NaCl, water, 70% ethanol, and 0.1 *N* acetic acid. The nitrogen content of his extracts varied from 0.4 to 7.7%, and two-thirds of the nitrogen remained in the residue. Although his work is on oat flour while our results are on the individual dry-milled fractions, the percentages of total nitrogen extracted by water and by 70% ethanol in his study are comparable with those in Table II. On the other hand, the percentage of total nitrogen extracted by 1 *M* NaCl and 0.1 *N* acetic acid was much higher in our studies. Our higher recovery of globulin may be a result of the higher concentration of NaCl used. In addition, there may be differences among the oats used for extraction. Our nitrogen recovery calculated from Table II was not far from the 82.7% value reported by Ewart. Starch gel electrophoresis in 8 *M* urea of his 70% ethanol extracts of oat flour showed three bands, the same number as we obtained from Wyndmere break flour. Five bands were observed in starch gel electrophoresis by Elton and Ewart (1962) from their oat flour dispersion in aluminum lactate buffer, which dissolved only 23% protein.

Waldschmidt-Leitz and Zwisler (1963) isolated albumin, globulin, and avenin from defatted oat flour and studied the amino acid composition. Because their nitrogen recovery was very low (less than 5%), their results may not be representative. For this reason, no comparison was made between their results and Table V. The amino acid composition of Wyndmere oats in Table IV agrees quite well with compositions reported by Hischke *et al.* (1968) for seven varieties of oat groats and with the mean amino acid composition of 289 oat groats as established by Robbins *et al.* (1971).

The availability of high-protein oats, the favorable solubility properties of oat proteins, and their well-known nutritive value indicate a bright future for low-cost, high-protein, and highly nutritious food products being made from oat fractions.

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Grain Sorghum Glutelin: Isolation and Characterization

Alfred C. Beckwith

Glutelin accounts for more than half of the total protein content in three grain sorghum hybrid flours. A procedure has been developed to isolate this major fraction of highly insoluble proteins. Changes in glutelin solubility, gel electrophoretic, and chromatographic properties accompanying the cleavage of protein disulfide bonds are described.

In describing the protein composition of three hybrid grain sorghums, Jones and Beckwith (1970) noted that less than half the total protein nitrogen in the flour fractions was extractable protein nitrogen. A method has now been developed for isolating the major protein fraction, hereafter called glutelin. This glutelin is not extracted from flour with common neutral or weakly acidic solvents. Basic solvents were not employed, to avoid possible chemical decomposition of macromolecules. Physical-chemical properties of the isolated glutelin were roughly determined, as well as those of the product left after disulfide bond disruption.

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MATERIALS AND METHODS

Guanidine hydrochloride (GHCl, Eastman Organic Chemicals white label grade) was recrystallized by the method of Kolthoff *et al.* (1957). When used in reaction solvents, GHCl was further purified by the procedure of Wu and Dimler (1964). β-Mercaptoethanol, also purchased from Eastman Organic Chemicals, was redistilled under nitrogen. The fraction boiling at 153–155°C was collected and used as a disulfide bond reducing agent. Acrylonitrile (Eastman's yellow label) was used as a sulfhydryl blocking agent without further purification. Sephadex G-150 crosslinked dextran was purchased from Pharmacia Fine Chemical Inc. Twice-recrystallized hog pancreas α-amylase came from the Mann Research Laboratories. The stated activity of the enzyme

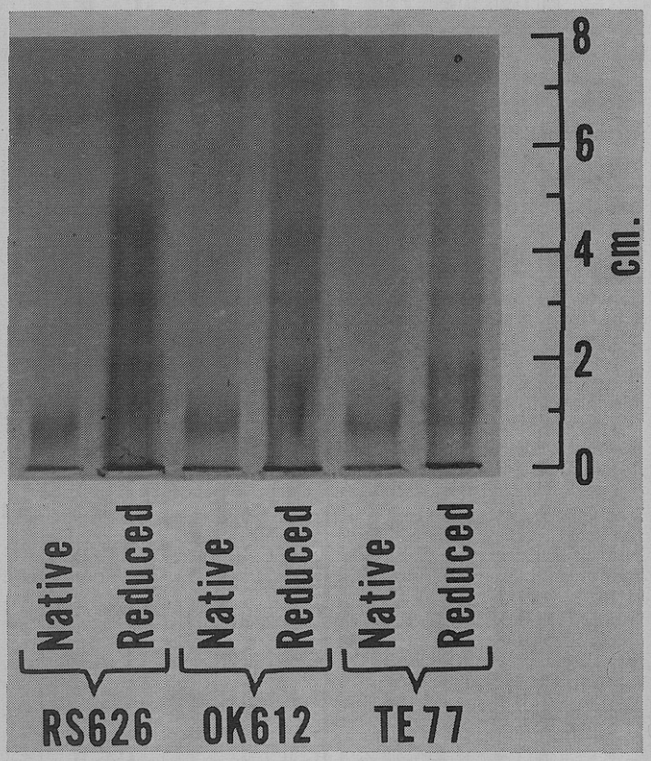

Figure 1. Electrophoretic patterns of native and reduced sorghum glutelins from three sorghum hybrids, RS-626, OK-612, and TE-77. Aluminum lactate–lactic acid buffer, 8 *M* urea, pH 3.1, 250 V, 4 hr

Table I. Selected Amino Acid Contents of Prolamins and Glutelins in Grain Sorghums

Amino acid	g/16 g of nitrogen	
	Prolamin	Glutelin
Lysine	0.1	2.1
Histidine	0.9	2.0
Arginine	1.0	3.6
Glutamic acid	30.0	23.3
Half cystine	0.4	1.2
Leucine	19.2	14.9

was about 620 units/mg. All other standard chemicals were reagent grade.

Protein Isolation. After globulins, albumins, and prolamins are removed from defatted sorghum flours (Jones and Beckwith, 1970) 40 g of the freeze-dried residue is dispersed in 500 ml of dimethyl sulfoxide (DMSO)–water (9:1, v/v) in a blender. The dispersion is then diluted with 310 ml of water and 90 ml of 0.2 *M* solution of sodium β-glycerophosphate–HCl buffer (pH 6.9). This dilution step is exothermic. From 3800 to 4000 units of α-amylase is introduced into the DMSO–water suspension after it was cooled to 30°C. With constant stirring the suspension is incubated in a 30°C constant temperature bath for 18–24 hr or until 0.1-ml aliquots, when diluted to 100 ml, give a negative blue amylose–iodine color. The crude protein dispersion is then further diluted with 1500 ml of water and centrifuged at 10°C and 10,400 $\times$ *g*. The insoluble glutelin cake is redispersed in water and again centrifuged. A final dispersion in water is dialyzed against large volumes of water to remove DMSO solvent at 4°C. The insoluble glutelin is then freeze-dried. Protein ($N \times 6.25$) is estimated by the standard semimicro-Kjeldahl procedure.

Amino Acid Analysis and Electrophoresis. The procedures followed to carry out gel electrophoresis and to perform amino acid analyses have been reported earlier (Jones and Beckwith, 1970).

Reduction and Alkylation. Samples of sorghum glutelin were dispersed in 6 *M* GHCl solutions buffered with tris(hydroxymethyl)aminomethane hydrochloride (pH 7.4) under a nitrogen atmosphere. On a basis of cystine residues determined by amino acid analyses, a 75–100 *M* excess of β-mercaptoethanol was added to reduce the glutelin disulfide bonds. Since the proteins were not soluble in this media, the reaction was allowed to proceed for 24 hr at 25°C. A two-fold excess of acrylonitrile was then added to react with all free sulfhydryl groups in the mixture (Weil and Seibles, 1961; Beckwith *et al.*, 1965). The reaction mixture was then acidified (pH 3) by adding glacial acetic acid. Insolubles were removed by centrifugation. All precipitates and solutions were separately dialyzed against large volumes of water with frequent changes to remove reagents before freeze-drying. Protein controls containing no mercaptoethanol or acrylonitrile were handled in a similar fashion as those samples treated with reducing agent.

Column Chromatography. Sephadex G-150 crosslinked dextran was equilibrated with 6 *M* GHCl solution for 5 hr at 75°C and then for 16 hr at 25°C. After gel fines were removed, a water-jacketed column (2.5 $\times$ 92 cm) was prepared with the G-150 dextran. Native or reduced-alkylated glutelin fractions which were soluble in 6 *M* GHCl were applied to columns in 2 ml of solvent (100 mg of protein). Columns were eluted at 25°C with 6 *M* GHCl at a flow rate of 10–11 ml/hr. Effluent fractions were collected in an LKB 7000 collector. An LKB Unicord II accessory monitored the ultraviolet absorbance of the effluent (at 280 nm).

RESULTS AND DISCUSSION

The isolation of insoluble glutelin proteins by means of enzymatic degradation of starch has been reported earlier (Paulis *et al.*, 1969). These workers used the method described here to isolate nondegraded corn glutelin proteins. The high concentration of DMSO used to disperse the defatted flour residue disrupts starch granules and the starch dissolves. In concentrated DMSO, however, the α-amylase has no activity, as determined in separate tests with potato amylose. After dilution of the DMSO with aqueous buffer some starch precipitates but the starch is readily hydrolyzed. On the basis of nitrogen recoveries compared to nonenzyme-treated controls, there appears to be no peptide nitrogen loss due to peptidase impurities in the amylase preparation. The sorghum glutelin isolates obtained after a single amylase treatment contain about 12–14% *N* or about 75–85% protein ($N \times 6.25$). Although specific tests were not made, the glutelin isolates probably contain nonstarchy carbohydrate impurities. Since the products are distinctly colored, undoubtedly protein pigment complexes are present.

Average amino acid composition of residues from the three sorghum hybrids studied earlier are given by Jones and Beckwith (1970). Here, Table I compares the average amounts of selected amino acids found in glutelin and prolamins, which account for more than 80% of the total proteins in sorghum flours.

As seen from Table I, glutelin contains appreciably more of the basic amino acids than does prolamin. Relative pro-

portions of glutamic acid with a polar terminal group and leucine with an aliphatic side chain remain high in sorghum glutelin, as they do in other cereal grain glutelins (Paulis *et al.*, 1969; Wu and Dimler, 1963). Later evidence suggests that the additional cysteine residues in sorghum glutelin probably form disulfide bonds, joining smaller protein units together.

Glutelin fractions from the three hybrid sorghums were neither soluble in aqueous media, in 8 *M* urea solutions, nor in DMSO–water systems. Only 23% of the glutelin nitrogen could be dissolved in neutral or weakly acidic 6 *M* GHCl solutions. At present it is not known what contribution protein–pigment complexes make to the poor solubility properties of these fractions.

Reduction of disulfide bonds in sorghum glutelin increases nitrogen solubility in neutral 6 *M* GHCl to 85–89%. Those reduced proteins soluble in GHCl solutions are also initially soluble in 8 *M* urea solutions, but unfortunately the urea solutions gel upon standing. As might be expected, this change in solubility upon disruption of disulfide linkages is accompanied by a change in the gel electrophoretic behavior (Figure 1).

The sharpest electrophoretic patterns in Figure 1 are for the RS-626 sorghum hybrid glutelin. The patterns in Figure 1 labeled native are for those proteins soluble in 6 *M* GHCl before reduction of disulfide bonds and alkylation of liberated sulfhydryls. The native proteins contain a small amount of material that migrates a short distance into the gel as two different electrophoretic species. The reduced-alkylated preparation contains considerable material trailing from the point of application as well as distinct electrophoretic components. Glutelins from the other two sorghum hydrids exhibit similar patterns as those for the RS-626 hydrid. All glutelin preparations shown in Figure 1 contain material remaining at the gel origin after disulfide bond reduction.

The column elution profile from Sephadex G-150 for the reduced-alkylated RS-626 glutelin appears as Figure 2. The elution volume for fraction I in Figure 2 is just slightly larger than the column void volume. This elution position is evidence that the reduced proteins still contain relatively large molecules. The elution profile for the unmodified glutelin soluble in 6 *M* GHCl is not shown, since it consisted of only a single broad peak. This single peak began eluting at the column void volume and continued over the entire volume required to remove the alkylated proteins.

The gel electrophoretic patterns for fractions I–V in Figure 2 and the pattern for the nonseparated reduced glutelin are reproduced in Figure 3. Fraction I contains no material migrating into the gel matrix during electrophoresis. In addition to material remaining at the origin, the electrophoretic patterns for fraction II shows faint bands at 4.8 and 5.2 cm in Figure 3. The striking feature in the pattern for fraction III is the presence of two intense, well-separated bands at 1.6 and 2.2 cm. There are also four other, much fainter, bands in fraction III. In 8 *M* urea solution at a concentration of 2% w/v, both fraction III and the nonseparated bands of reduced glutelin gel. Perhaps, therefore, the components of fraction III have a strong tendency to form aggregates, which could contribute to the intense staining at the origin in the gel pattern for this fraction. All other fractions, except V, at the same solids concentration remained liquid in the 8 *M* urea solvent.

Fraction IV appears to consist chiefly of a single electrophoretic component. Because of poor column separation this fraction has small amounts of material found in fraction III. Even though fraction V showed an ultraviolet absorbance in the elution profile (Figure 2), this fraction contained no material within 10 cm of the origin in electrophoresis that binds the amido black dye used for staining the gels. The column elution position of fraction V suggests that it consists of particles of relatively smaller size than found in the other fractions and some of these smaller molecules could be lost during the removal of GHCl.

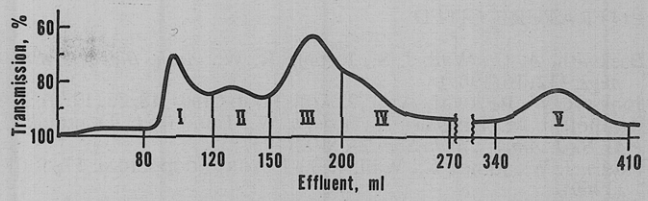

Figure 2. Elution profile for reduced-alkylated RS-626 glutelin from Sephadex G-150 (column is 2.5 × 92 cm), 6 *M* guanidine hydrochloride, 25°C, 10–11 ml/hr. Numerals in figure indicate fractions

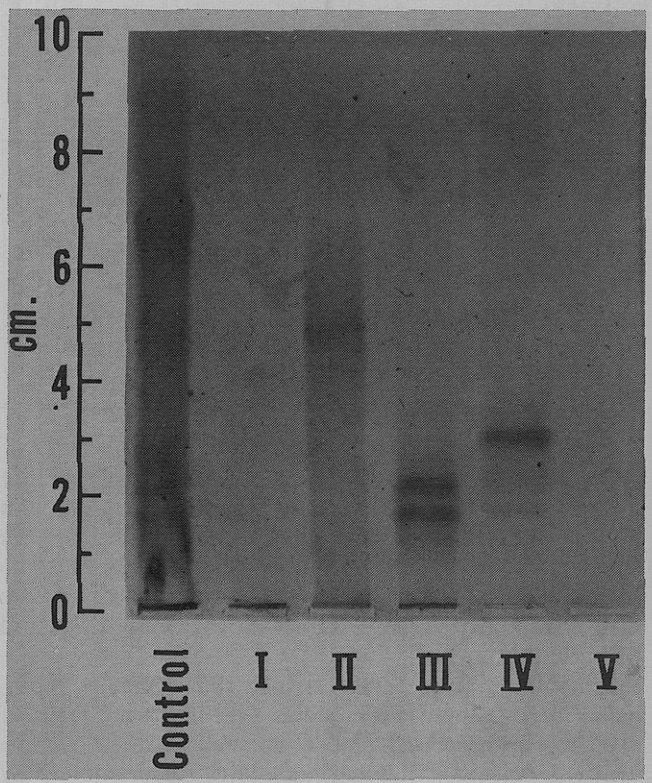

Figure 3. Electrophoretic patterns of reduced RS-626 glutelins (control) and RS-626 glutelin fractions I–V from Figure 2. Aluminum lactate–lactic acid buffer, 8 *M* urea, pH 3.1, 400 V, 4 hr

Quite likely, glutelin protein fractions of grain sorghum consist of smaller protein units joined together through disulfide linkages. A more thorough study of the physical–chemical properties of glutelin, however, must consider that sorghum contains some substances which can become irreversibly bound to the proteins. All column fractions isolated showed a certain amount of discoloration when dissolved in urea solvents; fraction I (Figure 2) displayed the greatest amount of discoloration. While amino acid analyses of fractions in Figure 2 have not been presented, such analyses have shown that fractions I–IV in Figure 2 have significant amounts of unknown acidic ninhydrin positive compounds. At present the exact nature of these complexes and means of preventing their formation remain unknown.

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Invertase of Germinated Barley

Neville Prentice

Invertase was present in soluble and insoluble form in barley axis, scutellum, shoot, and rootlet, but was absent or at a low level in the degermed caryopsis. The shoot tissue contained two soluble invertases which were isoelectric at pH 4.1 and pH 10.0, but the axis, scutellum, and rootlets contained only one soluble invertase, and this was isoelectric at pH 9.5. The soluble invertases have been examined also for pH optimum and stability, thermostability, molecular weight, and Michaelis constants; the insoluble enzymes have been examined for all these properties except molecular weight and isoelectric pH. The apparent molecular weights of all soluble invertases as determined by gel filtration were 92,000 ± 3000. K_m values for soluble and insoluble enzymes were in the range 2×10^{-3} *M* to 9×10^{-3} *M*.

One of the important processes in the germination of grain is the formation of sugars from endosperm carbohydrates for use by the developing seedling. In germinating wheat and barley seed, glucose is converted to sucrose in the scutellum (Edelman *et al.*, 1959) and sucrose is then transported to the axis, where it is rapidly utilized (Palmer, 1969).

Jones and Armstrong (1971) have shown that in germinating barley, α-amylase increases with increasing levels of applied gibberellic acid until a maximum level of enzyme activity is reached. This maximum level was determined by the level of maltose, glucose, and other low molecular weight substances near the aleurone cells. Removal of these sugars by way of sucrose synthesis in the scutellum and sucrose utilization in the rootlets and shoots should stimulate enzyme formation in the aleurone cells.

Radley (1969) has shown that if sucrose accumulates in barley scutellum, further breakdown of carbohydrate in the endosperm declines, since gibberellin production in the scutellum, and hence gibberellin-dependent enzyme synthesis in the aleurone cells, stops or continues at a low level.

Invertase (D-fructofuranoside fructohydrolase, E.C. 3.2.-1.26) of the barley kernel, an enzyme likely to be involved in sucrose utilization in the axis, has received little attention. Hoffmann and Günzel (1955) demonstrated increasing activity in aqueous extracts of tissue obtained at various times during germination. Most of the enzyme was in the seedling tissue. Nolte and Kirchdorfer (1954) viewed the increase in the water-soluble invertase activity to arise from the release of an extracellular enzyme by a hydrolytic cleavage of an intracellular one. Data obtained with three barley varieties indicated that activity of the soluble enzyme is influenced by nitrogenous fertilization of the plant, and that the level of activity is a varietal character. The results of Nolte and Kirchdorfer and of Radley suggest that invertase activity of a variety may be meaningful in relation to good malting quality for which optimal modification of endosperm constituents is necessary. Before such a relationship can be properly examined, a better characterization of the enzymes would be appropriate.

This paper describes some properties of soluble and insoluble invertase and the distribution of these enzymes in tissues of two barley varieties.

MATERIALS AND METHODS

Barleys and Malt Rootlets. The barleys were Larker, a midwest 6-row type, and Piroline, a 2-row type. Malt rootlets from kilned Larker malt were furnished by the Kurth Malting Co., Milwaukee, Wis.

Germination Procedures. (a) One-hundred-and-seventy grams (dry basis) of each barley was steeped in running tap water at 16°C to 45% moisture and germinated at 16°C in the dark until rootlet length was about 1 cm. This required 7 days for Larker and 6 days for Piroline. (b) Larker barley was washed thoroughly with sterile distilled water. Fifty kernels were placed in a sterile Petri dish which contained two sheets of Whatman No. 1 filter paper moistened with 5 ml of the water, and germinated as before for 96 hr. Twelve-hundred kernels were germinated and stored at −25°C.

Preparation of Tissue from Germinated Barleys. For the separation of component tissues, Larker and Piroline barleys were germinated by method (a). Kernels were thawed and separated into rootlet, shoot, degermed caryopsis, and embryo tissues. Piroline embryo was separated into axis and scutellum. All tissues were lyophilized.

For large quantities of rootlets, Larker was germinated by method (a) and lyophilized. For large quantities of shoots the Larker was germinated by method (b), and the shoots were excised and lyophilized.

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