Fig. 6.

The water-soluble fraction (A) comprised 36.8, 31.2 and 32.0% of fractions II, III and IV respectively. That is, over half of the components of the so-called albumin fraction obtained by the Osborne procedure would be classified other than albumin by the Meredith and Wren (1966) gel filtration technique. Approximately one third of the water-soluble fraction was eluted in the gliadin region (II) and a third in the low molecular weight region (IV).

Over half (59.7%) of the salt-soluble fraction (B) was eluted in the region over 100,000. It is suspected that this might arise from aggregation which could occur during dialysis and subsequent freeze drying used in the preparation of this fraction by the Osborne technique. The salt-soluble fraction also contained a considerable

TABLE 12. SEPHADEX G-150 CHROMATOGRAPHY OF OSBORNE FRACTIONS OBTAINED FROM A SOUND WHEAT FLOUR

Protein Fraction	I	II	III	IV
Mol. wt.	> 100,000	100,000-25,000	25,000-10,000	< 10,000
water-soluble, %	--	36.8	31.2	32.0
salt-soluble, %	59.7	23.8	16.5	--
alcohol-soluble, %	19.8	76.6	3.6	--
acetic acid-soluble, %	49.5	50.5	--	--
residue, %	57.0	43.0	--	--

Figure 6

Elution profiles of the five solubility protein fractions of the control flour.

Protein fractions are as follows:

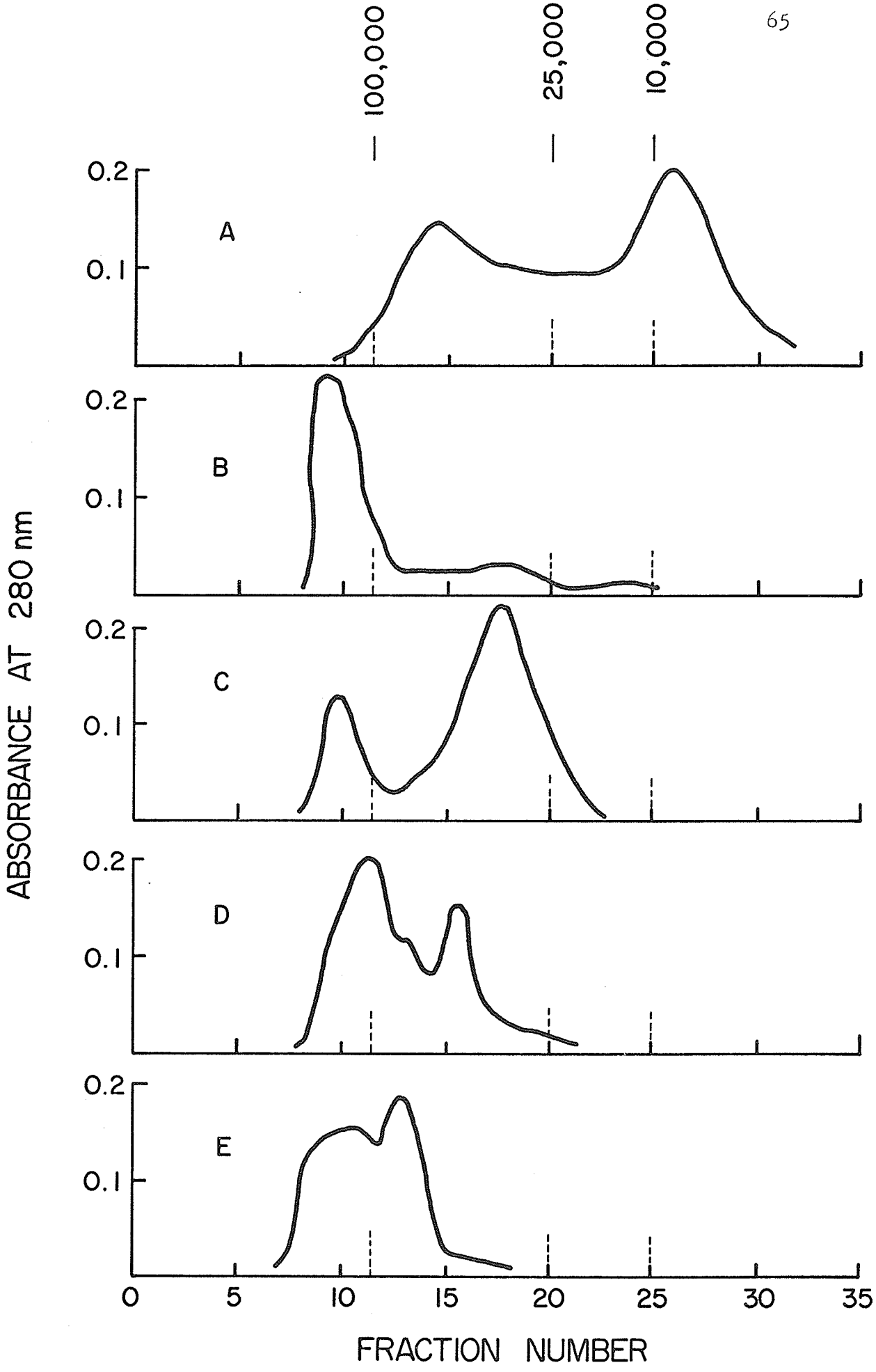
A. water-soluble

B. salt-soluble

C. alcohol-soluble

D. acetic acid-soluble

E. residue


100,000
25,000
10,000
A
B
C
D
E
0.2
0.1
ABSORBANCE AT 280 nm
0
5
10
15
20
25
30
35
FRACTION NUMBER

amount of fraction II proteins (gliadins) but no fraction IV proteins.

The alcohol fraction (C) was the most homogeneous in molecular weight of the five fractions obtained by the Osborne procedure. Fraction II proteins (gliadins) formed 76.6% of this fraction. In addition, this fraction contained a fair quantity of fraction I proteins (19.8%), a small amount of fraction III but no fraction IV.

Acetic acid-soluble fraction (D) contained equal amounts of fraction I and II while the residue proteins (E) had slightly more fraction I than II. It is seen that when the glutenin fraction by the Osborne technique is fractionated by gel filtration, approximately half of it is eluted in the Meredith and Wren gliadin region. The acetic acid-soluble and residue fractions had no fractions III and IV proteins. The salt-solution extraction of the Osborne procedure appears to be quite efficient in removing the low molecular weight components. However, these results show that the fractions obtained by the solubility fractionation are not equivalent to the same fractions obtained by gel filtration according to Meredith and Wren (1966). Accordingly, it would be highly desirable to redefine (and perhaps rename) the wheat endosperm proteins in order to prevent further confusion in the literature.

The results obtained here showing a wide range of molecular weights for endosperm proteins of the same solubility are in general agreement with those reported by many earlier workers. Beckwith et al. (1966) and Bernardin et al. (1967) reported, independently, that the molecular weight of wheat gliadins ranged from 25,000 to 50,000. Jones et al. (1965) obtained 31,000 for the molecular weight of γ-gliadin. Ewart (1969), Feillet and Nimmo (1970), and Fish and Abbott (1969) used

different techniques, including gel filtration, and obtained 26,250, 20,000, 12,900, 16,000 and 19,300 for the molecular weights of purified wheat albumins. Using the ultracentrifuge, Jones et al. (1961) obtained a weight-average molecular weight of 2-3 million for wheat glutenins. Their glutenin preparation also contained a significant quantity of small molecules (molecular weight of about 50,000). Kelley (1964) obtained a value of 75,000 for the molecular weight of a purified salt-soluble protein from wheat flour.

Solubility fractions of the flours from the soaked and sprouted wheats were also examined by Sephadex chromatography using AUC solvent. Their elution profiles are shown in Figs. 7 to 10. Distributions of the various fractions for each sample among the four molecular weight groups were determined and are tabulated in Appendix II.

The elution profiles for the soaked and sprouted samples were in general similar to those obtained for the control (compare Figs. 7 to 10 with Fig. 6). With increasing sprouting, there were gradual qualitative and quantitative changes in the profiles. These are discussed below.

Water-soluble fraction (A) showed a gradual decrease in the amount of fraction II and a concomitant increase in the amount of fraction III proteins. It appears that the separation of the albumins (fraction III) from the gliadins (fraction II) by extractions with salt solution is much more efficient when the flour is milled from sprouted wheat.

The salt-soluble fraction (B) showed a marked qualitative and quantitative change. Fraction I components decreased gradually to the point where the extract for the 8-day sample showed a major peak at the

Figure 7

Elution profiles of the five solubility protein fractions from soaked sample.

A to E refer to the same protein fractions as in Fig. 6.

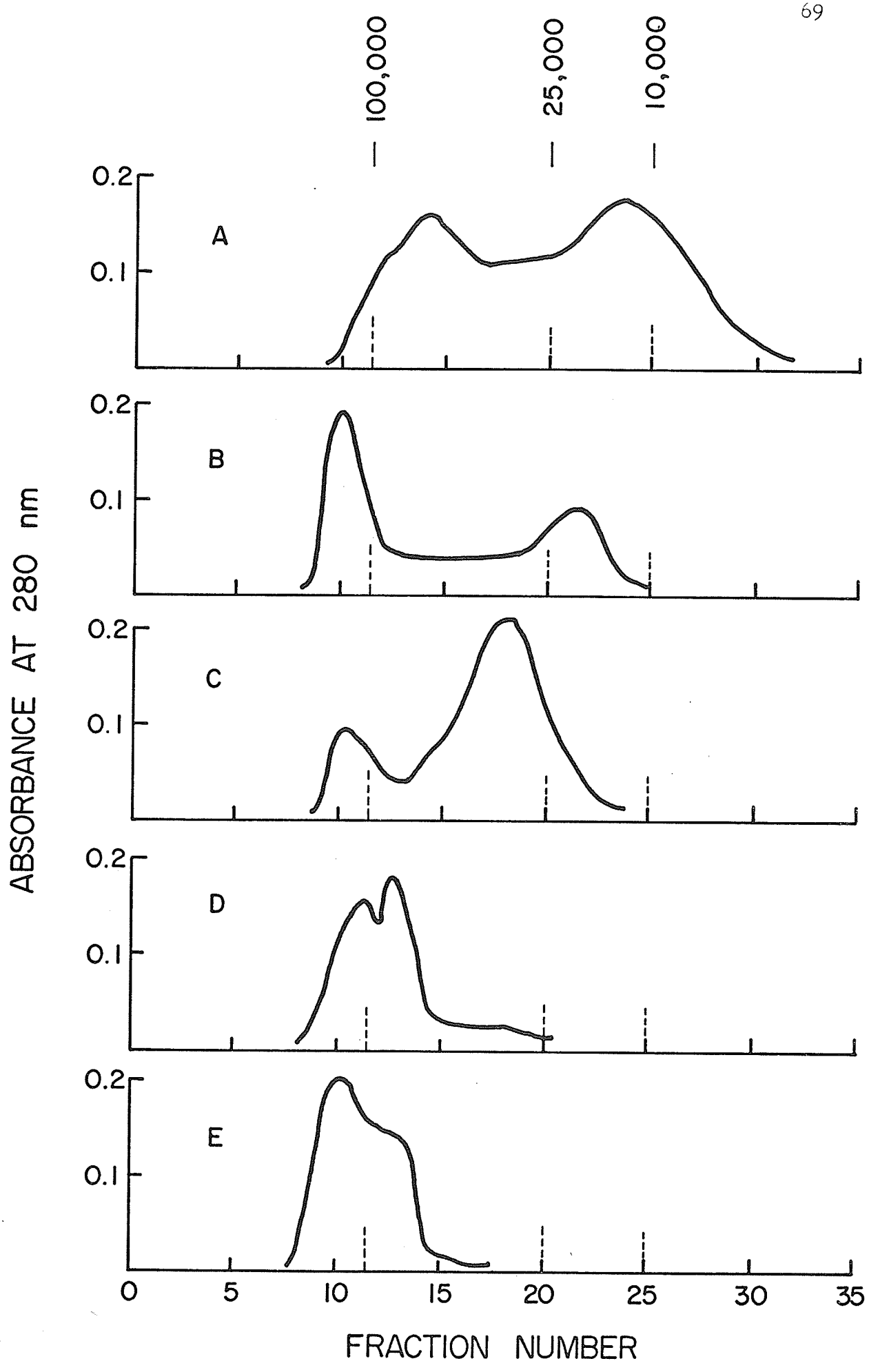

100,000
25,000
10,000
0.2
0.1
A
B
C
D
E
ABSORBANCE AT 280 nm
0
5
10
15
20
25
30
35
FRACTION NUMBER

Figure 8

Elution profiles of the five solubility fractions from 2-day sample.

The protein fractions are the same as those in Fig. 6.

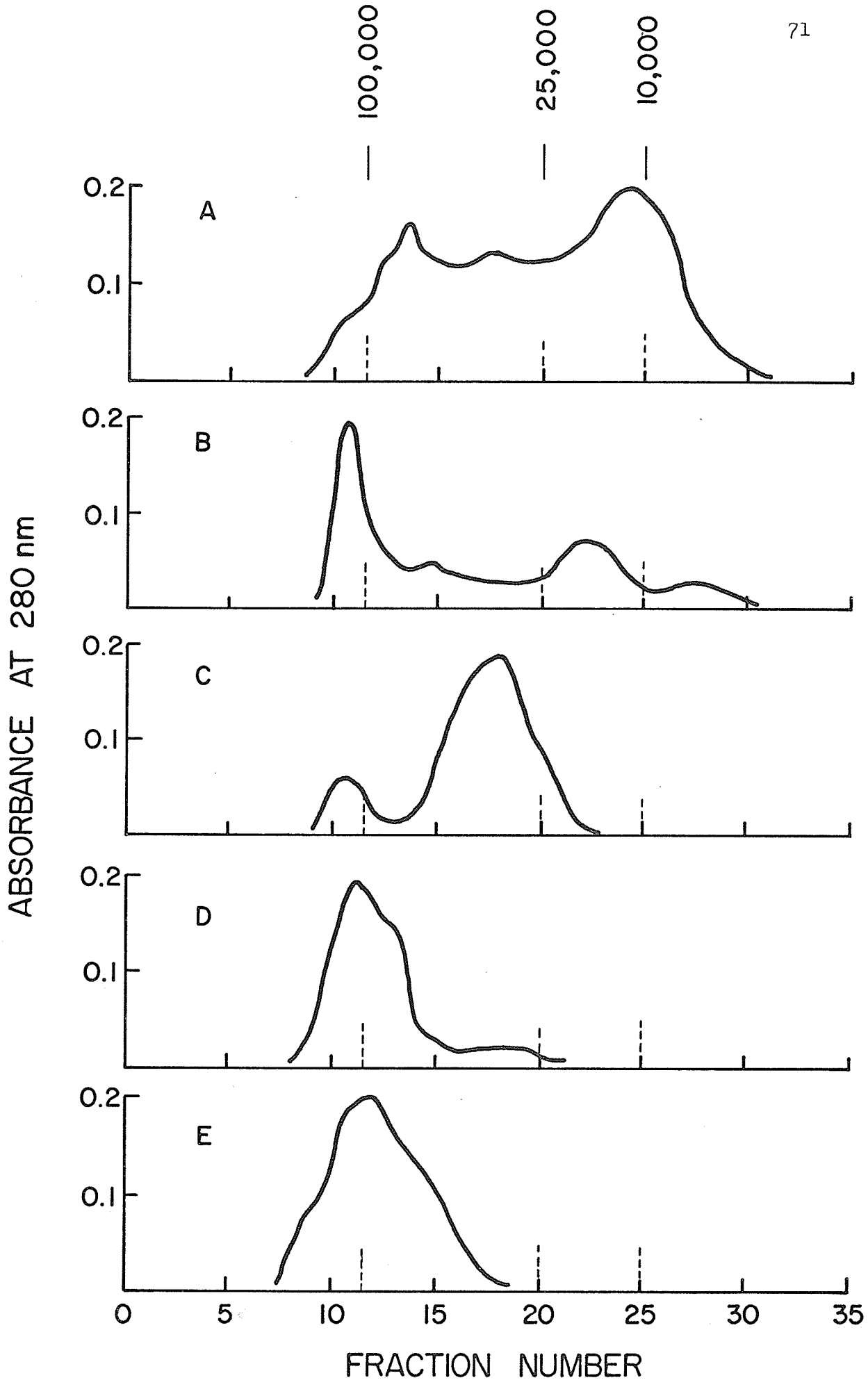

100,000
25,000
10,000
A
B
C
D
E
0.2
0.1
ABSORBANCE AT 280 nm
0
5
10
15
20
25
30
35
FRACTION NUMBER

Figure 9

Elution profiles of the five solubility protein fractions from 4-day sample.

The protein fractions are the same as those in Fig. 6.

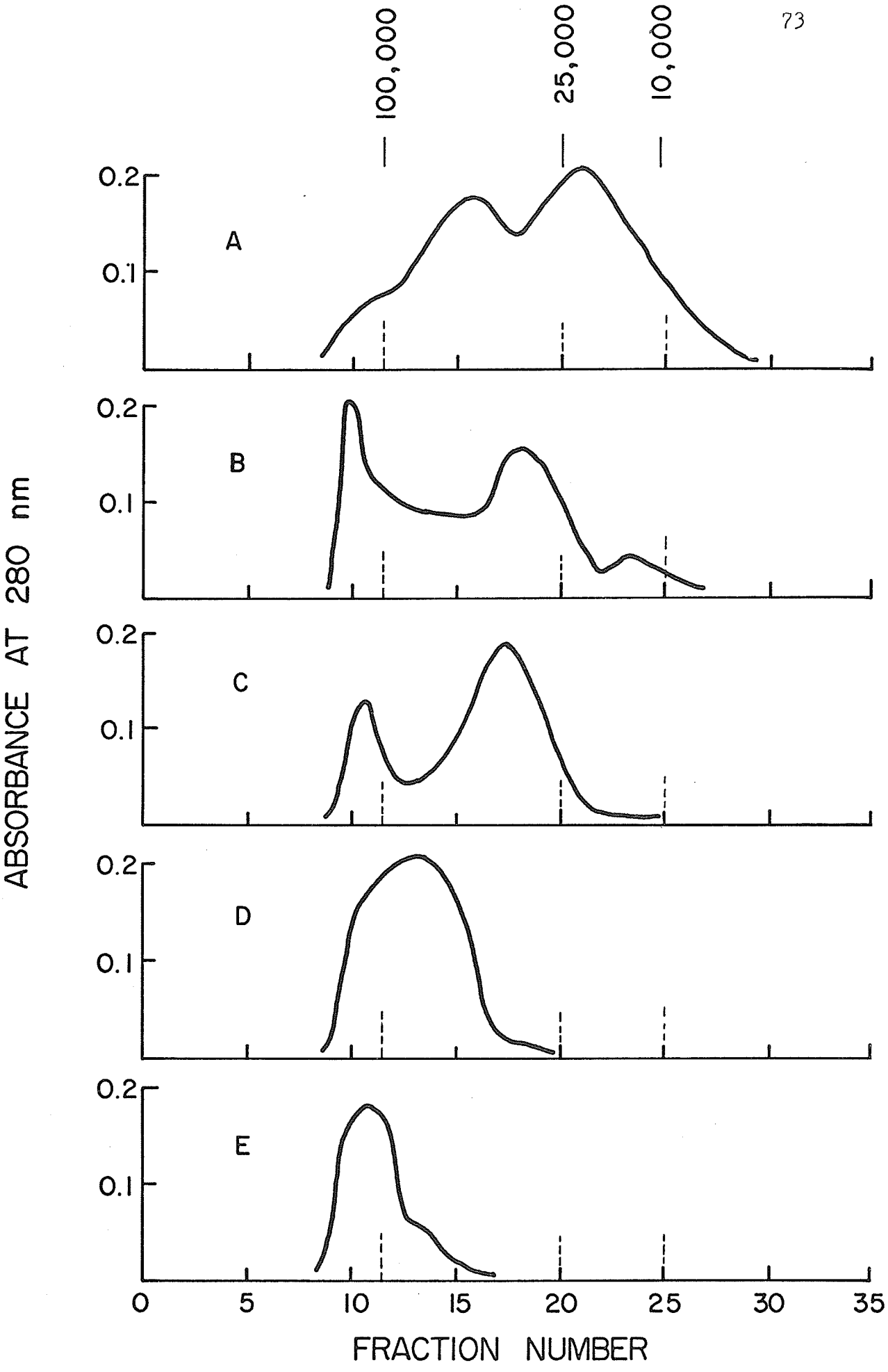
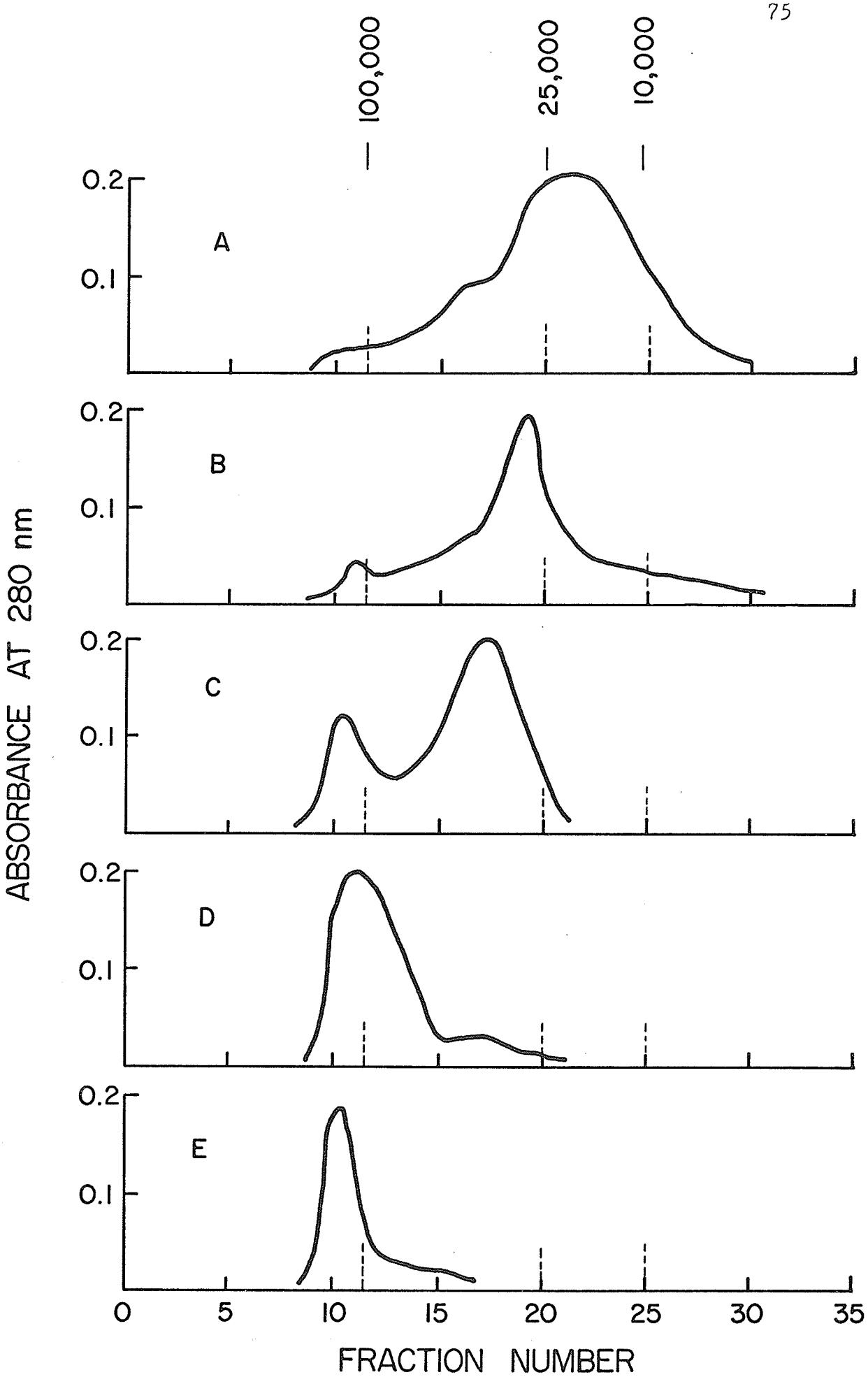

100,000
25,000
10,000
A
B
C
D
E
0.2
0.1
ABSORBANCE AT 280 nm
0
5
10
15
20
25
30
35
FRACTION NUMBER

Figure 10

Elution profiles of the five solubility protein fractions from 8-day sample.

The protein fractions are the same as those in Fig. 6.


100,000
25,000
10,000
A
B
C
D
E
0.2
0.1
ABSORBANCE AT 280 nm
0
5
10
15
20
25
30
35
FRACTION NUMBER

low molecular weight end of fraction II and only a small peak in the fraction I region.

There was little change in the profiles of the alcohol-soluble fraction (C) during sprouting. As the amounts of fractions I and III increased slightly fraction II decreased gradually (see Appendix II).

The profiles for the acetic acid-soluble (D) and the residue (E) fractions showed gradual qualitative changes with increasing sprouting. Elution peaks showed that these fractions became more homogeneous with sprouting. Both fractions were eluted in the same elution volume region and this did not change with sprouting.

Table 13 summarizes the distributions of proteins by fractions obtained from gel filtration for the five solubility fractions of each flour. The data were normalized so that the sum of the four fractions would equal the total nitrogen recovery shown in parenthesis for each flour.

As expected, the data show that the amounts of the high molecular weight components (fractions I and II) gradually decrease with sprouting while the amounts of the smaller components (fraction III and fraction IV plus dialyzate) increased. It is notable that fraction II proteins (gliadins according to Meredith and Wren) showed the greatest decrease. However, the solubility fractionation (see Table 9 above) showed that the gliadins (Osborne) remained essentially constant while the residue fraction decreased markedly. The calculated results of Table 13 showed the same trends with sprouting as did the data obtained by gel filtration of total AUC extracts of flour (see Table 10). Furthermore these results are in general agreement with those published by Coulson and Sim (1965),

TABLE 13. SUMMARY OF DISTRIBUTIONS OF SOLUBILITY FRACTIONS ACCORDING TO MOLECULAR WEIGHT

Fraction	I	II	III	IV	IV + dialysate
Mol. Wt. Range	> 100,00	100,000-25,000	25,000-10,000	10,000	< 10,000
Flour					
Control (94.0)*	29.9	55.7	5.6	2.8	8.8
Soaked (94.8)	35.1	50.5	7.7	2.5	7.7
Germinated					
2 days (89.0)	25.6	48.4	14.1	0.9	11.9
4 days (86.7)	27.1	46.3	12.4	0.9	14.2
8 days (80.7)	24.4	39.7	14.7	1.8	21.2

* Figures in parentheses represent total protein recovery in percent of the modified Osborne solubility fractionation.

Shorina and Vakar (1965) and Beresh (1969) who showed that the endosperm storage proteins (mainly gluten) are rapidly degraded during sprouting.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) has been used extensively in the investigation of both qualitative and quantitative differences in proteins. In the present study, this technique was used to ascertain if there were any qualitative changes in the endosperm proteins during sprouting. The results of these experiments will be presented in two subsections. The first subsection will deal with three of the four fractions obtained by the modified Osborne solubility fractionation procedure. The fourth fraction (the acetic acid-soluble) does not migrate into the gel, presumably because of its high molecular weight, and therefore cannot be resolved by the PAGE technique used. Subsection two will deal with the PAGE results for the four fractions obtained for two of the flours (control and 8-day sprouted) by the chromatographic fractionation on Sephadex G-150 using the AUC solvent.

Solubility Fractions

Figure 11 shows the electrophoretic patterns for the water-soluble fractions of the five flours used in this study. The patterns for the soaked and sprouted samples showed two minor bands in addition to the six bands of the control. These new bands are probably the same as the additional bands observed in the fast migrating components of Tris-HCl buffer extracts by Macko et al. (1967). Coulson and Sim (1965) using starch gel electrophoresis to examine total acetic acid extracts also observed a "few" new components of higher mobility that were formed during sprouting.

Figure 11

Electrophoretic patterns of water-soluble proteins.

The five patterns from top to bottom are for the following flours:

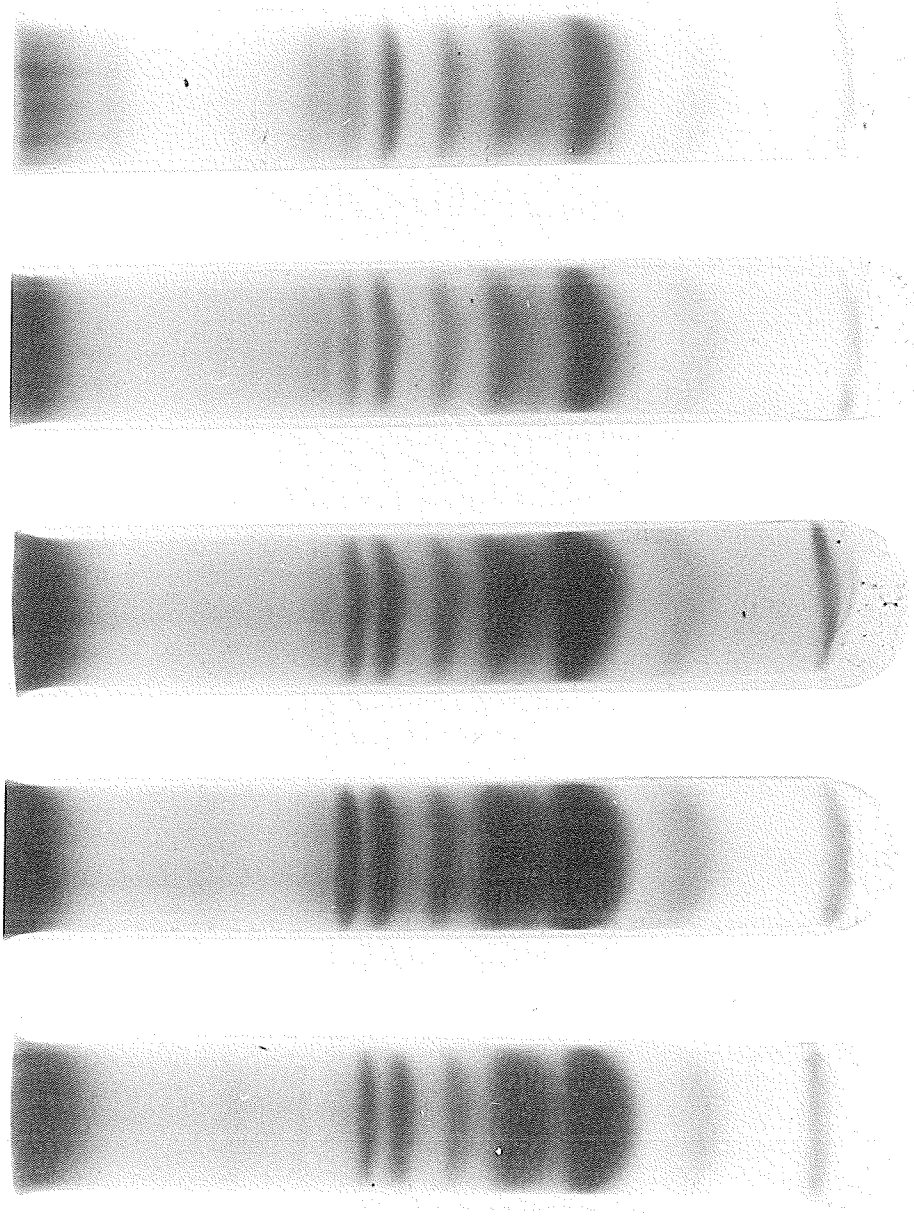
1 - 8 days

2 - 4 days

3 - 2 days

4 - soaked

5 - control



The electrophoretic patterns of the salt-soluble fraction are shown in Fig. 12. Bands of very low mobility disappeared gradually during sprouting while all the fast moving bands remained essentially unchanged.

There was little or no change in the electrophoretic patterns of the alcohol-soluble fractions (Fig. 13). As found by the two fractionation techniques, this fraction remained relatively unchanged during the sprouting period investigated in the present study.

Sephadex Chromatography Fractions

Electrophoretic patterns of all four fractions obtained for the control and 8-day sprouted sample by chromatographic fractionation on Sephadex G-150 using the AUC solvent are shown in Figs. 14 and 15 respectively. The patterns for the two samples are essentially the same and therefore results for the control only will be discussed and the minor differences noted. Fraction I comprised mainly proteins that did not enter the polyacrylamide gel. This is generally true of glutenin proteins. This fraction also had a number of minor slow-moving or gliadin-like components.

Fraction II comprised mainly slow-moving or gliadin-like components with small proportions of protein that did not enter the gel and some fast moving (albumin or globulin) components. Definition of this fraction as gliadin appears unjustified. It is definitely not as homogeneous electrophoretically as the alcohol-soluble fraction obtained by the Osborne procedure (compare pattern II in Fig. 14 and Fig. 13).

Fraction III comprised entirely fast moving bands with mobilities usually obtained for the albumins and globulins. Accordingly, reference

Figure 12

Electrophoretic patterns of salt-soluble proteins.

The five patterns from top to bottom are for the following flours:

1 - 8 days

2 - 4 days

3 - 2 days

4 - soaked

5 - control

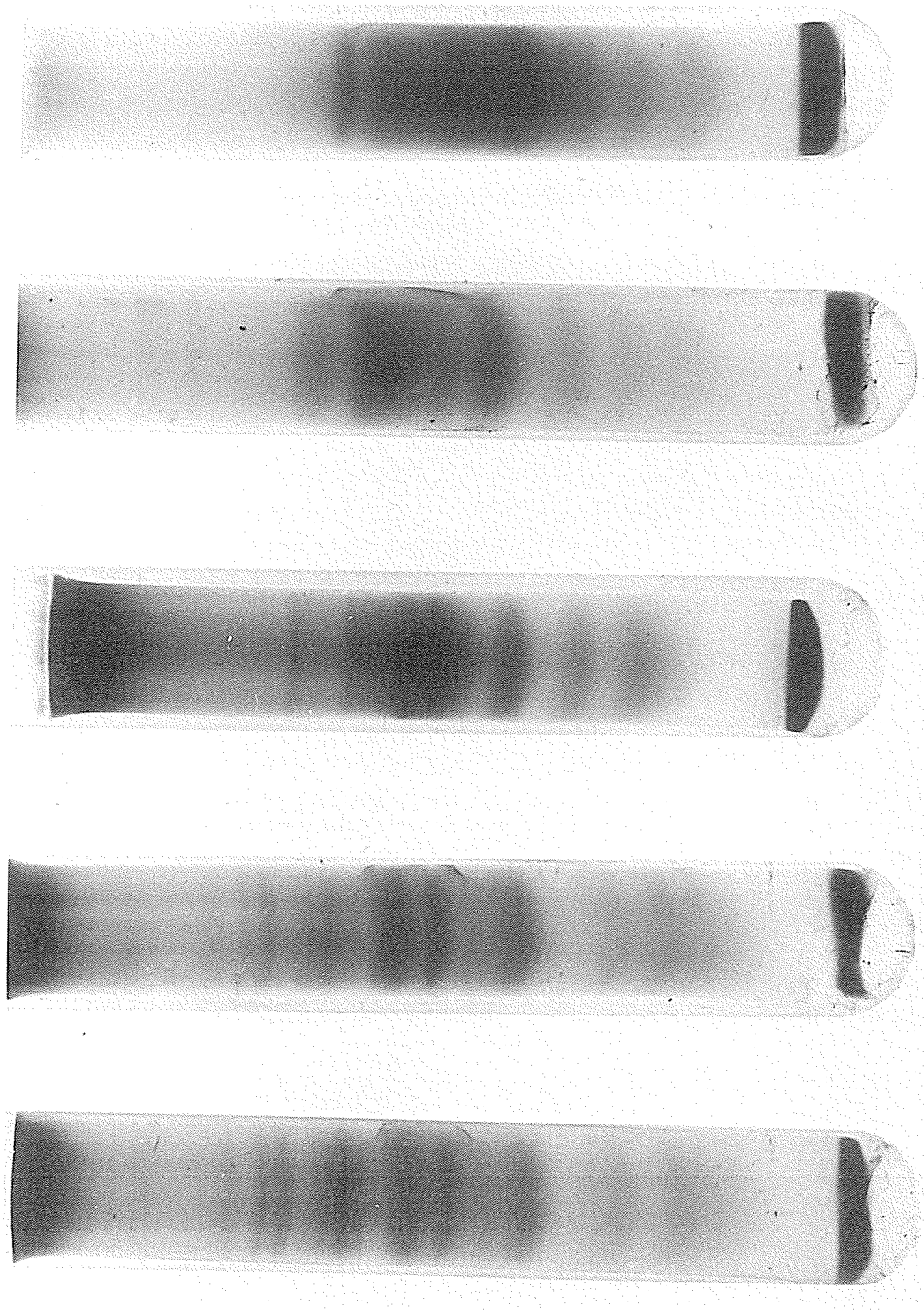


Figure 13

Electrophoretic patterns of alcohol-soluble proteins.

The five patterns from top to bottom are for the following flours:

1 - 8 days

2 - 4 days

3 - 2 days

4 - soaked

5 - control

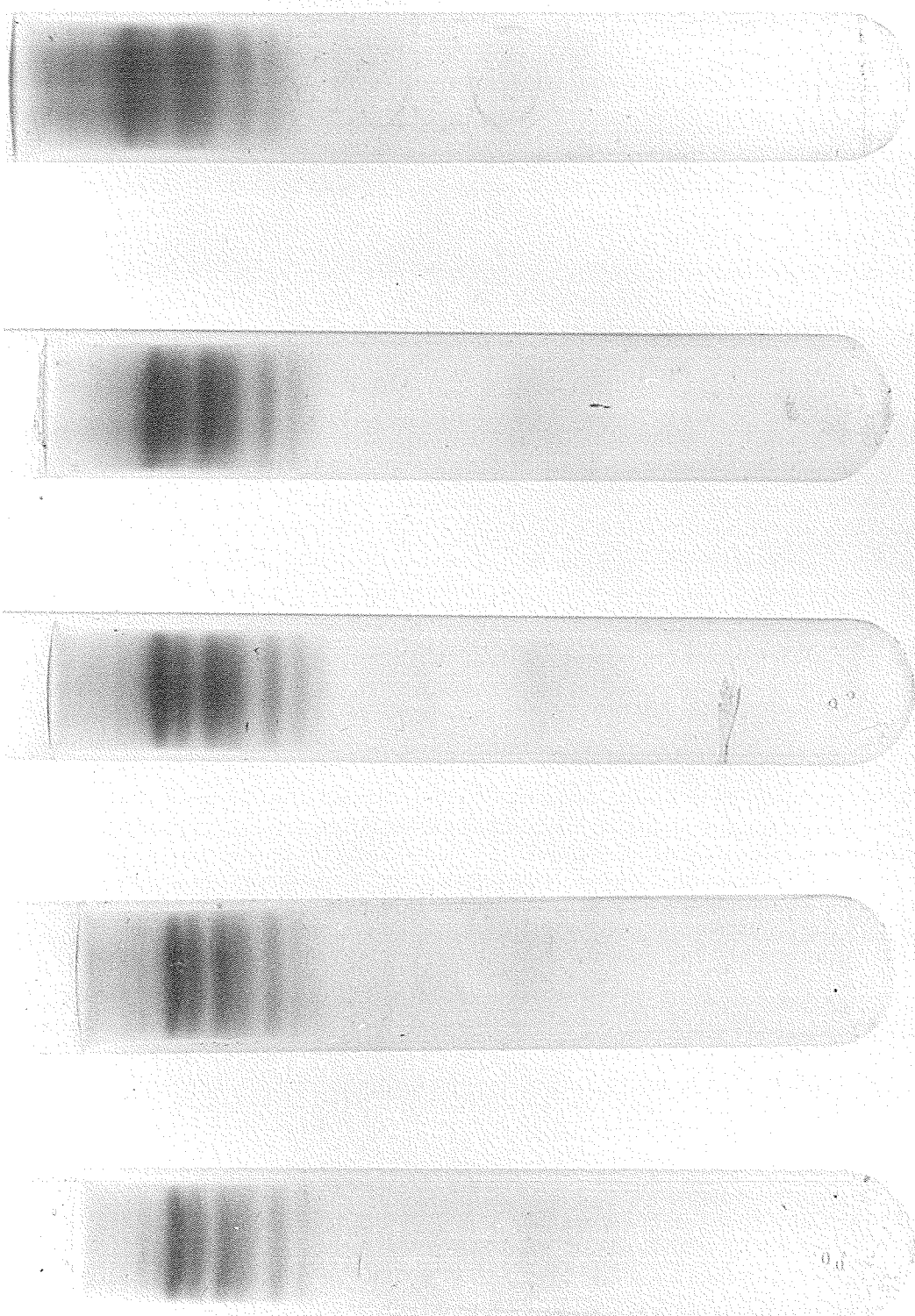


Figure 14

Electrophoretic patterns of the four gel chromatographic fractions for the control sample.

The four protein fractions are as follows:

I - >100,000

II - 100,000 - 25,000

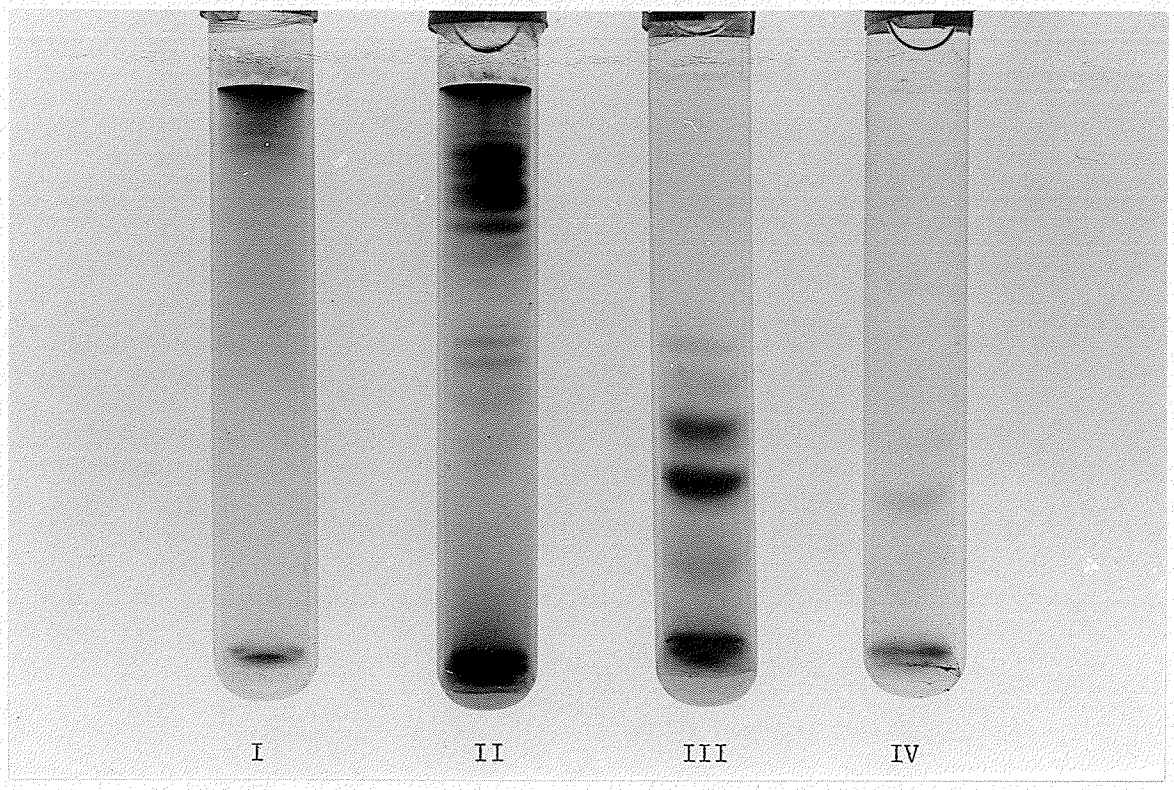
III - 25,000 - 10,000

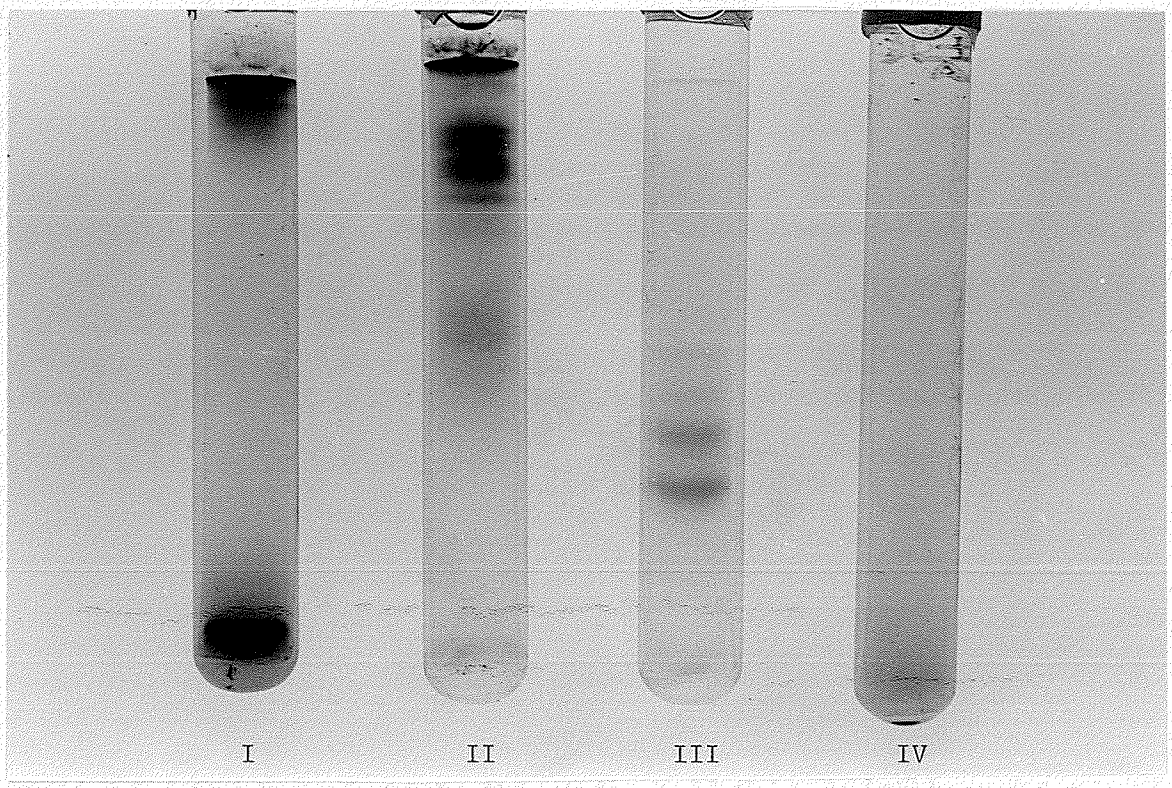
IV - <10,000

Figure 15

Electrophoretic patterns of the four gel chromatographic fractions for the 8-day sample.

The four protein fractions are the same as in Fig. 14.


I
II
III
IV


I
II
III
IV

to this fraction as albumin appears justified.

Fraction IV of the control flour contained one component of very high mobility. In mobility, this component appears similar to one of the major components of fraction III. The amount of protein in fraction IV was very low.

The first three Sephadex fraction of the 8-day sprouted sample had essentially the same patterns as the equivalent fractions of the control flour (compare Figs. 15 and 14). Fraction IV of the sprouted sample had no protein band that could be detected by the technique used. Presumably the small amount of material that absorbs at 280 nm (see Fig. 5) either moved very rapidly and off the gel or else it does not stain with amido black used to detect the protein bands.

It can be concluded from the results presented in this section that, during sprouting, the endosperm proteins are either very quickly degraded to very low molecular weight peptides and amino acids, or that the lower molecular weight degradation products are electrophoretically identical to components normally present in flour.

Proteolytic Activity

The rapid decrease in consistency during mixing of doughs from flours milled from sprouted wheats is generally attributed to the degradation of gluten proteins by the proteolytic enzymes that develop during germination and sprouting (Handford, 1967 and Redman, 1971). Accordingly, one of the objectives of the present study was to examine this cleavage of peptide bonds by proteinases. However, before the present work could be completed Beresh (1969) and Redman (1971) published evidence of peptide bond hydrolysis in glutens and doughs of

flours from sprouted wheat. The results of the present study are useful only in so far as they confirm the findings of Beresh and Redman.

Although the development of proteolytic activity during sprouting is well documented in the literature, it was of interest to measure the proteolytic activity with the changes in the proteins and amino nitrogen discussed above. These results will be presented in the first subsection that follows. The second subsection will deal with activities of the five solubility fractions obtained by the modified Osborne fractionation technique. The activities of the solubility fractions were measured to obtain information on the nature of proteinases in dormant and sprouted wheat.

Activity of Flours

Activities of two different crude enzyme extracts were determined. Extracts were prepared with ammonium sulfate solution (10% saturation) as recommended by Chua (1970) and acetate buffer (0.2 ionic strength and pH 3.8) as used by Wang and Grant (1969). Both extracts showed an exponential increase in activity with duration of sprouting (Table 14). Two-day soaking had no effect on the activity and two days of sprouting produced a relatively small increase. Sprouting beyond two days produced an exponential increase in activity. Activity of the extract from the 4-day sample is 5-7 times the activity of the 2-day extract and activity of the 8-day extract is more than two times the activity of the 4-day extract. The rate of increase in activity with sprouting is essentially the same for the two extracts. However, the activity of the acetate buffer extracts is approximately two times the activity of the ammonium sulfate extracts.

The results obtained in the present study are in agreement with published data. Mounfield (1936) showed that the activity of aqueous

TABLE 14. PROTEOLYTIC ACTIVITY OF FLOUR EXTRACTS OBTAINED WITH TWO SOLVENTS

	Activity			
	10% Sat'd ammonium sulfate		Acetate buffer (pH 3.8)	
	A[1]	B[2]	A[1]	B[2]
Control	1.5	1.0	3.4	2.2
Soaked	1.4	0.9	3.5	2.2
Germinated				
2 days	1.6	1.1	4.6	3.2
4 days	11.0	7.8	21.6	15.3
8 days	25.0	17.9	52.5	37.8

[1] Activity in (μmoles tyrosine/g. flour/min.) x 10^3

[2] Activity in (μmoles tyrosine/mg. protein (N x 5.7)/min.) x 10^5

extracts of ground whole wheat increased six fold in four days and ten fold in seven days of sprouting at room temperature. The correlation between the proteolytic activities presented here with changes of the proteins and amino nitrogen will be discussed later in the General Discussion section.

Activity of Solubility Fractions

Most of the proteolytic activity of the flour was in the water-soluble fraction (Table 15). The activity of this fraction from the 8-day sample was approximately 10 times the activity for the same fraction from the control sample. Although this is somewhat lower than the increase in activity obtained for the two flour extracts discussed above, the agreement between the two sets of results is considered to be quite acceptable in view of the difference in the two enzyme preparations (flour extract versus water-soluble fraction). It is quite possible that the water-soluble fraction could have lost a portion of its activity during the lengthy extraction and dialysis periods used in the Osborne fractionation.

In addition to the activity of the water-soluble fraction, the salt-soluble fraction contained considerable activity. However, the activity of this fraction increased very little with increasing sprouting. It appears that the proteinases that develop during sprouting are mainly of the water-soluble type. However, approximately half of the activity of the sound wheat was in the salt-soluble fraction.

The alcohol-soluble fraction had essentially no proteolytic activity as determined by the assay method used in the present study. This observation appears contrary to the results of Kaminski and Bushuk (1969) who showed that this fraction contained proteolytic activity that could be readily detected by the highly sensitive starch gel

TABLE 15. PROTEOLYTIC ACTIVITY OF FRACTIONS OBTAINED BY SOLUBILITY FRACTIONATION

	Specific activity, (μmoles tyrosine/mg.protein/min.)x10^5				
	Water Soluble	Salt Soluble	Alcohol Soluble	Acetic acid Soluble	Residue
Control	10.3	14.7	--	1.5	2.9
Soaked	10.1	14.7	--	2.0	1.3
Germinated					
2 days	12.7	12.4	--	2.1	1.1
4 days	29.6	17.7	trace	2.9	1.7
8 days	115.0	26.5	trace	2.8	0.9

electrophoretic technique. The discrepancy could arise from the difference in the sensitivity of the two methods used for detecting proteolytic activity.

Both the acetic acid-soluble and residue proteins contained a small amount of proteolytic activity. This is probably due to contamination of these fractions by water-soluble proteins.

The sum of the activities of the solubility fractions is approximately 60% of the activity of the more active (acetate buffer) extract of each flour. These results are not surprising since a loss of activity during the multi-step fractionation is not unexpected.

α-Amylase Activity

The rapid increase in the amount of softening of doughs during fermentation from flours milled from sprouted wheat is generally attributed to the degradation of starch by the α-amylases that develop during germination and sprouting. At extremely high levels of activity the detrimental effects are apparent quite early in the breadmaking process, actually at the dough stage. The introduction of mechanical dough development to baking technology with the concomitant tendency for higher starch damage has resulted in a substantial decrease in the level of sprouted wheat that can be tolerated in the mill mix.

Accordingly, it was of interest to measure the amylase activity of the flours used in the present study and to correlate this activity with the changes in starch and free sugars which will be discussed later.

Changes of α-amylase activity of flours from the control and sprouted wheats are shown in Table 16. The activity for the soaked sample is 750 times the activity of the control. During the entire

TABLE 16. CHANGES OF α-AMYLASE ACTIVITY OF FLOURS FROM SPROUTED WHEAT

	α-Amylase Activity (relative)
Control	1
Soaked	750
Germinated	
2 days	2,060
4 days	11,400
8 days	27,000

sprouting period investigated there was an exponential increase in activity. These results are in general agreement with published results of others. Olered and Jonssen (1970) and MacGregor (1971) independently found over a thousand-fold increase in activity during sprouting.

Another examination of α-amylase activity was carried out with the PAGE technique by incorporating the substrate into the gel. The results are shown in Fig. 16. The pattern for the control sample showed that the amylase activity was located at the very top of the lower gel. Soaking and sprouting of wheats produced two new major and other minor components on the zymogram of amylases. These observations are in agreement with those of Kruger and Tkachuk (1969) and Olered and Jonssen (1970). Kruger and Tkachuk (1969) detected three major and one minor α-amylase component in malted wheat with the same technique. Olered and Jonssen (1970) used agarose as the support material for their electrophoretic technique and found three areas with α-amylase activity for malted wheats.

Changes in Starch and Free Sugars

Starch Damage and Free Sugars

The data pertinent to this section are summarized in Table 17. α-Amylase activities are repeated in this table from Table 16 for discussion purposes.

Flour milled from sound Manitou wheat contained a relatively high percentage of damaged starch, 22.5 units by the procedure of Farrand (1964). This value is normal for hard red spring wheat flour milled on a Buhler experimental mill. Two days soaking of the wheat in distilled

Figure 16

Zymograms for α-amylases extracted from the five flours.

Zymograms from top to bottom are identified as follows:

1 - 8 days sprouted

2 - 4 days sprouted

3 - 2 days sprouted

4 - soaked

5 - control

6 - substrate blank

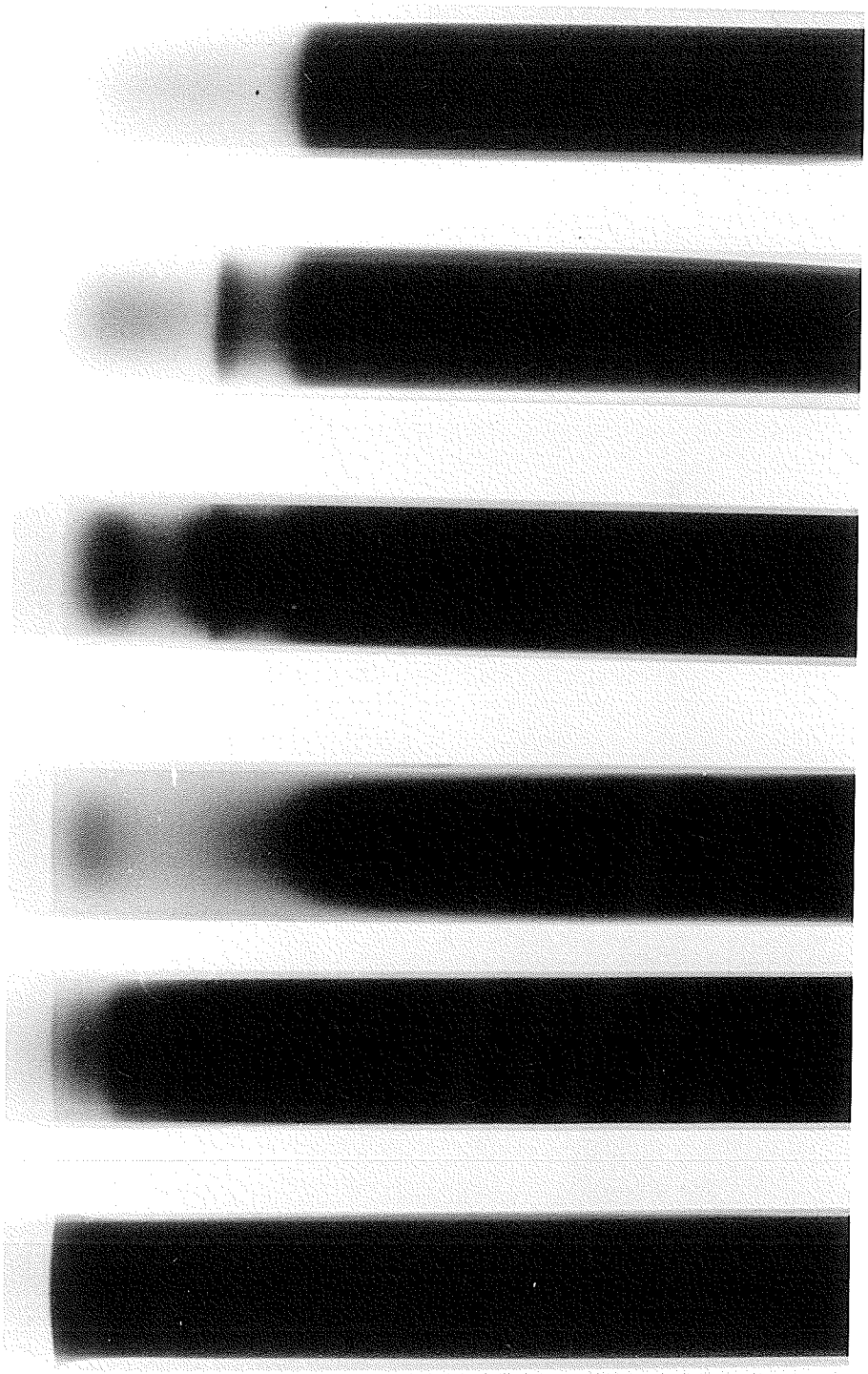


TABLE 17. CHANGES OF STARCH DAMAGE, FREE SUGARS, PARTICLE SIZE INDEX AND α-AMYLASE ACTIVITY OF FLOURS DURING SOAKING AND SPROUTING

	Starch damage, (Farrand Units) Method A[1]	Method B[2]	Free Sugars g. glucose /g. flour	P.S.I.	α-Amylase Activity (relative)
Control	22.5	25.7	3,120	17.3	1
Soaked	0.0	5.9	3,600	43.8	750
Germinated					
2 days	14.4	20.3	3,960	33.2	2,060
4 days	34.8	30.3	8,460	27.0	11,400
8 days	44.4	59.7	21,600	30.7	27,000

[1] Method of Farrand (1964), in Farrand units.

[2] Method of Williams and Fegol (1969), in calculated Farrand units.

water reduced the starch damage to zero. In milling technology, this is equivalent to a transformation of the hard vitreous endosperm of hard wheat to the mellow mealy endosperm of soft wheat. Starch damage values for soft wheats by the Farrand procedure fall in the range 0 to 5 units.

Starch damage increased rapidly with germination and sprouting of the soaked sample. The starch damage of the 2-day sample was somewhat lower than that of the control but the values of the 4- and 8-day samples were much higher.

Table 17 also shows the starch damage values obtained by the iodine staining procedure of Williams and Fegol (1969) and calculated in terms of Farrand units. As found by these authors, their method gave slightly higher values than the Farrand method. However, the relative order of the samples is the same by the two procedures. One exception is the 4-day sample which gave a lower value by the Williams and Fegol method than the Farrand method.

Microscopic evidence will be presented below which indicates that the type of starch damage in the control flour is quite different from the damage in the three sprouted samples. It seemed that the latter type of damage, referred to as "enzymic" damage, resulted largely from the enzymic erosion of the granules during sprouting. Accordingly, it was of interest to correlate the degree of starch damage with α-amylase activity of the samples. Although the number of samples is not ideal for a statistical correlation, it is apparent that the increase in starch damage of sprouted wheat parallels the increase in α-amylase activity (see Table 5). Both sets of values for starch damage are positively

correlated to α-amylase activity at the 1% level of significance.

A further confirmation of the high activity of α-amylase in wheat endosperm during sprouting can be obtained by determining the free sugar content of the flours. The increase in free sugars of the soaked and the 2-day samples over that of the control was small but quite definite. A marked exponential increase was obtained for the 4- and 8-day samples. This increase paralleled the increase in α-amylase activity and starch damage obtained for the same samples. The free sugar content and α-amylase activity are also positively correlated at the 1% level of significance (see Table 5).

Ordinary Light Microscopy

Starch from the control flour showed some "mechanically" damaged granules similar to those reported by Williams (1969). Only a few damaged granules were observed in the starch from the soaked wheat.

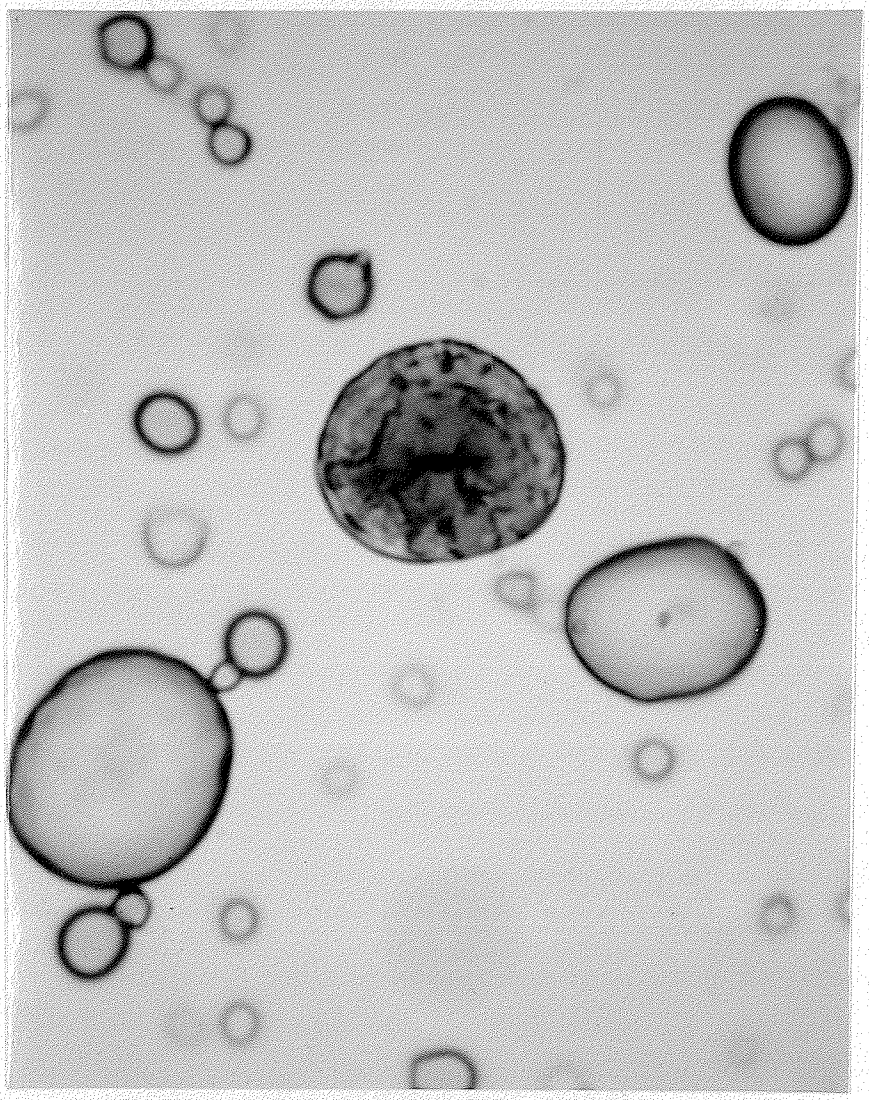
A vastly different type of damaged starch was observed in all three germinated samples (Figs. 17A and 17B). This type of damage, referred to as enzymic damage, was first observed by Sandstedt (1954) who incubated starch granules with malt extracts at room temperature. It is different from the various types of damage discussed by Williams (1969). Granules that suffered enzymic damage showed light and dark areas after staining with iodine. When the same granules were viewed under polarised light, the birefringent cross was still visible through the light and dark patches indicating that the original semicrystalline structure of the granule was not disrupted by the enzymic action.

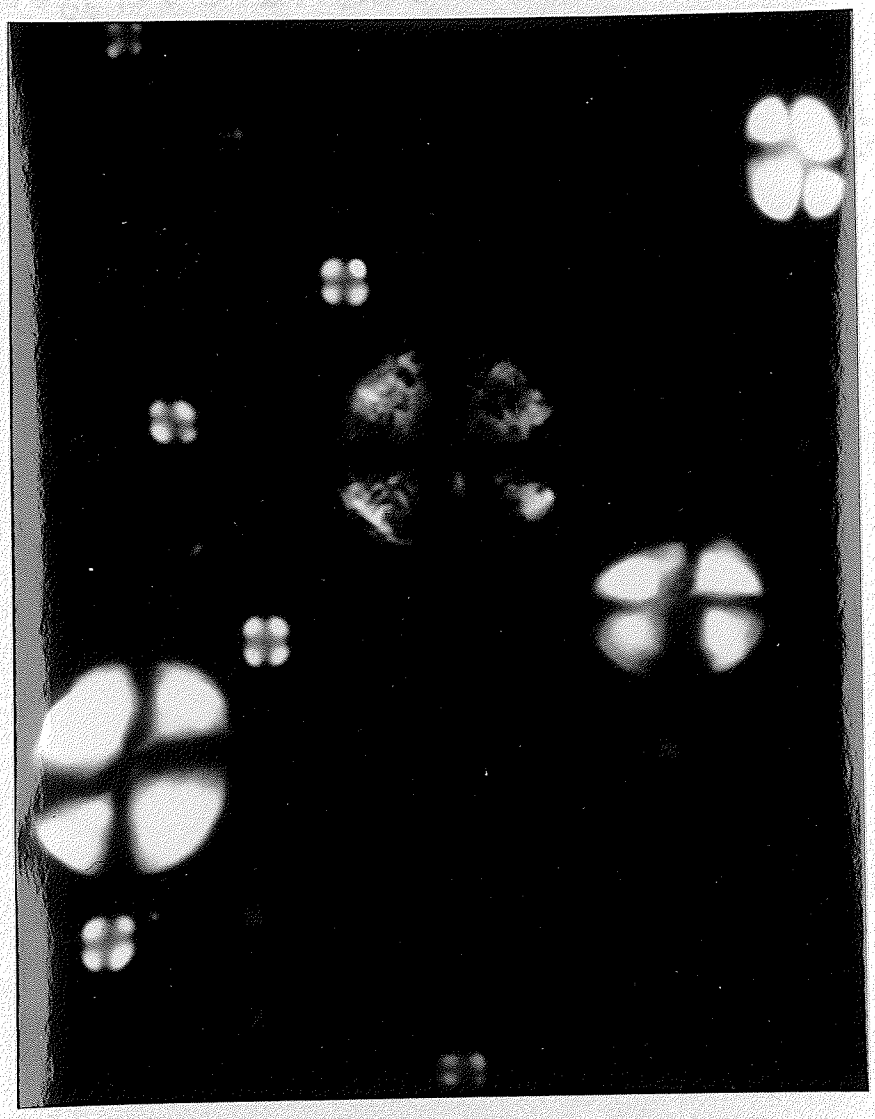
Figure 17A

Washed-out wheat starch granules after 8 days of germination (x 800)

Figure 17B

Fig. 17A viewed under polarized light conditions (x 800)





Scanning Electron Microscopy

Scanning electron microscopy was used to further study the nature of the enzymic damage inferred from analytical data and ordinary light microscopy. Three types of specimens were examined: 1) cracked wheat grains, 2) flour, and 3) starch granules. The results of these experiments are discussed in the following subsections.

Cracked Wheat. Figures 18 and 19 show the open surfaces of cracked control and soaked wheats respectively. These photomicrographs show starch granules of varying size embedded in an amorphous-appearing material. Similar results were published by Aranyi and Hawrylewicz (1969). These workers suggested that the amorphous material was proteinaceous. There is no evidence in the photographs of Figs. 18 and 19 of any damage of starch granules, either enzymic or physical.

Figure 20 shows the internal surface of a cracked grain of a wheat kernel germinated for four days. Starch granules show various sizes and two different shapes, lenticular shaped or A type and smaller spherical or B type granules. Both shapes were previously described by Buttrose (1963). One A type granule extensively eroded at the edge (groove erosion) can be seen in Fig. 20. There is also some evidence of spot surface erosion.

The open surface of a cracked seed, germinated for eight days, is shown in Fig. 21. This photomicrograph shows both large and small granules severely eroded. Presumably this erosion is caused by enzymic action. Two different types of erosion were observed on the large or A type granules. Type 1 is the erosion that produces a groove on the edge of the granules as mentioned above, and Type 2 is the random

Figure 18

Scanning electron micrograph of a cracked kernel of the control sample (x 1,840).

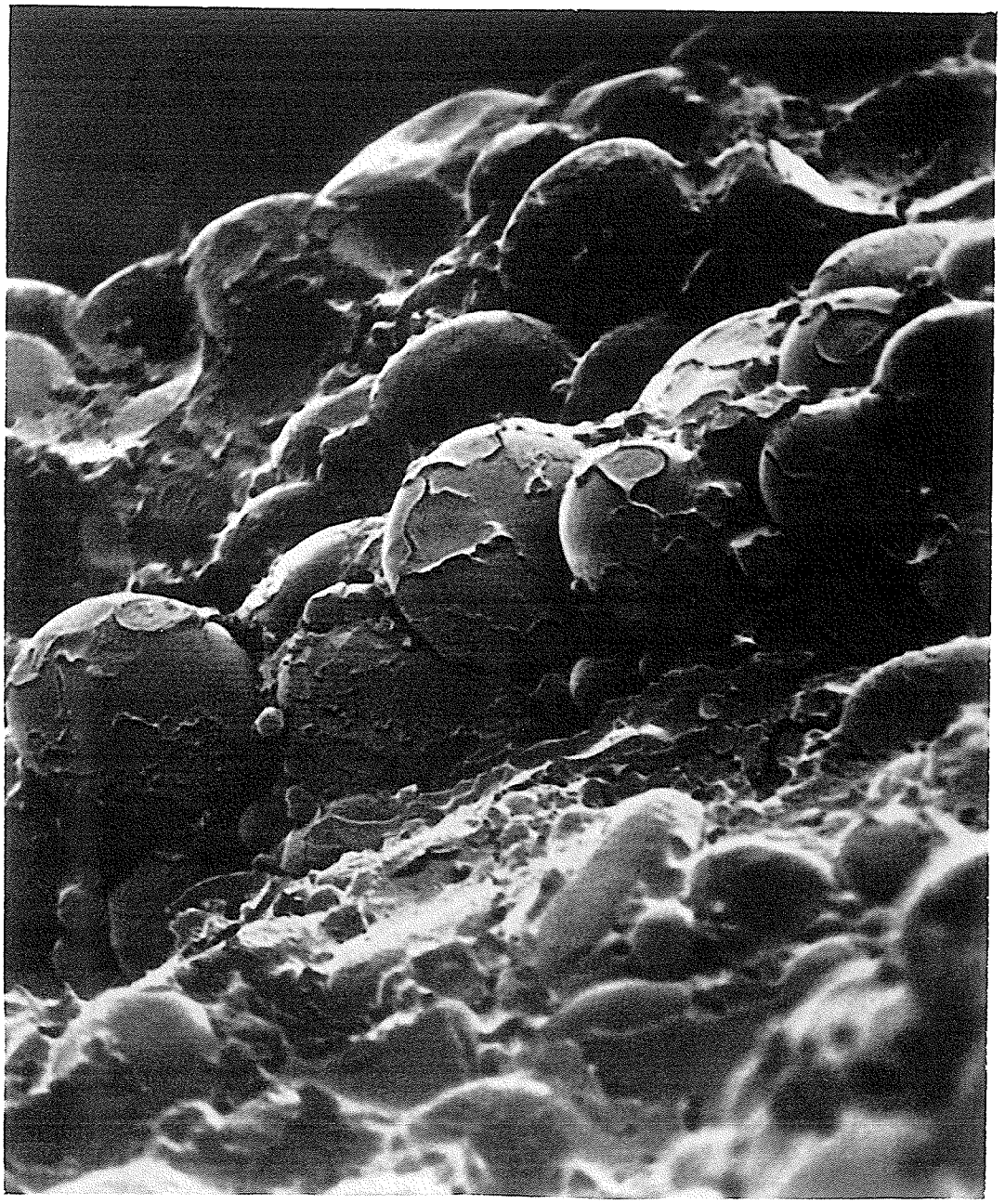


Figure 19

Scanning electron micrograph of a cracked kernel of the soaked wheat (x 670).

Figure 20

Scanning electron micrograph of a cracked kernel of the 4-day sprouted wheat (x 3,170).

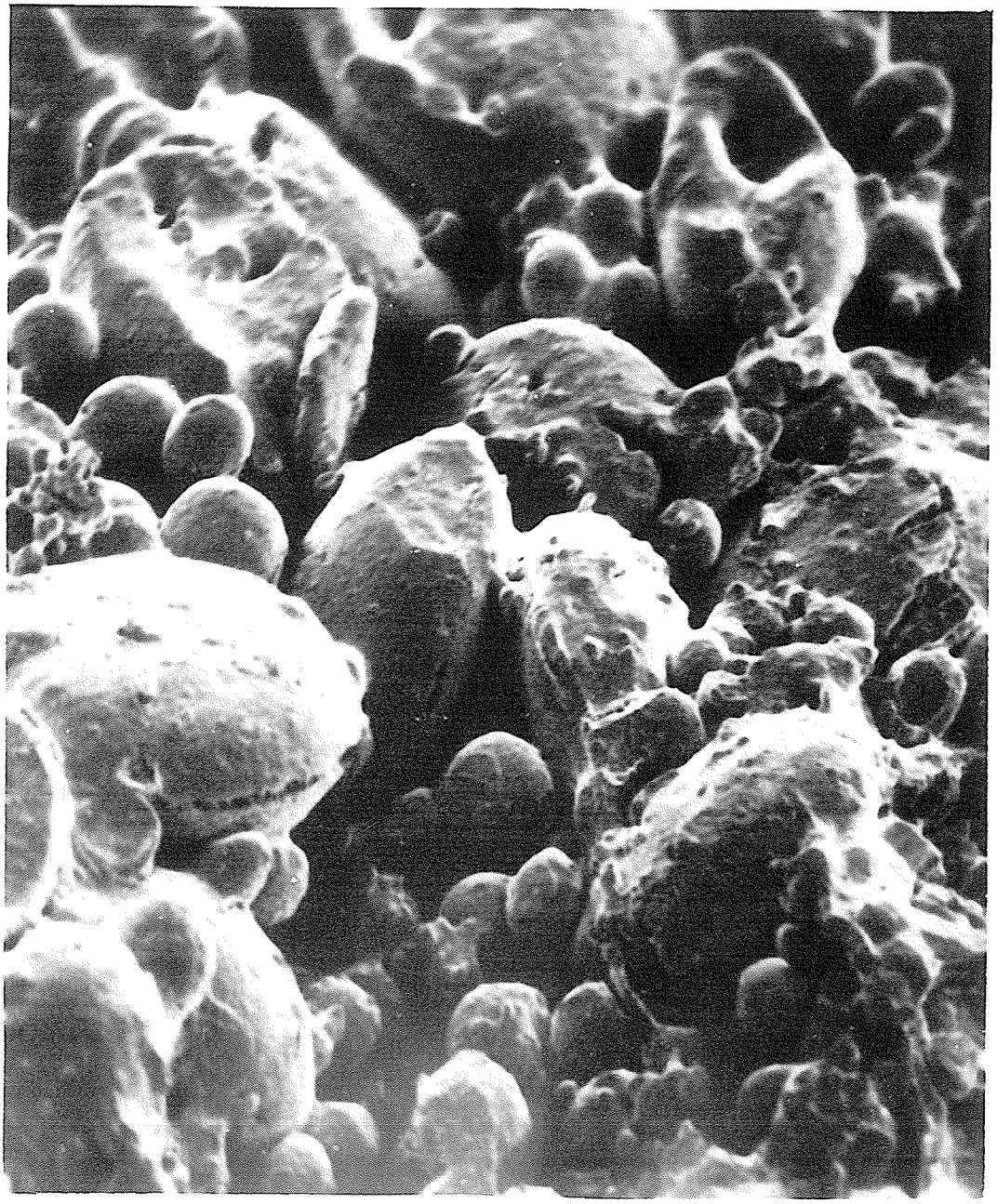
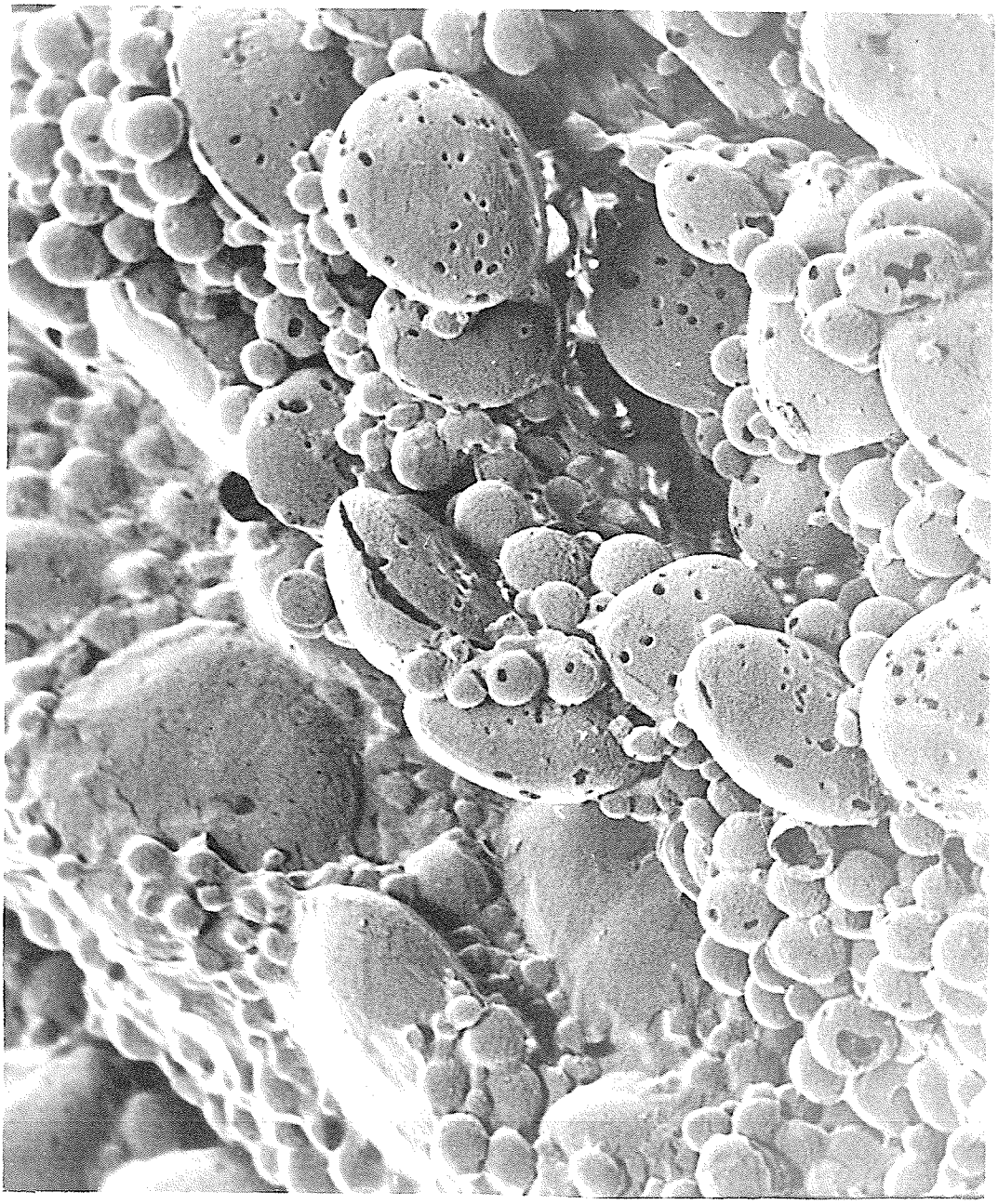


Figure 21

Scanning electron micrograph of a cracked kernel of the 8-day sprouted wheat (x 1,500).



appearance of small holes on the granule surface. The smaller granules show only the second type of erosion. Both types of erosion were reported previously by Evers and McDermott (1970) and are produced by the action of wheat α-amylase on washed-out wheat starch. They examined the mode of attack of α-amylase on a few individual granules and found that where a shallow groove was initially present in the granule, the enzyme attack generally occurred there. Penetration of the enzyme into the granule seemed to occur exclusively at this point which was probably less resistant to enzyme degradation.

Figure 22 shows a cracked starch granule found on the open surface of the kernel sprouted for 8 days. This granule showed extensive internal erosion. The periphery of the granule appears quite intact except for a few holes but its center is quite extensively disrupted to the point where the core is completely hollow. Again this result is similar to that published by Evers and McDermott (1970) who incubated wheat starch with wheat amylase extract in a buffer solution.

In general, the amylase attack seemed to concentrate only at specific areas on the granules, especially in the earlier stages of germination. This may be related to the distribution of the amylase in wheat endosperm. The moisture content of the germinating seed is probably too low to facilitate free diffusion of the enzyme.

Flour Photomicrographs. Figure 23 shows a photomicrograph of flour particles from the control wheat sample. The larger granules showed extensive surface cracking which probably resulted during milling from pressure of the rollers. The amorphous material which covers the surface of most of the granules is readily evident.

Figure 22

Scanning electron micrograph of a cracked starch granule from the 8-day sprouted wheat (x 10,000).

Figure 23

Scanning electron micrograph of flour milled from the control (sound) wheat (x 1,940).

Figure 24 shows a portion of a flour particle milled from soaked wheat. It shows the arrangement of large and small granules and the amorphous film on their surface. Some of the granules show evidence of enzymic attack.

Undisrupted fragments of endosperm were observed in the photomicrographs of flour from 2-day germinated wheat (Figs. 25 and 26). Figure 25 shows the arrangement of large and small starch granules embedded in amorphous-appearing material. A closer view of the same specimen is shown in Fig. 26. A very definite thin film covering the surface of a number of granules can be seen in this photomicrograph. Figure 27 shows another photomicrograph of flour milled from 2-day sprouted wheat. Some of the granules in this picture show evidence of enzyme erosion.

Figure 28 is a photomicrograph of a flour particle milled from the wheat sprouted for four days. Extensive erosion can be seen on the small granules. The larger granules suffered only slight erosion.

A photomicrograph of flour from wheat sprouted for 8 days (Fig. 29) showed extensive erosion on both large and small starch granules. There are deep grooves on the large granules and holes on the surfaces of both the large and small granules.

Starch Granules. A further examination of the erosion of starch granules during sprouting was carried out on granules washed out from flours. These results are presented in this subsection.

Figure 30 shows starch granules washed out from the control sample. Cracks on the surface of the large granules are readily evident. As mentioned previously these cracks are probably produced by milling.

Figure 24

Scanning electron micrograph of a flour particle from the soaked wheat (x 3,250).

Figure 25

Scanning electron micrograph of a flour particle from the 2-day sprouted wheat (x 870).

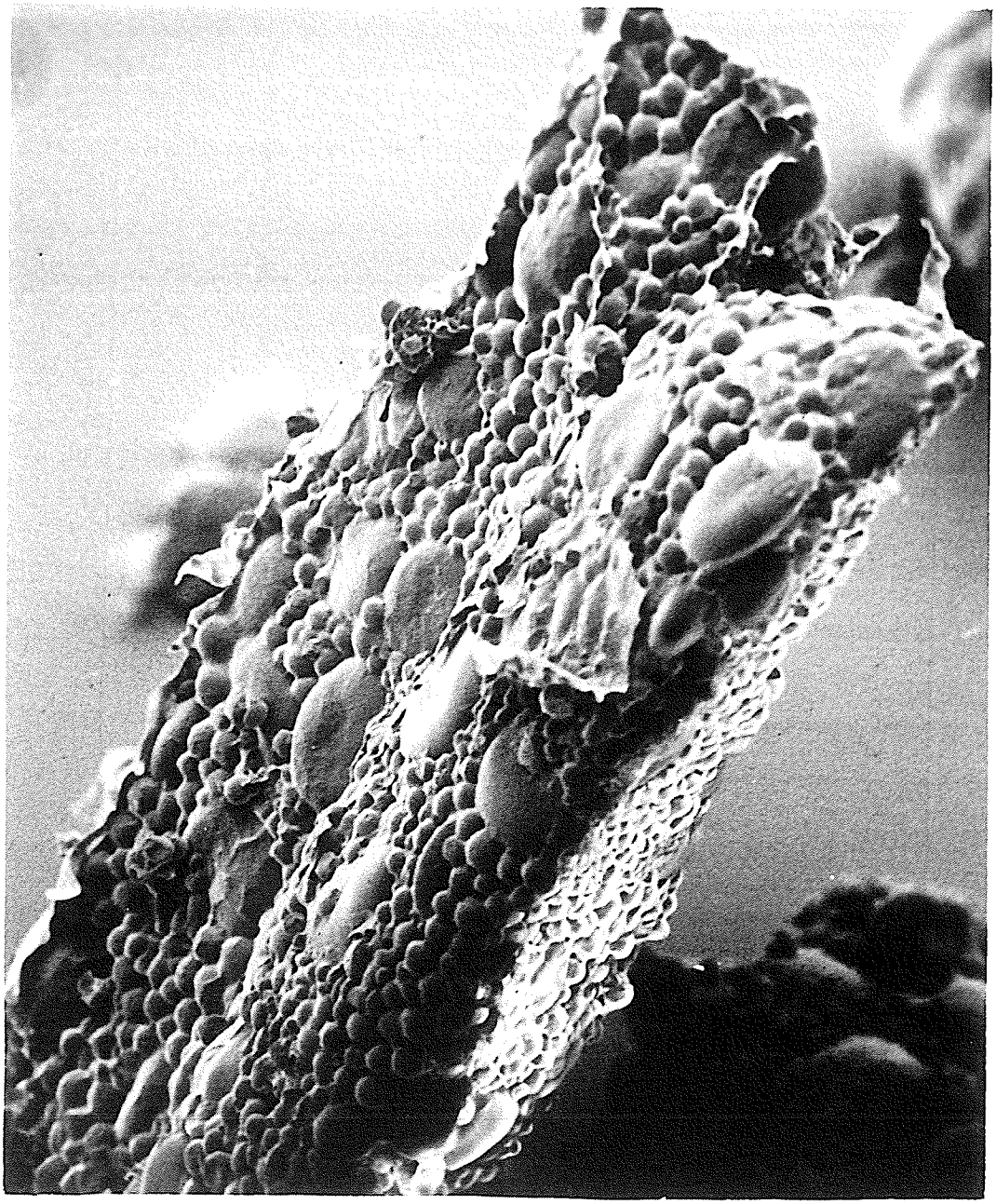


Figure 26

Scanning electron micrograph of a portion of the flour particle shown in Fig. 25 at a higher magnification (x 1,840).

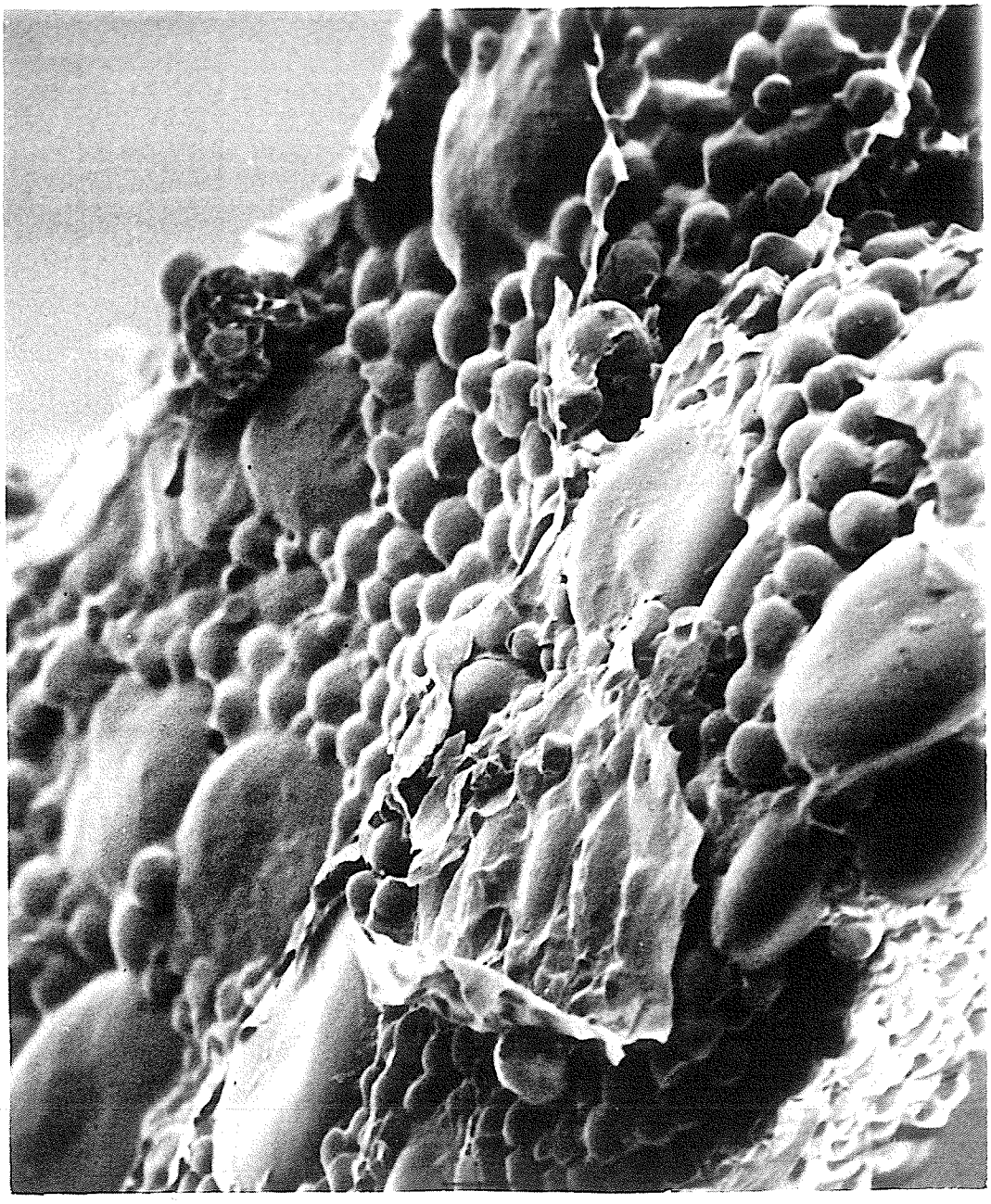


Figure 27

Scanning electron micrograph of flour milled from the 2-day sprouted wheat (x 2,000).

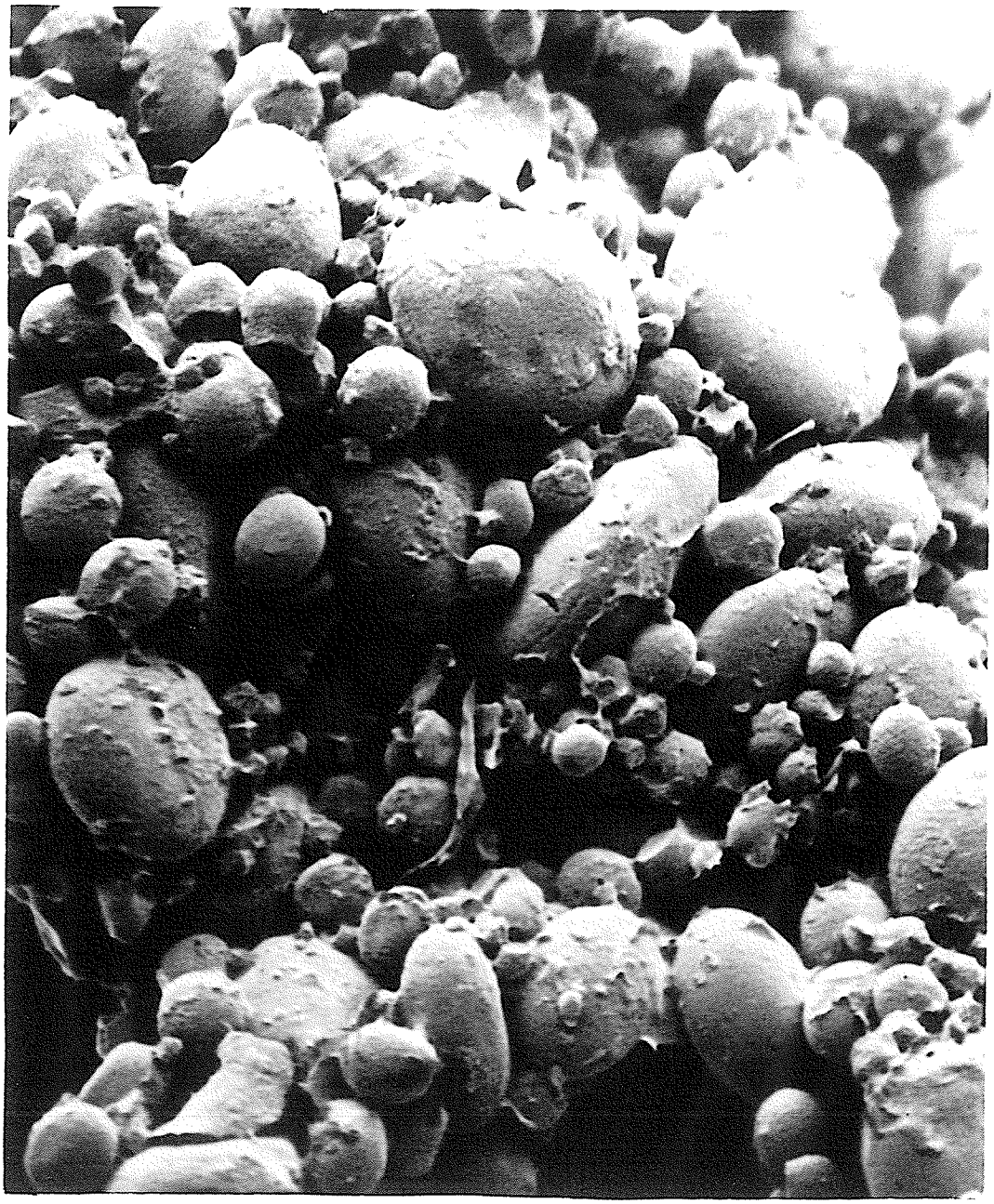


Figure 28

Scanning electron micrograph of a flour particle milled from the 4-day sprouted wheat (x 1,840).

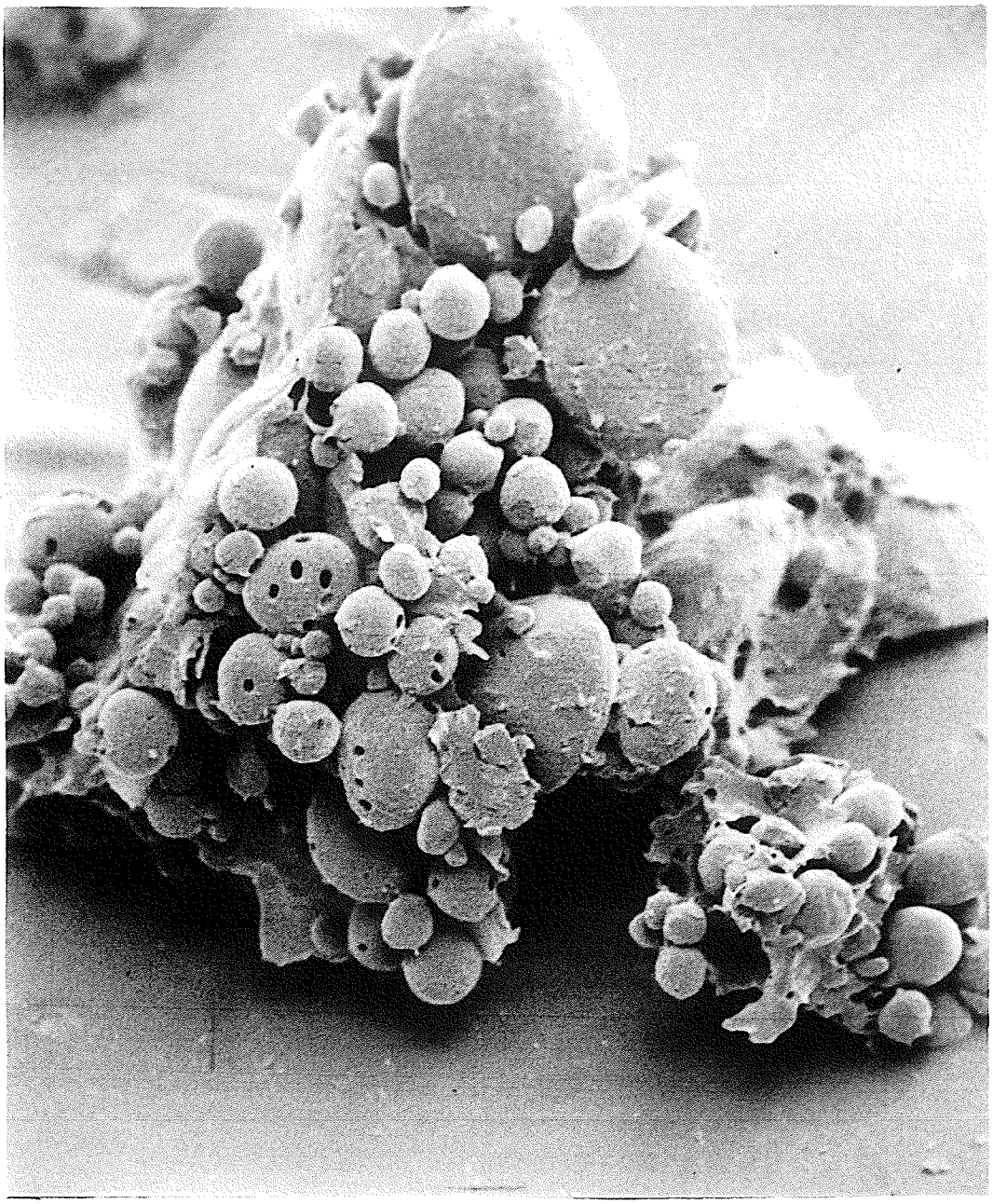
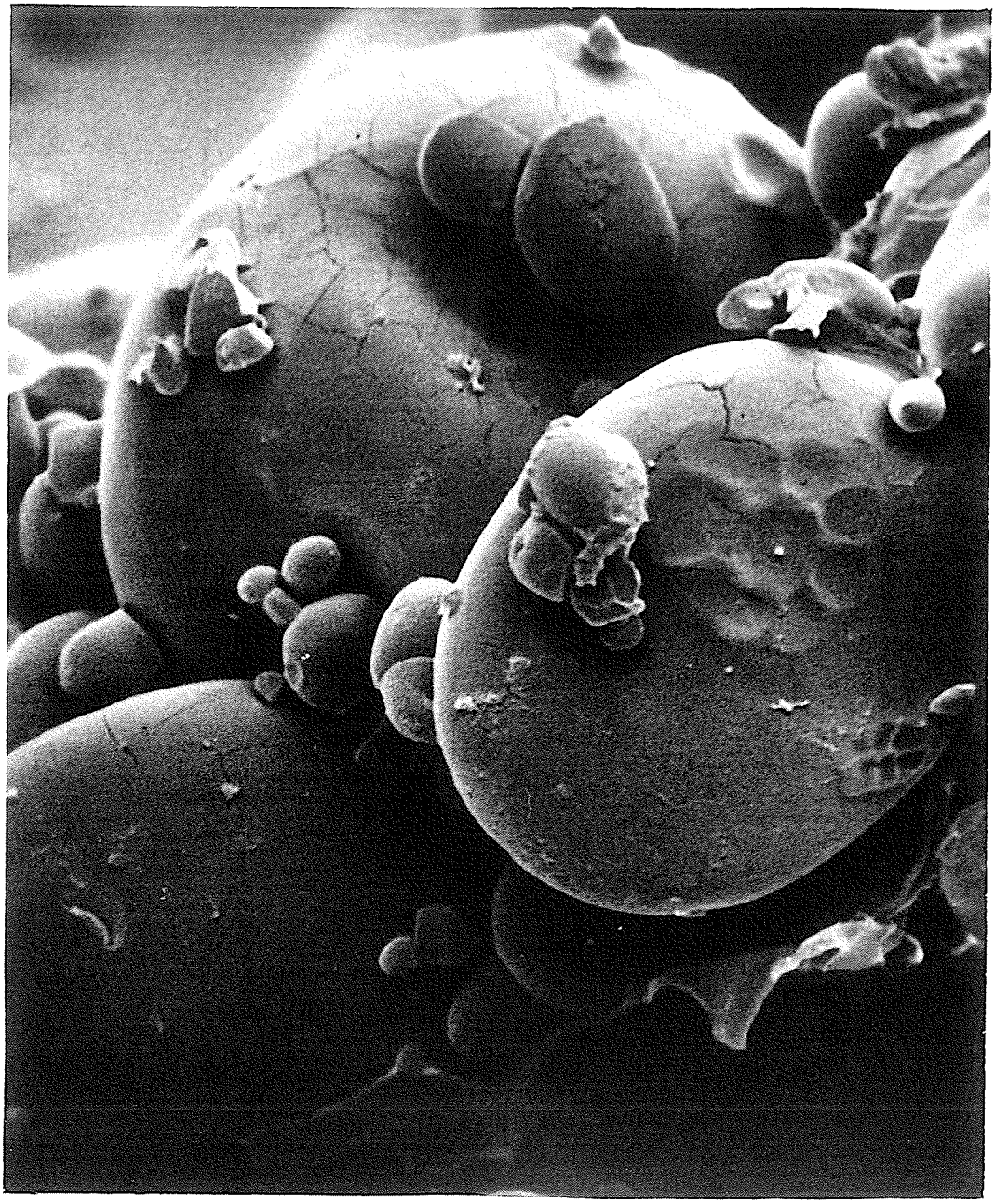


Figure 29

Scanning electron micrograph of a flour particle from the 8-day sprouted wheat (x 1,790).

Figure 30

Scanning electron micrograph of starch granules washed out from the control flour (x 3,500).



Some of the very large granules showed depressions on the surface. These marks are likely due to the pressure from small granules created during the maturing of the grain.

The granules from the soaked sample showed slight groove and surface erosion (Fig. 31).

Figures 32 and 33 show photomicrographs of starch granules from the 8-day sample. A severely eroded granule can be seen in Fig. 32. The whole surface of the granule is covered with deep small holes. Figure 33 shows a side view of a severely eroded granule. This is an extremely good picture of the erosion at the groove region. Both severely and mildly damaged starch granules can be seen in this sample. This again confirms the suggestion made earlier that the amylase attack is concentrated at certain specific areas of the granules probably because the enzyme is located at these points in the endosperm. The type of erosion pattern shown here is different from that reported by Evers et al. (1971). These workers incubated raw wheat starch with glucoamylase of fungal origin in an aqueous solution and found the starch was attacked by the enzyme uniformly over the entire granule surface. However, results of the present study are in general agreement with earlier SEM observations of Evers and McDermott (1970) on the action of wheat α-amylase on washed-out wheat starch granules.

Using SEM, it is possible to obtain absolute dimensions of starch granules. The size of granules from the flours used in the present study ranged from 5 to 23 μ. These values are the same as those reported by Klassen (1970). He obtained by ordinary microscopy the size distribution pattern for the same variety of wheat as that used in the present

Figure 31

Scanning electron micrograph of starch granules washed out from the flour of the soaked sample (x 4,000).

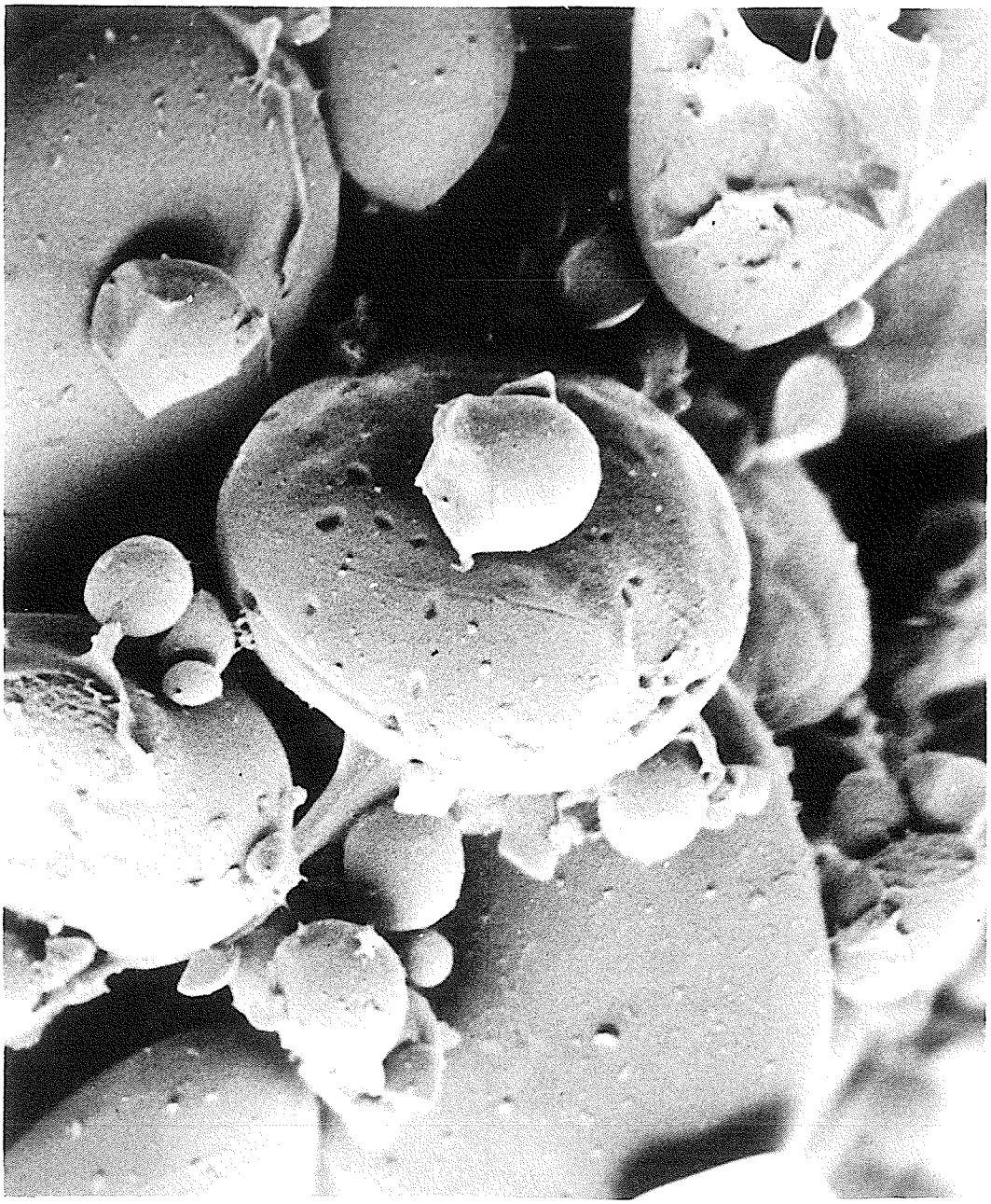


Figure 32

Scanning electron micrograph of a randomly eroded starch granule from the 8-day sprouted wheat (x 3,370).

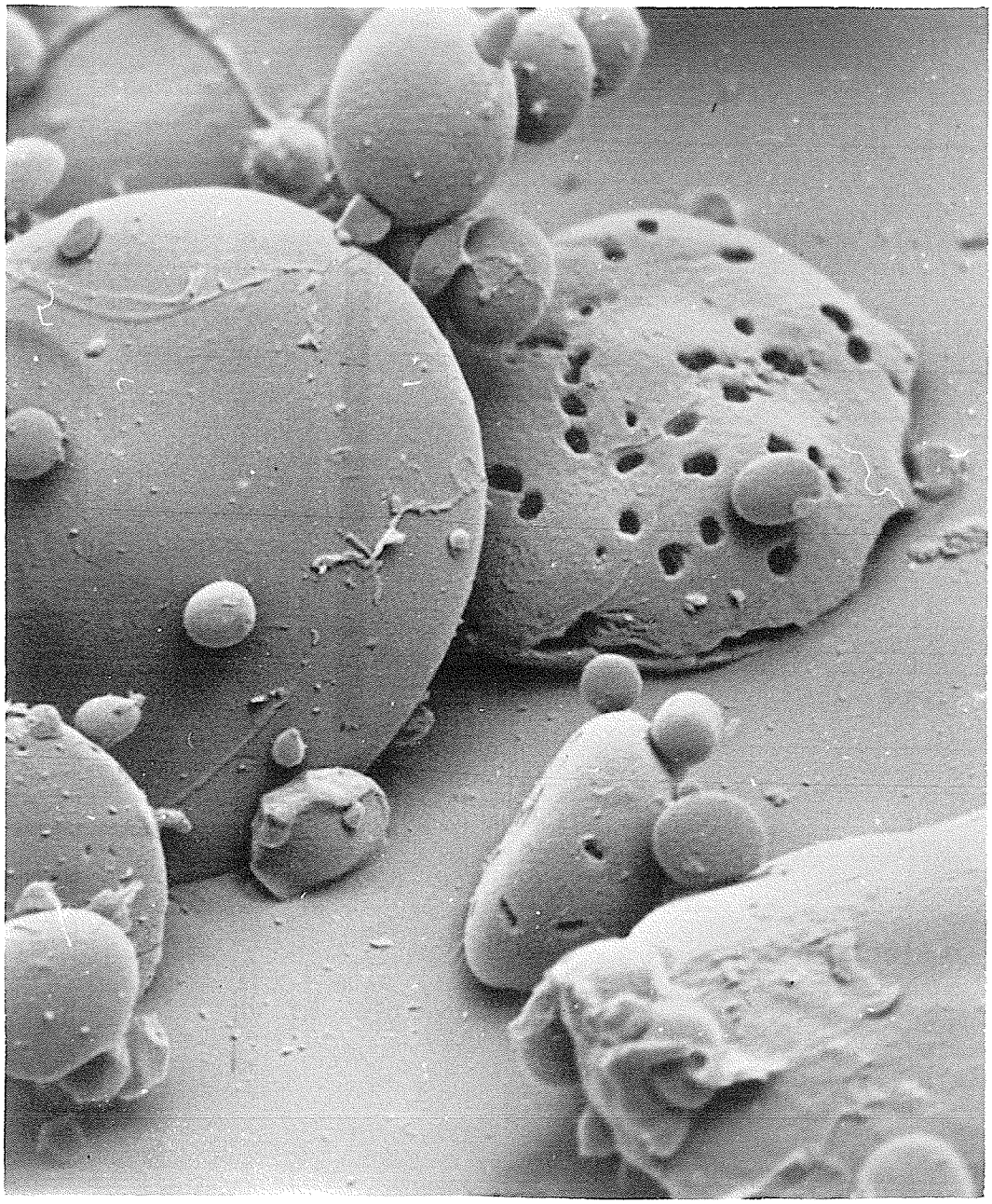


Figure 33

Scanning electron micrograph of a starch granule showing groove and surface erosion (x 8,680).

study (Manitou) and found that 76.5% of the granules fell within the range of 10 to 27 μ..

GENERAL DISCUSSION

One variety of Canadian hard red spring wheat (Manitou) was germinated and sprouted under controlled laboratory conditions and then used to study the effects of sprouting on some of the important (from the utilization viewpoint) technological and biochemical properties.

Milling quality was assessed on the experimental Buhler mill. Flour yield and color were markedly lowered by the sprouting. Ash content of the flours from the three sprouted wheats was actually lower than the ash of the control. Presumably this decrease resulted partly from the leaching during the soaking prior to sprouting and partly because of the utilization of the inorganic ions in the metabolism of the young wheat plant.

As is well known, baking quality was extensively lowered during sprouting (Bushuk et al., 1966). If this damage is not too extreme, it can be corrected by adjusting processing parameters such as water absorption, fermentation time and proofing time. However even with these adjustments, the quality of the bread obtained from flour milled from sprouted wheat is usually only marginal.

The poor baking quality of flour from sprout-damaged wheat has been generally attributed to the development of high α-amylase activity. Presumably this leads to a decrease in the water holding capacity of the starch and this factor, in turn, gives rise to sticky doughs and even sticky crumb in the bread if the α-amylase activity is extremely high. The present study showed that during sprouting of wheat, other endosperm components that are important to baking quality undergo changes. These

changes could also contribute to the deterioration of baking quality.

Starch, which forms the substrate for the α-amylase, undergoes definite chemical and physical changes during sprouting. Results obtained with ordinary and scanning electron microscopy showed that marked changes can occur in the structure of the starch granules. The large lenticular A type granules showed two types of damage: 1) erosion at the equatorial groove and 2) random circular surface erosion. A type granules also showed evidence of extensive internal erosion which could only be seen by examining cracked granules. B type granules showed only the random circular surface erosion. This enzymic damage was analyzed as so-called starch damage by two techniques used for this purpose (Farrand, 1966 and Williams and Fegol, 1969). The importance of starch damage in baking quality is well documented in the literature (c.f. Bushuk et al., 1966). As anticipated, the degradation of starch was followed by a parallel production of free sugars. Both the degree of starch damage and the amount of free sugars depended on the α-amylase activity (see Figs. 34 and 35).

At the early stages of sprouting, the large granules were attacked before the smaller granules. Also, granules that are located in the kernel adjacent to the aleurone layer of the wheat kernel appeared to be more extensively eroded than those located deeper inside the kernel. It has been shown that in dormant wheat, the concentration of various enzymes is higher in the aleurone layer than in the inner endosperm (Engel, 1945). Presumably this distribution would also apply to α-amylase in sprouted wheat.

The most important component of flour in relation to its baking

Figure 34

Starch damage versus α-amylase activity.

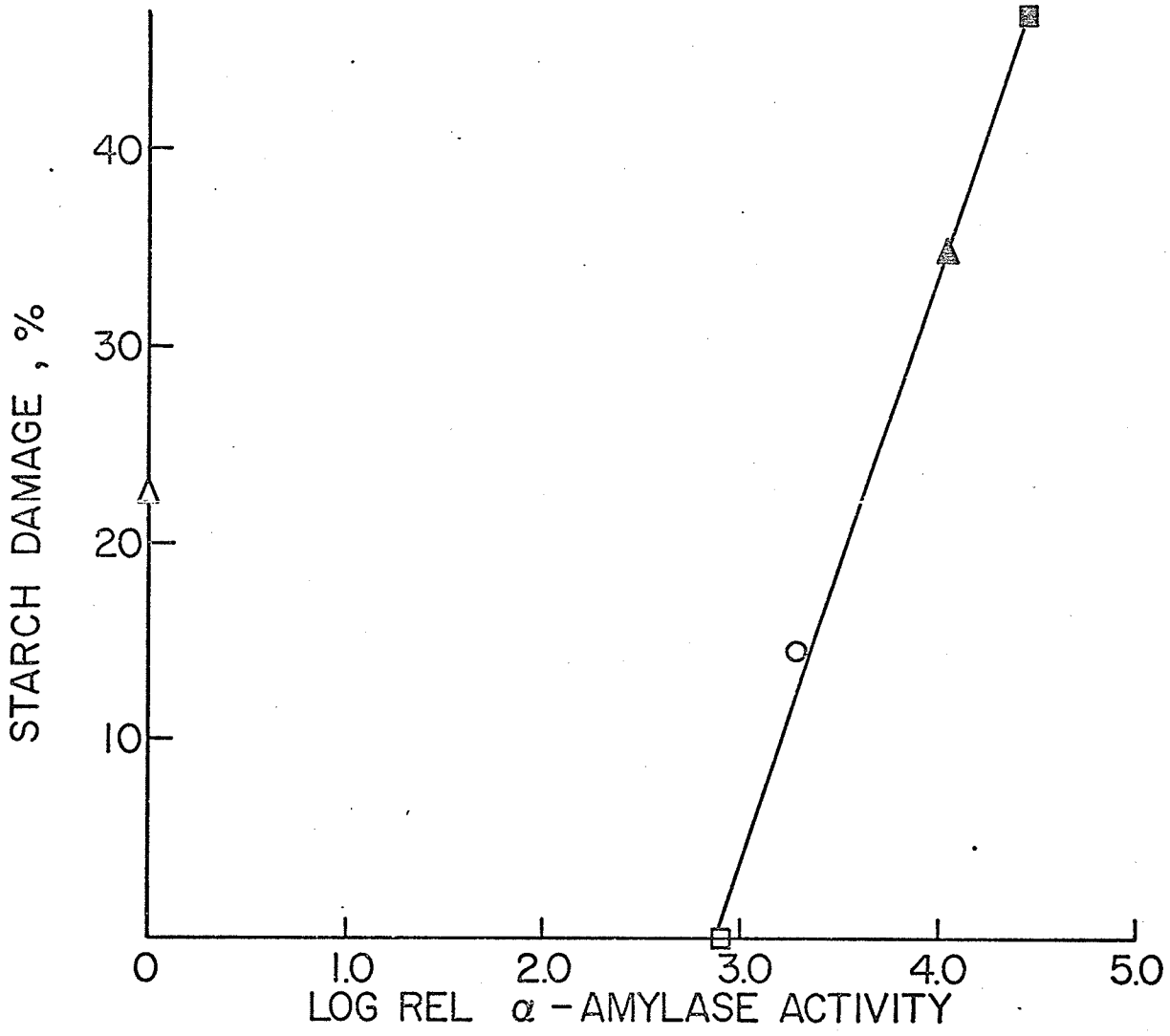
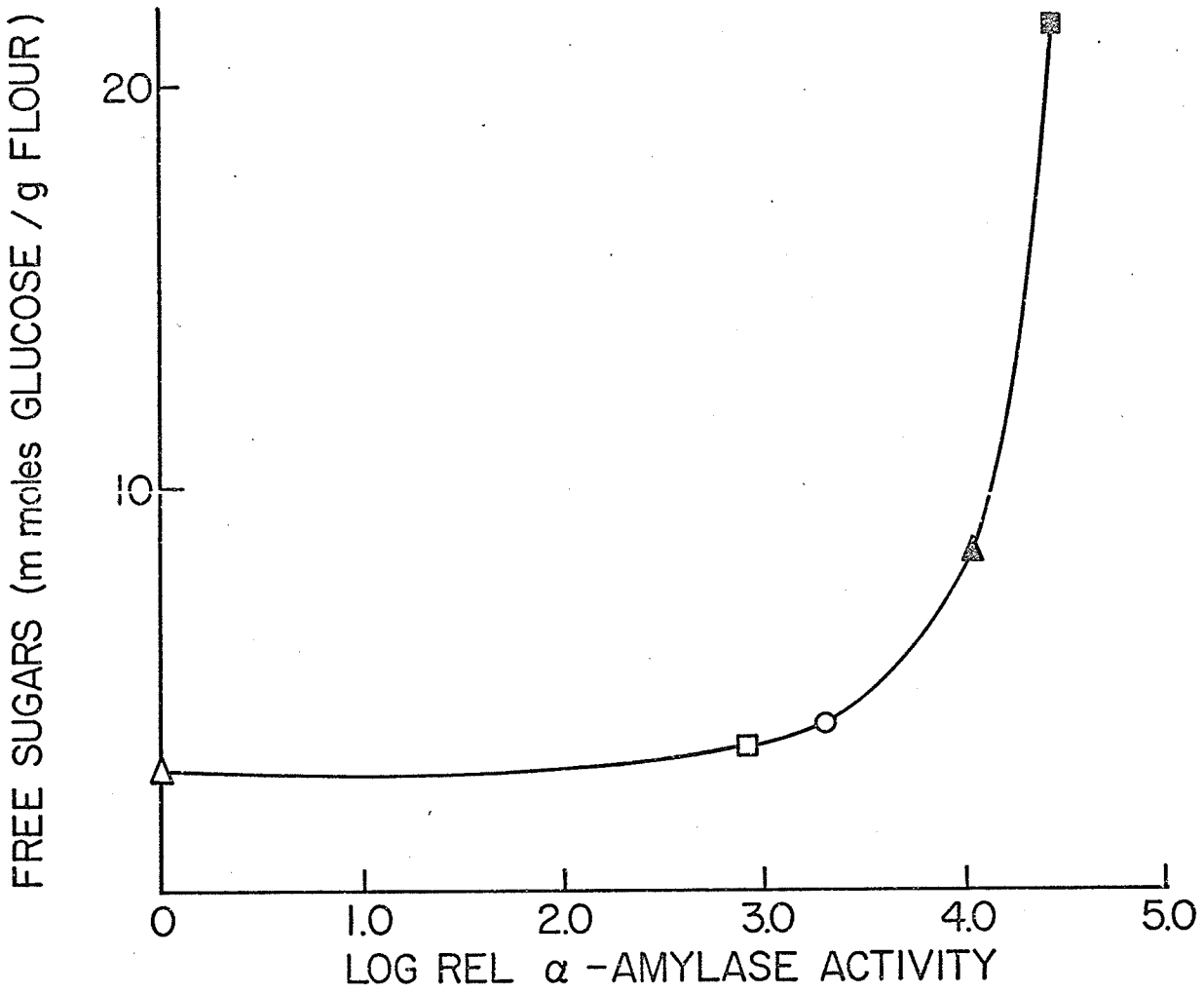

STARCH DAMAGE , %
40
30
20
10
0
1.0
2.0
3.0
4.0
5.0
LOG REL α - AMYLASE ACTIVITY

Figure 35

Free sugars versus α-amylase activity.


FREE SUGARS (m moles GLUCOSE / g FLOUR)
20
10
0
1.0
2.0
3.0
4.0
5.0
LOG REL α -AMYLASE ACTIVITY

quality is the protein. The present study showed that this component undergoes marked changes during sprouting which could have a further (to α-amylase) detrimental effect on baking quality. First of all, there was a small loss of protein during sprouting as indicated by the protein content of the flour. Secondly the solubility properties are markedly affected (see Table 9). The extensive decrease in the amount of the residue component is probably the most important change in relation to baking quality. Orth (1971) has shown that baking quality of 26 normal wheats of different type, expressed in terms of loaf volume, was directly related to the amount of residue protein.

The observed changes in the solubility properties can be explained in terms of hydrolysis of peptide bonds by the increased proteolytic activity that develops during sprouting (see Table 15). Hydrolysis of peptide bonds during sprouting was confirmed by two forms of experimental evidence. Firstly, gel filtration chromatography showed a gradual decrease in the amount of the high molecular weight fraction ($>100,000$) and a concomitant increase in the two low molecular weight fractions ($< 25,000$). Secondly, the number of free amino groups increased rapidly with sprouting. For each sample, both the decrease in molecular weight of the proteins (decrease in the residue, or the $>100,000$ fraction) and the increase in amino nitrogen were related to the proteolytic activity (see Figs. 36 - 38). It is technologically significant that the high molecular weight component of the endosperm proteins was degraded preferentially to the other components. The heavy component is essential for the formation of gluten that will have optimum gas retaining capacity in a loaf of bread.

Figure 36

Residue versus proteinase activity.

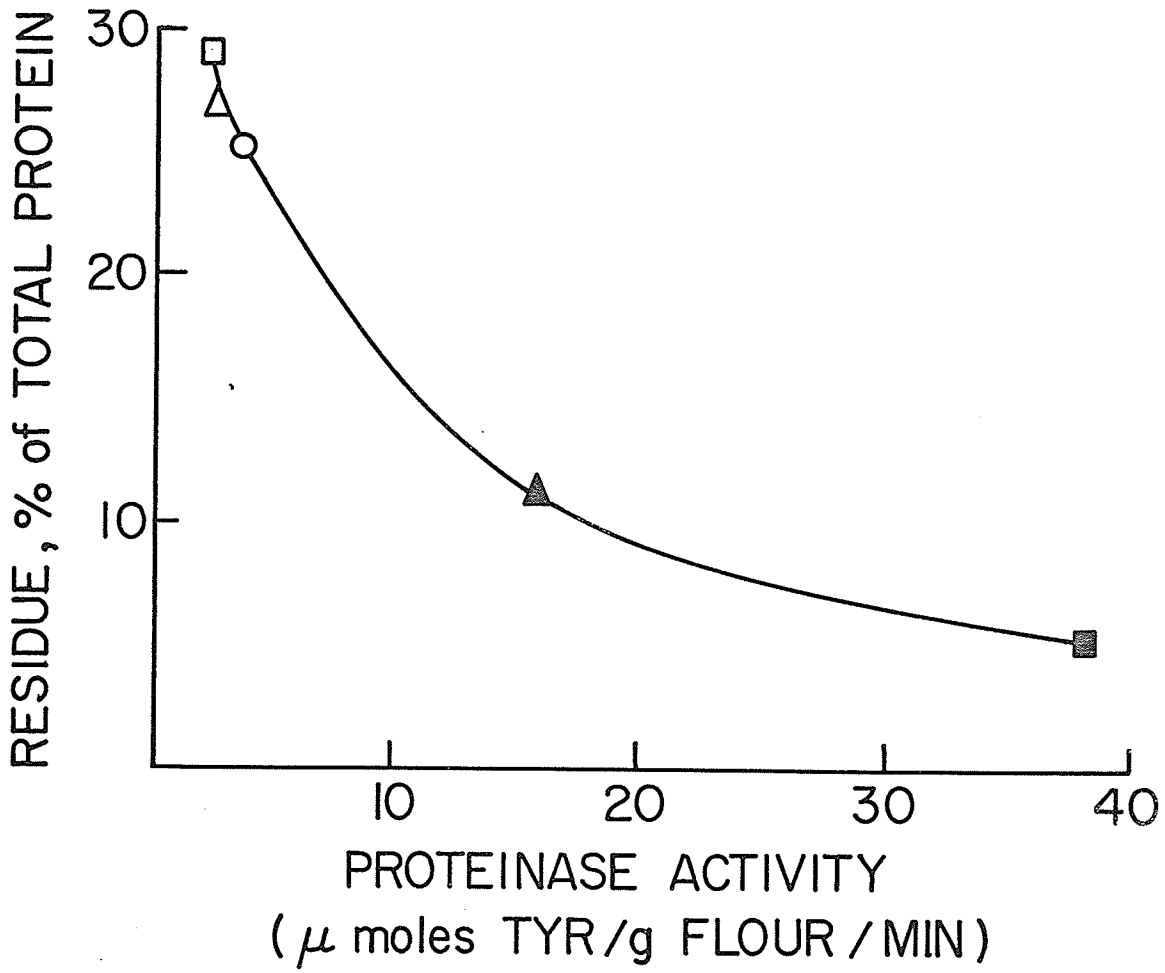

RESIDUE, % of TOTAL PROTEIN
30
20
10
10
20
30
40
PROTEINASE ACTIVITY
(μ moles TYR/g FLOUR/MIN)

Figure 37

Fraction >100,000 M.W. versus proteinase activity.

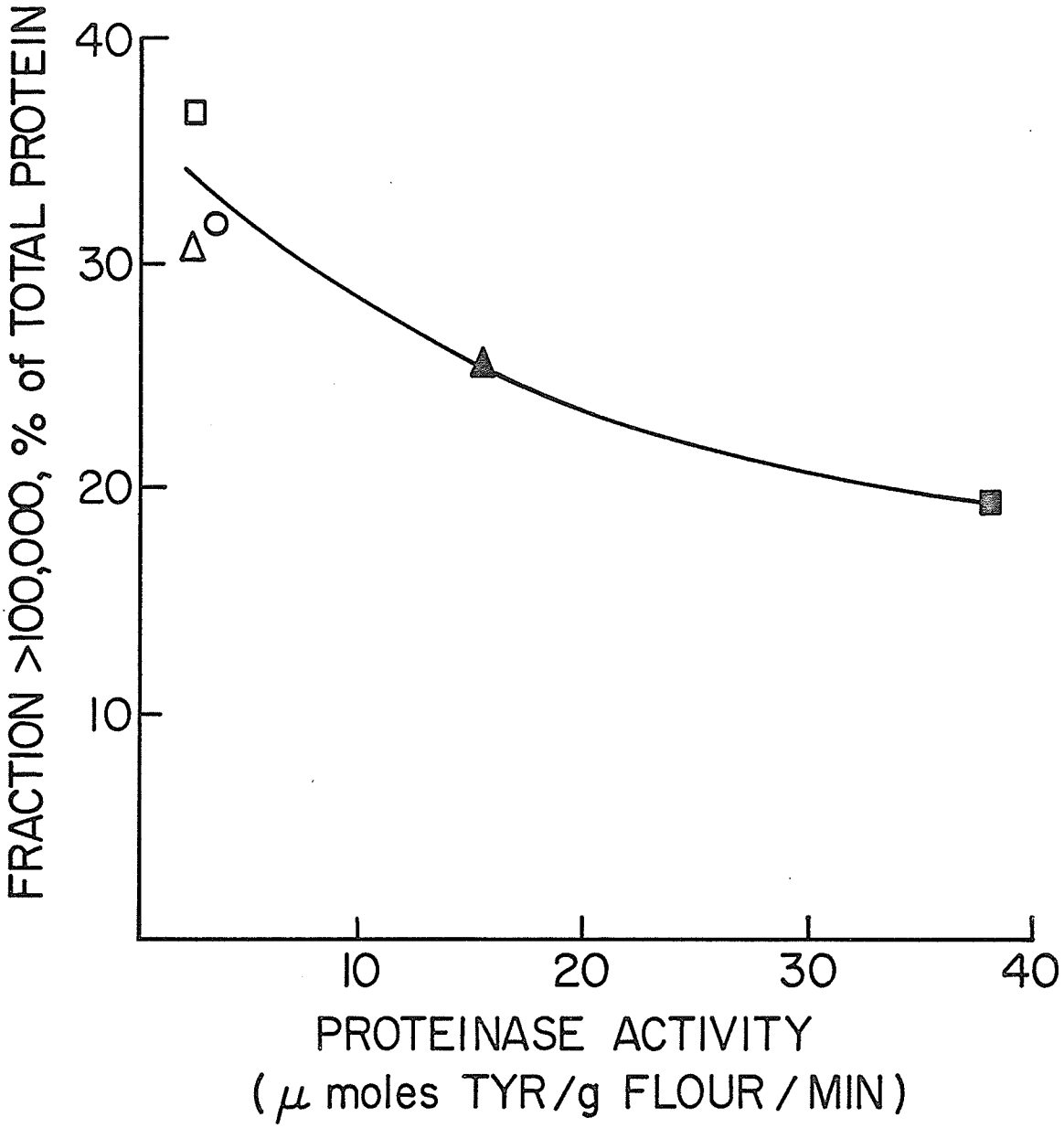
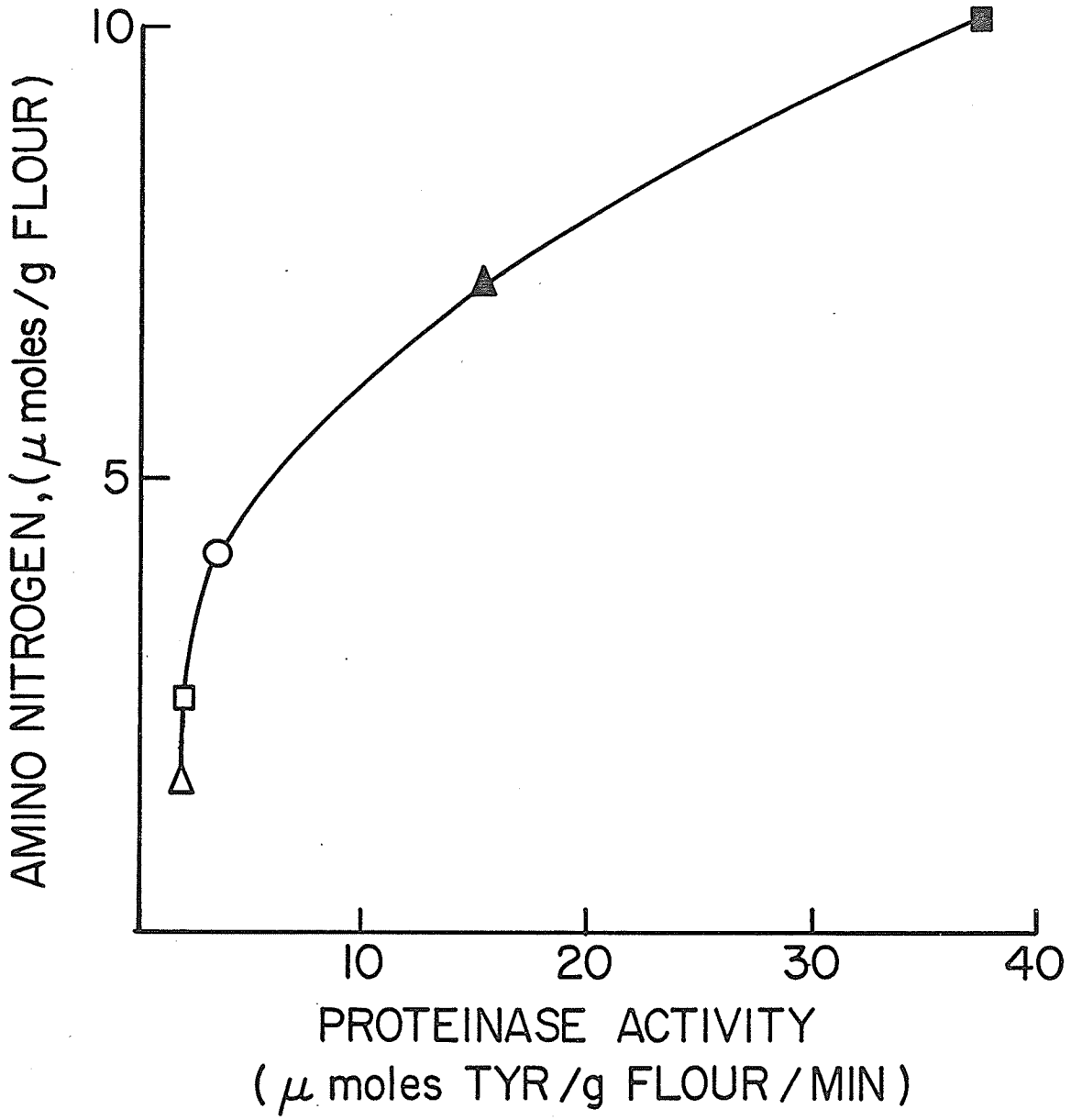

FRACTION >100,000, % of TOTAL PROTEIN
40
30
20
10
10
20
30
40
PROTEINASE ACTIVITY
(μ moles TYR/g FLOUR/MIN)

Figure 38

Amino nitrogen versus proteinase activity.


AMINO NITROGEN, (μ moles / g FLOUR)
10
5
10
20
30
40
PROTEINASE ACTIVITY
(μ moles TYR / g FLOUR / MIN)

Other minor analytical changes in the proteins during sprouting were observed. Amino acid compositions showed significant decreases in the proportions of arginine and proline. Analyses of sulfhydryl (SH) and disulfide (S-S) groups showed a slight decrease in SH and a small increase in S-S. The changes in SH and S-S could be technologically significant because of the functional role of these groups in dough.

The present study confirms the recently published work from the Soviet Union (Beresh, 1969) which showed that degradation of gluten proteins during sprouting occurs as a result of cleavage of peptide bonds. No evidence was obtained in the present study of the breakdown of S-S bonds as postulated by Shorina et al. (1967). Accordingly, it is concluded that the breakdown of the gluten complex during sprouting starts with cleavage of peptide bonds. Subsequently this could lead to a breakdown of secondary (ionic, hydrogen, hydrophobic) bonds that are known to contribute to the physical structure of gluten.

To bring the present study to a more useful practical conclusion, it would be of interest to extend it in the following directions. It would be useful to know the nature and the functional role of the low molecular weight protein components that are produced during sprouting. Also it would be worthwhile to extend this study to wheat sprouted under natural field conditions. Finally, from the wheat breeding point of view, it would be useful to study a number of different varieties to determine if there are any genotypic variations in the changes that occur in the endosperm that are pertinent to breadmaking quality.

SUMMARY AND CONTRIBUTIONS TO KNOWLEDGE

1. Sprouting of wheat had a detrimental effect on its milling quality; the yield of flour decreased and color deteriorated. These observations confirm and extend previously published information.
2. Breadmaking quality of flour from sprouted wheat was considerably poorer than that of flour from dormant wheat; the extent of deterioration of quality increased with increasing degree of sprouting.
3. α-Amylase activity of the flour increased about 27,000 fold during 8 days of sprouting under controlled conditions; this is in general agreement with published information.
4. Proteolytic activity increased 17 fold during 8 days of sprouting; this increase is somewhat higher than previously reported.
5. During sprouting of wheat, there were extensive chemical and physical changes in the starch.
6. Starch molecules were degraded, presumably by the action of amylases, to sugars. This change was confirmed by showing the rapid increase of free sugar content during sprouting.
7. Ordinary light and scanning electron microscopes were used to observe the physical changes during sprouting in the starch granules.
8. Erosion of starch granules, referred to as enzymic damage, occurred and the extent of erosion increased with sprouting.
9. Two types of surface erosion were observed:
   (a) equatorial or groove erosion of the large or A type granules, and
   (b) random spot surface erosion of both the large A type granules and the smaller B type granules.

10. The large granules showed extensive internal erosion; during progressive sprouting, the large granules were attacked before the small granules.
11. For a specific degree of sprouting, the granules located in the kernel adjacent to the aleurone layer were more extensively eroded than those located deeper inside the kernel.
12. The amount of "enzymic" starch damage, as determined by the Farrand and Fegol and Williams tests, and the amount of free sugars increased with increasing α-amylase activity of the flour.
13. Marked changes in the properties of the endosperm protein occurred during sprouting. Firstly, there was a small but significant loss of protein as indicated by the total protein content of the flours milled from the wheats. Secondly, the solubility properties are markedly affected.
14. Solubility fractionation showed an extensive decrease in the amount of insoluble or residue protein. It was concluded that this change was particularly detrimental to breadmaking quality.
15. It was shown that the number of total free amino groups increased during sprouting. This is taken as evidence of proteolytic cleavage of peptide bonds.
16. The gradual decrease during sprouting in molecular size of the endosperm proteins was confirmed by fractionation of AUC extracts of the flour on Sephadex G-150.
17. The decrease in the amount of the high molecular weight protein component in flour and the increase in the number of amino groups were directly related to the increase in proteolytic activity.

18. Amino acid compositions showed that during sprouting there was a differential disappearance of arginine and proline.

19. Analyses of SH and S-S groups showed a 30% decrease in the former and a 10% increase in the latter when dormant wheat was sprouted for 8 days.

20. So far as the writer is aware, points 5 to 19 inclusive are new contributions to knowledge in cereal chemistry.

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APPENDIX I. PROTEIN SOLUBILITY DISTRIBUTION FOR GROUND WHOLE WHEATS

Protein fractions	Control	Soaked	Germinated		
			2 Days	4 Days	8 Days
Water-soluble, %	11.2	9.2	8.1	10.3	9.3
Salt-soluble, %	9.8	9.4	10.2	12.1	12.1
Ethanol-soluble, %	31.6	33.4	32.6	28.8	24.0
Acetic acid-soluble, %	3.9	3.6	14.1	20.5	17.6
Residue, %	39.0	41.5	28.9	19.9	19.7
Acetic acid-soluble + residue, %	42.9	45.1	43.0	40.4	37.3
Recovery, %	95.5	97.1	93.9	91.6	82.7

APPENDIX II

MOLECULAR WEIGHT DISTRIBUTION OF SOLUBILITY FRACTIONS BY GEL FILTRATION ON SEPHADEX G-150 USING AUC SOLVENT

Fraction Mol. wt.		I 100,000	II 100,000 -25,000	III 25,000 -10,000	IV 10,000
Water-soluble	control	-	36.8	31.2	32.0
	soaked	5.3	45.9	33.0	15.8
	2 days	6.2	46.4	32.2	15.2
	4 days	6.3	45.0	39.5	9.2
	8 days	4.0	34.0	47.0	15.0
Salt-soluble	control	59.7	23.8	16.5	-
	soaked	33.0	37.5	29.5	-
	2 days	29.0	37.2	33.8	-
	4 days	16.4	56.5	27.1	-
	8 days	2.0	65.0	30.0	3.0
Alcohol-soluble	control	19.8	76.6	3.6	-
	soaked	12.4	79.6	8.0	-
	2 days	14.5	68.0	17.5	-
	4 days	22.9	69.0	8.1	-
	8 days	26.0	64.7	9.3	-
Acetic acid-soluble	control	49.5	50.5	-	-
	soaked	53.5	46.5	-	-
	2 days	50.7	49.3	-	-
	4 days	51.8	48.2	-	-
	8 days	51.0	49.0	-	-
Residue	control	57.0	43.0	-	-
	soaked	51.2	48.8	-	-
	2 days	55.1	44.9	-	-
	4 days	55.8	44.2	-	-
	8 days	51.0	49.0	-	-