AMINO ACID COMPOSITION OF THE FRACTIONATED PROTEINS
OF SORGHUM GRAIN AND HIGH-LYSINE CORN

by 45

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INTRODUCTION

Published literature on the amino acid composition of various types of proteins in sorghum grain is limited as compared to that for corn and wheat. Knowledge of cereal proteins is important because of their unique physical properties and contributions to kernel structure. The amino acid content of various protein fractions aids in establishing the effects protein and its constitutients have on nutrition, processing, and feed formulation. This research program was designed to further the knowledge in these areas by investigating soluble and insoluble protein fractions of sorghum grain, opaque-2 corn and their respective amino acid compositions.

The protein fractions were obtained from five sorghum grain samples and opaque-2 corn. Proteins of each grain were fractionated by use of a modified Mendel-Osborne method (1) to obtain five protein fractions defined as water-soluble (albumins), salt-soluble (globulins), alcohol-soluble (prolamines), alkali-soluble (glutelins), and insoluble materials (scleroproteins). Amino acid analyses of both the whole grain and the various protein fractions were established by acid hydrolysis and separation by ion exchange chromatography. The following amino acids were determined: lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine.

REVIEW OF LITERATURE

Extraction and Fractionation of Cereal Proteins

The extraction and fractionation of cereal grain proteins has been an effective method for evaluating composition and nutritive value of the proteins. Osborne and Mendel (1) reported 22% of the total protein of corn with which they worked to be soluble in 10% potassium chloride solution, 41% soluble in 90% alcohol, 31% soluble in 0.2% potassium hydroxide solution, and 6% as insolublo and lost. Spitzer et al. (2) reported 5.27% of the total nitrogen of corn to be present as amides, 21.61% as globulin (soluble in 10% sodium chloride solution), 29.41% as zein (soluble in 90% alcohol) and 42.85% as glutelin. These data indicate considerable disagreement as to the distribution of various proteins in corn.

Ether-extracted ripe corn was fractionated by Zeleny (3) with successive extractions with water, molar-sodium chloride solution, hot 80% ethyl alcohol, and 0.2% potassium hydroxide solution. One-gram samples were shaken for one hour with each of the above solutions, filtered and washed three times with the respective solvent. Samples were air-dried after each extraction. Filtrate and washings from each extraction were combined and total nitrogen established by the Kjeldahl method. Results on the successive extractions were 6.28, 7.82, 41.97, and 16.92% respectively, with 27.01% classified as nitrogen not peptized. Zeleny (3) theorized that the nitrogenous substances not peptized by the various solvents consisted of seed coat protoins that were difficult to separate from the insoluble carbohydrate material with which they were associated.

Nagy et al. (4), Jimenez (5), and Mertz et al. (6) have studied extraction and fractionation of corn protoins using various modifications of the classical Osborne method (1). The method utilizes a dilute salt solution to remove albumins and globulins, an aqueous ethyl alcohol solution to extract zein, and a dilute alkali solution to dissolve glutelins. These workers seldom recovered more than 80 to 90% of the total soluble protein from corn.

Defatted commercial cornmeal ground to pass a 60-mesh sieve was used by Foster et al. (7) to determine soluble fractions of protein soluble in distilled water, 5% sodium chloride solution, pH 10 carbonate buffer, and pH 7.8 phosphate buffer, respectively. The extractions were performed in 250-milliter centrifuge tubes using 100 milliters of extracting fluid. Separation was made by centrifugation at 2000 r.p.m. Extractions were compared both at room temperature and in the cold room. The results indicated water extracts approximately 11% of the total protein, either at room or cold temperature. Extraction with 5% sodium chloride solution yielded an additional 11% of the protein, while the carbonate buffer following water and salt extraction removed 4.9% of the protein.

The Mertz and Bressani (8, 9) method of protein extraction gave a more complete extraction of protein from corn or sorghum grain than modifications of the Osborne method, but also caused considerable denaturation. The proteins of corn germ and corn endosperm were almost completely extracted by adjusting a slurry of 1 part corn product to 2 parts water to pH 9 with dilute sodium hydroxide. The slurry was alternately frozen and thawed 3 times and diluted with 25 parts more water. Following dilution, 15 parts copper sulfate and 2 parts sodium sulfite were added, and after solution of these salts the pH was adjusted to 12 with sodium hydroxide. After stirring for 3 hours the supernatant from centrifuging was either dialyzed or the proteins precipitated by addition of copper sulfate solution to pH 6.0, for endosperm proteins. About 90% of the total nitrogen of the extract was recovered as dry (lyophilized) product.

This copper fractionation method (8) was used by Mertz and Bates (10) to determine the distribution of soluble nitrogen in ground, defatted <u>opaque</u>-2 corn endosperm and ordinary dent endosperm. The soluble nitrogen distribution for <u>opaque</u>-2 corn was 35% acid-soluble, 26% alcohol-soluble (zein), and 29% alkali-soluble (glutelin). They attributed the increased content of lysine in opaque-2 corn endosperm to the reduced ratio of zein to glutelin, plus the increased lysine content of the acid-soluble and zein fractions.

A Russian scientist, Tsurkan (11) reported the composition of nitrogeneous compounds to be best classified by extraction successively with 5% sodium chloride, 0.2% sodium hydroxide, and finally the Mertz and Bressani (8) reagent.

Only limited published literature on the types of proteins in sorghum grain was available, Unger and Morris (12), Wall (13), Virupaksha and Sastry (14), and Rooney and Clark (15). Unger and Morris (12) extracted with 60% aqueous alcohol a zein-like protein known as kafirin from the endosperm and from the wet-milling gluten fraction of sorghum grain. These workers found kafirin (prolamine fraction) represented about 83% of the endosperm protein and contained a high proportion of glutamic acid.

Virupaksha and Sastry (14, 16) studied the soluble fractions of dehulled, defatted endosperm flour of sorghum grain. Solvents used for the protein fractionation were distilled water to extract the albumin, 1% sodium chloride solution for globulin, 60% aqueous ethanol for prolamine, and 0.1% sodium hydroxide solution for glutelin. One-hundred-milligram samples were shaken for 2 hours with 2.0 milliters of each solvent in a "wrist action" shaker. Extraction was made at room temperature for albumin, globulin, and glutelin and at 60°C for prolamine. The fraction was then centrifuged and washed with the solvent used for extraction. Kjeldahl nitrogen was established on the extract and its respective washing.

The results of Virupaksha and Sastry's (14) soluble fractionations indicated that prolamine and glutelin were the principal proteins of the sorghum endosperm, while albumin and globulin together represent less than 12% of the total endosperm protein. These workers reported the distribution of protein fractions in sorghum endosperm to be similar to that of corn endosperm proteins. The percentage of protein solubilized by Virupaksha and Sastry (14) for five different varieties of sorghum grain ranged from 80.7 to 103.4%. Increases in the protein content of the sorghum grain was attributed mainly to an increase in the prolamine fraction of the grain. The same trends in the prolamine content of other high-protein varieties of cereals have been reported by Bressani and Mertz (17) and Cagampang et al. (18). The prolamine (kafirin) fraction of sorghum grain is extremely low in lysine content. Thus, an increase in the kafirin portion of high-protein sorghums explains the actual decrease in the percentage of lysine in the protein. Similar trends have been reported by Waggle and Deyoe (19) for the entire protein of sorghum grain. One Indian variety of sorghum grain, 160-Cernum, was found to deviate from the normal relationship in that it had a high lysine content in addition to containing 17-18% protein. This variety had the highest ratio of glutelin to prolamine of the five sorghum varieties fractionated.

McIntyre and Kymal (20) found that extracting rice proteins using a series of extractions with water, 5% sodium chloride, 70% alcohol, and 0.2% sodium hydroxide left about 25% of the protein undispersed. These workers indicated that a majority of this remaining protein could be dissolved by use of a detergent solution.

Jones and Gersdoff (21), Teller and Teller (22), and Fellers et al. (23) reported various types and quantities of protein (according to solubilities in various solvents) in branny materials of wheat. Albumins, globulins, prolamines, glutelins, and alkaline-insoluble proteins were reported. An appreciable amount (9-25%) of nonprotein nitrogen compounds were found to exist in the bran of wheat. Teller and Teller (22) reported that the proportion of nonprotein nitrogen was very constant and amounted to nearly one-tenth of the total nitrogen present.

Factors Affecting Extraction and Fractionation of Cereal Proteins

Albumins. Various physical factors affect the amount of protein extracted with water. Nagel et al. (24) utilized a shaking technique with fat-free soybean meal to determine the effect of particle size, solvent-meal ratio, temperature, and time. Particles that would pass through 100-mesh screen were satisfactory for extracting nitrogenous compounds. In regards to a water to meal ratio, a 100 to 2.5 ratio by weight was found to give satisfactory extraction. Though the temperature effect can be quite significant, little variation in extracted material was found between 15 and 35°C. Length of shaking time for these water extracts was not critical. These workers found one minute's shaking for three successive times would disperse as much protein as 3 hours for each extraction. Coarser meal size yielded more water-soluble protein with longer shaking time, probably due to the longer time required by the solvent to saturate the unbroken cells.

Foster et al. (7) indicated water extraction of corn to be incomplete after 24 hours. Nagy et al. (4) reported a stirring procedure to be almost as effective for extraction as shaking, and that low temperatures did not lower extraction.

Globulins. Nagy et al. (4) reported the amount of salt-soluble protein extracted to be independent of salt (sodium chloride) concentration (5-10%), but it was increased about 2% by grinding the material from 60- to 100-mesh. Similar results obtained by O'Hara and Saunders (25) indicated that solutions of sodium chloride greater than 0.5N had little effect on the solubility of the protein.

Using wheat flour, Pence et al. (26) extracted albumins and globulins with sodium chloride, magnesium sulfate, and potassium sulfate solutions. These workers reported sodium chloride in 0.5N solution to be the best single salt to use for the simultaneous extraction of the largest amounts of both albumins and globulins.

In comparing albumins and globulins of wheat, Pence and Elder (27) observed globulins to have a significantly greater nitrogen content than albumins. The distinguishing characteristic of the albumins was a tryptophan content more than three times larger than that of the globulins, whereas the globulins had an arginine content nearly double that of the albumins.

Prolamines. Incomplete solubility of zein preparations in aqueous ethanol was noted by Chittenden and Osborne (28). The insolubility was thought to result from use of high temperature, repeated contact with different solvents, or a large number of extraction steps. Craine et al. (29) indicated that the physical form in which the product was obtained might cause low solubility. For instance, precipitation of zein from an alcoholic solvent by addition of water or an organic solvent often yielded a taffy-like mass.

Early work by Showalter and Carr (30) indicated that the proportion of zein to total protein was greater when the total nitrogen content of corn was higher. These workers found an increase in zein was accompanied by a corresponding decrease in the glutelin (alkali-soluble) fraction. Using 90% alcohol as the extracting solvent, they reported 8.1% zein in corn containing 15.7% protein, and 2.2% zein in corn with 8.0% protein. Similar studies by Hansen et al. (31) using 70% ethanol indicated corn (dent) endosperm at the 13% protein level contained 7.1% zein while sweet corn endosperm contained 6.1% zein.

Nagy et al. (4) reported low extraction values after 24 hours shaking unless the alcohol was buffered with sodium acetate. The increased solubilizing action of alcohol containing sodium acetate was probably due to absorption affinities of proteins known to be dependent upon pH. These workers considered the alcohol-soluble fraction to be least affected by slight variations in experimental techniques.

Using several samples of corn meal, Hansen et al. (31) reported 70% ethanol extracted the same amount of protein as did 85% ethanol buffered with 0.5% sodium acetate.

Removal of total lipids from wheat or wheat flour may affect the amount of protein soluble in alcohol. Utilizing a method of column separation of proteins developed by Maes (32), Maes (33) found that the fraction soluble in isopropyl alcohol decreased about 20% for flour and 10% for wheat when the cereal material was defatted. However, total protein extractable from flour with distilled water, 40% isopropyl alcohol, 3.85% lactic acid, and 0.5% potassium hydroxide was not affected by defatting. Solubility of protein from corn and sorghum grain in isopropyl alcohol was negligible (33).

Zein was defined by Craine et al. (29) as the protein fraction extractable from corn with aqueous alcohols. This fraction was soluble in water containing a high concentration of urea, at pH 11 and above, or in the presence of anionic detergents. The extreme conditions necessary for solubility were partially due to the amino acid composition of the protein, which had high contents of glutamine, leucine, and proline residues, but low levels of basic amino acids. Turner et al. (34) reported that zein as extracted from corn consisted of several protein species including at least one that was a disulfide-linked aggregate of smaller components. These workers believed that differences in composition of this protein might have resulted from variations in extraction and treatment procedures used to obtain zein.

A recognized authority on the alcohol-soluble proteins of cereal grains, Mosse (35), concluded zeins were made of two main protein fractions, which were apparently strongly associated with small protein or polypeptide molecules (containing lysine). Disulfide bridges and a variable amount of pigment (a part of which was firmly bound to polypeptide chains) were also reported to be partially responsible for an association-dissociation process in zein.

Hansen et al. (31), Frey (36), Mitchell et al. (37), Sauberlich et al. (38), and Glynn et al. (39) observed high-protein corn contained a greater proportion of zein in the total protein than did low-protein corn. These workers found no tryptophan in zein and relatively little valine or lysine. Leucine increased in proportion in the total protein as protein percentage in corn increased, whereas no apparent trend was observed for isoleucine. These investigations indicated that corn high in protein and thus high in zein may be of lower nutritional value than low-protein corn unless supplemented. The low nutritional value of zein has been studied by numerous workers including Osborne and Mendel (1) and Kligler and Krehl (40). Results have indicated that zein will not support satisfactory growth even when supplemented with the essential amino acids in which it is deficient. Later work by Dimler (41), Jimenez (5), and Mosse (35) substantiated that alcohol-soluble proteins are deficient in essential amino acids. Dalby (42), Dimler (43), Jimenez (5), and Nelson (44) indicated that corn high in lysine is low in alcohol-soluble protein as compared to ordinary dent corn. The latter found zein of ordinary dent corn to represent 54.2% of the total soluble protein of the endosperm, and twice that present in opaque-2 (25.1%) and floury-2 (28.7%).

Wall (13) briefly reviewed work by Dr. P. S. Sarma (Bangalore, India) on the different types of proteins based on solubility characteristics in high and low protein varieties of grain sorghum in India. As the protein content of the grain increased, the 70% ethanol-soluble protein fraction increased more than either the water-soluble or salt-soluble protein fractions. This was not particularly desirable since the alcohol-soluble fraction would be most deficient in lysine.

Glutelins. Glutelin obtained from corn by use of a modified procedure of Jones and Csonka (45) was used by Csonka (46) to determine the cystine, tryptophan, and tyrosine contents. Of the three amino acids studied, cystine was the only one affected by the alkali used in the preparation of glutelins. The other two, tryptophan and tyrosine, are stable in alkali. Use of alkali in extracting glutelins from cereals resulted in an undetermined loss of cystine. The glutelin of corn was reported to contain 0.516% cystine, 2.07% tryptophan, and 4.99% tyrosine on a moisture and ash-free basis.

Whole glutelin was prepared from ground defatted corn by a modified copper extraction and fractionation method of Lloyd and Mertz (47). They found whole glutelin to be quite different from zein in amino acid composition. Zein was low in glycine, lysine, and tryptophan, whereas the glutelins contained about 5% glycine, 4% lysine, and 1% tryptophan. In addition, glutelins contained more aspartic acid, arginine, cystine, and valine and less glutamic acid, isoleucine, leucine, proline, and serine than zein. It was concluded that the glutelins had a higher biological value than zein, assuming equal digestibility.

Dimler (41) viewed current methods of extracting glutelins to be somewhat inadequate due to the difficulty of getting complete extraction and because of the considerable chance for chemical modification during extraction and isolation. The molecular weight of glutelin was reported as predominantly a very high molecular weight protein in which protein or polypeptide units were held together by disulfide linkages.

Amino Acid Composition of Related Cereal Grains

The effect of protein content on sorghum grain amino acid content and distribution was reported by Waggle and Deyoe (19). The samples of higher protein content were found to contain the greatest quantities of amino acids. Amino acids higher in concentration in the protein of high protein grain were glutamic acid, proline, alanine, isoleucine, leucine, and phenylalanine. The concentration of lysine, histidine, arginine, threonine, and glycine in the protein tended to decrease as the protein content increased. Percentages of aspartic acid, serine, valine, and methionine in the protein were relatively unaffected by protein level of the grain.

Bressani and Rios (48) and Bressani and Mertz (17) reported that the levels of the essential amino acids in maize and sorghum grain were very similar, and both grains were said to be deficient in lysine and tryptophan.

The amino acid contents of opaque-2 and ordinary dent corn were compared by Cromwell et al. (49). The lysine and tryptophan levels of opaque-2 corn were 104 and 67% greater, respectively, than those for ordinary dent corn. Expressed as percentage of the protein, opaque-2 corn had more aspartic acid, glycine, valine, cystine, tryptophan and basic amino acids, lysine, histidine and arginine, but had less threonine, serine, glutamic acid, proline, alanine, methionine, isoleucine, leucine, tyrosine and phenylalanine when compared with ordinary dent corn.

Sauberlich et al. (38) studied the amino acid composition of corn samples ranging in protein content from 6.8 to 12%. The amount of all the amino acids increased with an increase in protein content of the corn. However, considerable differences were noted in the rate of increase among the individual amino acids.

Similar studies on amino acid composition of wheat by Hepburn and Bradley (50) indicated high protein samples of wheat to be higher in glutamic acid, phenylalanine, and proline. Histidine, isoleucine, and tyrosine had the least variation with nitrogen level of wheat, whereas the remaining amino acids indicated the reverse trend to varying degrees. These workers concluded tho total amino acid contribution of any given sample of wheat was determined primarily by the amount of protein it contained; a high-protein wheat would contribute greater total amounts of each amino acid than would a wheat of much lower protein content.

Amino Acid Composition of Protein Fractions

Jimenez (5) studied the amino acid composition of four endosperm fractions including ordinary dent corn (+), opaque-2 (O_2), floury-2 (fl_2), and O_2fl_2. Two major protein fractions, ethanol-soluble (zein) and sodium hydroxide-soluble (glutelins), were studied and two minor protein fractions, water-soluble (albumins) and sodium chloride-soluble (globulins). Jimenez (5) observed that globulins, zein, and glutelins had different amino acid compositions, but that no major variations were observed for most of the amino acids among different genotypes. The lysine content of the albumins was reported to be 40-fold greater than in the zein in O_2, fl_2, and +. The globulins had the highest lysine content of the four fractions, approximately 50-fold greater than in zein. Glutelins, the major soluble fraction, of O_2 and fl_2 endosperms were relatively high in lysine as compared to normal and fl_2 which had a lysine content similar to that of the albumins. Results indicated that most of the lysine content in the endosperm was contributed by the glutelins in all threo genotypes.

Similar studies by Mosse (35) compared the alcohol-soluble proteins of floury-2 and opaque-2 maizes. Floury-2 zein was lower in alcoholic amino acids (serine and threonine) and higher in methionine and lysine, whereas opaque-2 zein was lower in valine, and higher in arginine, glycine, and cystine.

Dimler's (43) comparison of the amino acid composition of zein and glutelin fractions of corn proteins indicated zein to be higher in leucine, phenylalanine, isoleucine, alanine, and glutamic acid. Glutelin was highest in lysine, tryptophan, methionine, cystine, glycine, histidine, and arginine, whereas serine, tyrosine, proline, aspartic acid, valine, and threonine contents were quite similar.

Studying three different types of corn, Boundy et al. (51) reported the amino acid composition of various protein fractions obtained using a stirring technique. Solvents used included distilled water, 0.5M sodium chloride solution, 70% ethanol solution, and 0.1N sodium hydroxide solution. Though Boundy et al. (51) found some significant differences in the amino acid contents of the globulins, zeins, and glutelins in the three corns, none were sufficiently marked to indicate a major deviation from protein type. The globulins were reported to be highest in the basic amino acids and glycine. Globulins had a lower content of glutamic acid, proline, alanine, and leucine than zeins. The amino acid content of the glutelins appeared to be between that of globulins and zeins, except for the higher content of histidine and proline in the glutelin fractions.

The amino acid composition of the various protein fractions described by Virupaksha and Sastry (14) was determined on two high-protein varieties of sorghum grain. These workers found the globulin fraction had a better distribution of all essential related amino acids than was present in either the original endosperm meal or the prolamine fraction.

The amino acid composition of the prolamine and glutelin fractions studied by Virupaksha and Sastry (14) indicated the amounts of tyrosine, histidine, arginine, glycine, and cystine present in the glutelin fraction to be several times higher than the levels of these amino acids in the prolamine fraction. Serine and aspartic acid values were about 62 and 35% higher, respectively, in the glutelin fraction, whereas tyrosine and alanine were approximately 40 and 33% lower. The amounts of glutamic acid, proline, valine, isoleucine, leucine, and phenylalanine were about the same in both fractions.

The same study reported the concentration of glutamic acid, valine, leucine, and phenylalanine in the endosperm meal to be higher than that in the individual protein fractions. This was thought to be due to some residual protein fraction which was not extracted that had a high level of those amino acids.

Results of Virupaksha and Sastry (14) also indicated that changes in protein composition which would increase the prolamine fraction would result in a decrease in the lysine content, and an increase in the glutelin fraction would result in an increase in the lysine level of the seed.

MATERIALS AND METHODS

Samples

Five samples of sorghum grain of the 1966 crop were obtained from performance test plots of the Agricultural Experimental Station, Kansas State University. Samples used were grown in Riley and Colby county under similar soil and fertility conditions. The hybrids grown in Riley county included Asgrow's Rica, RS-625, and TE-66, hereafter referred to as MO-6, MO-81, and MO-88 respectively. Colby county hybrids included Asgrow's Rica and TE-66, hereafter referred to as CO-6 and CO-88 respectively. These five sorghum samples ranged in crude protein content from 8.3 to 14.8%, where CO-6 was 8.3%

protein; CO-88, 8.1%; MO-6, 12.8%; MO-81, 13.5%; and MO-88, 14.8%. The protein content of the opaque-2 corn was 9.8%.

High-lysine corn (opaque-2) was grown in Northeast Kansas during 1967. The seedstock was obtained from the University of Illinois.

Sample Preparation

The sorghum grain and opaque-2 corn were cleaned of dirt and foreign material by use of a Carter Dockage Tester. Duplicate samples of each lot of sorghum grain or opaque-2 corn were ground on a Mikro-Sample Mill to pass through a screen size of 0.02 inches. The uniformily mixed samples were stored at room temperature in air-tight containers.

Kjeldahl Nitrogen

The nitrogen content of previously cleaned samples was established in duplicate using the macro-Kjeldahl method (52). Determined nitrogen was converted to crude protein by multiplying by the factor 6.25.

Aliquots of the respective protein fractions, lyophilized products, and the dry residual material were analyzed for nitrogen by the micro-Kjeldahl procedure (52). Percentage of nitrogen was multiplied by the factor 6.25 to obtain the percentage of crude protein.

Protein Fractionation Procedures

Fractionation and extraction of duplicate samples were performed utilizing modifications of procedures of Osborne and Mendel (1, 53) and Nagy et al. (4). Samples of sorghum grain and opaque-2 corn were subjected to successive extractions with each of the following solvents: distilled water to extract the albumin, 5% sodium chloride solution for globulin, 80% ethanol plus 0.2% sodium acetate solution for prolamines, and 0.2% sodium hydroxide solution for glutelins. The residual proteins (scleroproteins) were retained for analysis.

Water Extraction. Distilled water (100 ml) was added to a three gram sample in a 250 ml centrifuge tube. The stoppered tubes were rotated longitudinally for 24 hours at room temperature. Suspensions were then centrifuged at 2000 R.P.M. for 15 minutes and the supernatant decanted. Thirty ml of distilled water was added to the centrifuge tube and stirred with a glass rod to resuspend the insoluble material. The insolubles were washed twice according to this procedure. The supernatant liquid was stored at 4°C until lyophilized.

Salt Extraction. Insoluble residue from the water extraction was combined with a 5% (w/v) solution of sodium chloride by use of the same extraction procedures. Prior to lyophilization, the supernatant was dialyzed continously for 48 hours against four changes of distilled water at 4°C.

Alcohol Extraction. The same extraction technique used for the prior two solvents was used in extracting the insoluble residue with a mixture of 18.8% water, 80% ethanol, and 0.2% sodium acetate (v/v/w). The lyophilized material was redissolved in a smaller volume of the ethanol-acetate mixture to facilitate quantitative removal from the lyophilizing vessel. The liquid samples were stored at 4°C. Aliquots of the sample were used in estimating crude protein content and amino acid composition.

Alkali Extraction. Following successive extraction with distilled water, sodium chloride solution, and ethanol-acetate solution, the insoluble residue was extracted with 0.2% (w/v) sodium hydroxide solution. Aliquots of the supernatant liquid or of lyophilized product from the alkali extraction were used for analyses.

Insoluble Residue. The residual material remaining after extraction with the four previously mentioned solvents was transferred quantitatively to a tared container. The wet residue was dried in an air oven at 110°C (5 hours). Dry residue was quantitatively recovered and analyzed for crude protein and amino acid composition.

Amino Acid Analysis

Acid hydrolysis was used in the preparation of the whole grain and protein fractions for amino acid analysis. Either whole grain, aliquots of the liquid fractions, or lyophilized fractions were placed in a 15 x 150-mm. test tube. The top of the tube was narrowed and an excess of constant boiling 6N HCl was added. The tube was placed in a dry ice-alcohol bath and sealed in vacuum ($27\frac{1}{2}$ inches mercury). Then the contents of the tubes were hydrolyzed at 110°C for 22 hours. Following hydrolysis samples were filtered through a fritted disc funnel to remove insoluble humins. Hydrochloric acid was removed from the hydrolyzates by evaporation in vacuum, and each residue was made up to a known volume in 0.2N sodium citrate buffer, pH 2.2. Hydrolyzed samples prepared for analysis were stored at -20°C until used. Amino acid analyses of the hydrolyzates were made following the procedures of Spackman et al. (54) and Moore et al. (55) by means of a Beckman 120 automatic amino acid analyzer.

RESULTS AND DISCUSSION

Results of Protein Fractionation

The percentages of total nitrogen extracted from the five sorghum grain samples and opaque-2 corn with each of the solvents are shown in Table I. The table also includes the protein content of the whole grain and the percentage of insoluble protein remaining after the extraction process.

The percent of soluble protein extracted from the five sorghum grain samples varied from 26.4 to 40.0%, whereas 86.1% of the opaque-2 corn protein was solubilized. In order to check those results, the percent protein remaining in the insoluble material was used to calculate the percent of total protein recovered. Total protein recovered ranged from 84 to 98.6% in the five sorghum grain samples, whereas 96.7% of the total protein was recovered from opaque-2 corn.

The data indicated opaque-2 corn protein to be more easily solubilized by this extraction technique than that of any of the five sorghum grain samples. The glutelin and prolamine fractions represented the two major soluble-protein fractions in sorghum grain. This agreed with reports by Jimenez (5) utilizing opaque-2 endosperm and with Virupaksha and Sastry (14) utilizing sorghum grain endosperm. Though glutelin was found to be the major soluble-protein fraction of the opaque-2 corn kernel, only small differences were observed between the quantities of globulin and prolamine. This agreed with preliminary work on the same sample. Jimenez (5) reported the zein of normal corn endosperm to be twice that present in opaque-2 corn endosperm.

TABLE I

Results of the fractionation of protein from sorghum grain and opaque-2 corn by the modified Mendel-Osborne method.

Fraction	Opaque-2 Corn	CO-6	CO-88	MO-6	MO-81	MO-88
Albumin[a]	9.0	4.9	4.5	4.5	2.2	2.7
Globulin[a]	11.2	5.8	3.5	4.3	3.6	2.5
Prolamine[a]	9.5	8.5	5.0	7.5	4.3	3.9
Glutelin[a]	56.4	20.8	17.9	14.9	17.4	17.3
Total soluble protein[a]	86.1	40.0	30.9	31.2	27.5	26.4
Insoluble residue[a]	10.6	58.6	62.6	52.8	62.3	59.3
Total protein recovered	96.7	98.6	93.5	84.0	89.8	85.7
Initial protein of grain, %	9.8	8.3	8.4	12.8	13.5	14.8

[a]Data reported as percent of total protein.

Amino Acid Composition of Whole Grain

The amino acid composition of the whole grain samples used in protein fractionation is given in Table II. The composition of the five sorghum grain samples was in close agreement with that reported by Deyoe and Shellenberger (56) and Waggle and Deyoe (19), whereas the amino acid composition for opaque-2 corn was very similar to values reported by Cromwell et al. (49).

The lysine and arginine content of opaque-2 corn was nearly double that of the sorghum grain, whereas sorghum grain was approximately one-third lower than opaque-2 corn in histidine, aspartic acid, glycine, and cystine. Concentrations of alanine and leucine were about one-third higher in sorghum grain, whereas only slight increases in glutamic acid, isoleucine, and phenylalanine were observed over the levels found in the opaque-2 corn. The amounts of threonine, serine, proline, methionine, and tyrosine were similar in the two grains.

As to the effect protein content had on the amino acid composition of sorghum grain, the concentration in protein of lysine, histidine, arginine, and glycine tended to decrease as the protein content increased. Concentrations of glutamic acid, alanine, and leucine tended to increase as the protein content increased.

TABLE II

Amino acid composition of sorghum grain and opaque-2 corn used in protein fractionation (grams of amino acid per 100 grams of protein).

Amino Acids[a]	Opaque-2[b] Corn	CO-6	CO-88	MO-6	MO-81	MO-88	Sorghum Average
Lysine	4.14	2.32	2.08	1.81	1.61	1.59	1.88
Histidine	3.52	2.32	1.99	1.97	1.90	2.17	2.07
Ammonia	2.12	2.83	3.04	3.05	2.85	3.17	2.98
Arginine	6.56	3.76	3.36	3.30	3.12	3.10	3.32
Aspartic acid	9.00	6.48	6.37	6.41	6.04	6.60	6.38
Threonine	3.97	3.26	3.11	3.11	2.92	2.93	3.06
Serine	4.71	4.49	4.34	4.47	4.21	4.03	4.31
Glutamic acid	17.38	20.87	21.70	22.74	23.94	22.20	22.29
Proline	8.44	7.82	7.58	7.97	7.95	7.91	7.84
Glycine	4.93	3.14	3.04	2.76	2.57	2.53	2.81
Alanine	6.50	9.19	9.60	9.70	9.79	9.96	9.65
Cystine[c]	2.50	1.82	1.62	1.60	1.58	1.53	1.63
Valine	4.77	4.61	4.54	2.80	4.08	4.70	4.14
Methionine[c]	1.85	1.61	1.40	1.53	1.56	1.61	1.54
Isoleucine	3.32	3.90	3.94	3.99	3.74	3.92	3.90
Leucine	9.28	13.12	13.25	14.04	13.44	14.22	13.62
Tyrosine	3.82	3.98	3.90	4.10	3.89	4.03	3.98
Phenylalanine	4.15	5.06	5.05	5.09	4.95	5.18	5.07
Recovery of amino acids, %	99.55	103.10	106.79	103.75	108.88	111.08	106.72
Crude protein in grain, %	9.8	8.3	8.4	12.8	13.5	14.8	11.6

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[b]Average of duplicate amino acid analyses.
[c]Cystine and methionine values estimated by performic acid oxidation.

Amino Acid Composition of Protein Fractions

Tables III-VII contain the amino acid compositions of the various protein fractions for _opaque_-2 corn and the five different samples of sorghum grain. In general, the albumins (Table III), globulins (Table IV), prolamines (Table V), glutelins (Table VI), and scleroproteins (Table VII) had differing amino acid compositions, but no major variations were observed for most of the amino acids among different sorghum grain samples. This was reported to be true with work on different genotypes of corn by Jimenez (5) and by Boundy et al. (51).

Sorghum grain samples used in this study ranged from 8.3 to 14.8% in protein content, and represented three different hybrids differing in protein content. Thus the feasibility of discussing hybrid differences statistically was limited. However, the purpose of the study was to compare various protein fractions of _opaque_-2 corn with similar fractions of sorghum grain, for which sufficient sorghum grain samples had been studied to obtain representative data.

Some major trends were observed due to differences in protein content. The proline content of the albumin fraction tended to increase as the protein content of the grain increased, while the leucine content tended to decrease with increased protein content. The lysine and glycine content of the insoluble protein of sorghum grain tended to decrease as the protein content increased, whereas the amounts of leucine and glutamic acid tended to increase with increased protein content.

TABLE III

Amino acid composition of the water-soluble fraction of sorghum grain and <u>opaque</u>-2 corn (grams of amino acid per 100 grams of protein).

Amino Acids[a]	Opaque-2[b] Corn	CO-6	CO-88	MO-6	MO-31[b]	MO-88[b]	Sorghum Average
Lysine	5.41	5.28	4.54	5.58	3.36	3.58	4.47
Histidine	2.55	1.66	2.01	2.09	1.76	1.79	1.86
Ammonia	2.23	1.10	2.74	1.68	3.06	3.80	2.48
Arginine	6.38	6.09	6.78	4.33	4.91	4.33	5.29
Aspartic acid	9.79	12.92	11.68	10.53	11.12	11.02	11.45
Threonine	4.95	6.04	5.30	5.21	6.26	5.91	5.74
Serine	4.59	5.26	5.80	4.61	5.23	5.23	5.23
Glutamic acid	14.79	10.90	15.06	14.81	15.81	16.39	14.59
Proline	10.70	4.88	5.27	7.02	6.44	8.80	6.48
Glycine	7.74	7.40	6.94	7.33	8.25	8.47	7.68
Alanine	8.14	9.28	8.20	9.01	9.07	9.64	9.04
Cystine[c]	1.64	0.61	1.26	1.28	0.34	1.00	0.90
Valine	4.95	7.64	2.74	7.21	3.48	3.44	4.90
Methionine[c]	1.12	0.63	1.19	1.24	0.20	0.55	0.76
Isoleucine	3.51	4.66	4.37	3.57	4.32	3.26	4.04
Leucine	5.30	7.85	7.35	6.93	6.49	5.55	6.83
Tyrosine	2.77	3.30	3.54	3.96	3.48	3.12	3.48
Phenylalanine	2.60	3.85	4.31	3.63	3.30	2.99	3.62
Recovery of amino acids, %	81.44	66.31	61.57	63.69	55.04	49.52	59.23
Crude protein in grain, %	9.8	8.3	8.4	12.8	13.5	14.8	11.6

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[b]Average of duplicate amino acid analyses.
[c]Cystine and methionine values estimated by performic acid oxidation.

TABLE IV

Amino acid composition of the sodium chloride fraction of sorghum grain and opaque-2 corn (grams of amino acid per 100 grams of protein).

Amino Acids[a]	Opaque-2[b] Corn	CO-6	CO-88	MO-6	MO-81	MO-88	Sorghum Average
Lysine	5.98	5.35	4.52	4.59	4.33	4.25	4.61
Histidine	3.12	2.10	1.97	2.21	2.13	2.14	2.11
Ammonia	1.76	1.98	1.81	2.06	2.11	1.73	1.94
Arginine	9.05	8.23	9.32	9.41	9.13	9.75	9.17
Aspartic acid	9.16	9.29	10.54	9.62	9.88	9.55	9.78
Threonine	4.37	4.99	5.09	5.03	4.85	5.11	5.01
Serine	5.10	5.17	5.14	5.45	5.56	5.21	5.30
Glutamic acid	14.60	13.51	13.07	13.80	14.48	13.72	13.72
Proline	4.31	4.94	5.30	5.20	5.00	7.17	5.52
Glycine	5.89	6.06	5.91	6.02	6.16	4.25	5.68
Alanine	6.81	6.81	7.23	6.67	6.98	4.51	6.44
Cystine[c]	8.64	2.09	1.38	2.63	2.11	2.34	2.11
Valine	5.80	6.11	5.90	4.93	5.30	4.82	5.41
Methionine[c]	6.42	1.46	0.89	1.53	1.36	1.47	1.34
Isoleucine	4.03	4.34	4.54	4.21	3.84	4.46	4.28
Leucine	7.37	7.66	8.09	7.56	7.32	7.89	7.71
Tyrosine	3.62	3.67	3.35	3.39	3.32	3.60	3.47
Phenylalanine	4.60	4.23	4.11	4.28	4.15	4.42	4.24
Recovery of amino acids, %	87.79	78.63	77.12	86.81	79.37	81.48	80.68
Crude protein in grain, %	9.8	8.3	8.4	12.8	13.5	14.8	11.6

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[b]Average of duplicate amino acid analyses.
[c]Cystine and methionine values estimated by performic acid oxidation.

TABLE V

Amino acid composition of the ethanol-acetate mixture fraction of sorghum grain and opaque-2 corn (grams of amino acid per 100 grams of protein).

Amino Acids[a]	Opaque-2 Corn	CO-6	CO-88	MO-6	MO-81	MO-88	Sorghum Average
Lysine	0.53	0.40	0.75	0.32	0.48	0.30	0.45
Histidine	1.18	0.83	0.80	0.89	0.81	0.79	0.82
Ammonia	2.47	3.39	2.95	3.07	2.63	2.85	2.98
Arginine	1.95	1.91	2.21	1.61	1.82	1.62	1.83
Aspartic acid	5.64	7.05	7.45	6.87	6.20	6.72	6.86
Threonine	2.81	2.54	2.68	2.68	2.50	2.52	2.58
Serine	4.98	4.08	3.95	4.13	3.72	3.75	3.93
Glutamic acid	22.81	25.04	24.08	27.62	23.99	24.72	25.09
Proline	9.67	9.93	8.45	5.75	9.25	8.88	8.45
Glycine	1.82	1.80	2.14	1.61	1.77	1.62	1.79
Alanine	8.34	10.09	9.73	10.87	9.74	9.94	10.07
Half cystine[b]	1.13	---	---	0.33	---	---	---
Valine	3.62	4.60	4.66	1.94	4.57	4.59	4.07
Methionine[b]	0.61	0.52	1.10	0.86	2.09	1.39	1.19
Isoleucine	3.92	3.07	3.61	4.11	3.79	4.19	3.75
Leucine	17.21	15.32	15.76	16.36	16.06	15.94	15.89
Tyrosine	4.77	4.28	4.36	4.85	4.75	4.52	4.55
Phenylalanine	6.54	5.16	5.32	6.12	5.83	5.66	5.62
Recovery of amino acids, %	122.34	68.21	70.20	92.31	92.64	103.91	85.45
Crude protein in grain, %	9.8	8.3	8.4	12.8	13.5	14.8	11.6

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[b]Cystine and methionine values were estimated by acid hydrolysis.
[c]Amino acids with missing values were not determined.

TABLE VI

Amino acid composition of sodium hydroxide fraction of sorghum grain and opaque-2 corn (grams of amino acid per 100 grams of protein).

Amino Acids[a]	Opaque-2 Corn	CO-6[b]	CO-88	MO-6	MO-81	MO-88	Sorghum Average
Lysine	2.93	2.31	3.03	2.51	2.84	2.98	2.73
Histidine	3.28	2.80	3.02	3.24	3.41	3.25	3.14
Ammonia	1.84	2.64	2.41	2.89	2.11	2.17	2.44
Arginine	6.54	4.21	5.52	5.06	6.10	5.57	5.29
Aspartic acid	6.99	7.63	8.29	7.65	7.86	7.96	7.88
Threonine	3.78	3.89	4.23	4.02	4.20	4.35	4.14
Serine	4.00	4.38	5.17	4.68	4.98	4.67	4.78
Glutamic acid	19.39	18.52	18.28	19.16	18.60	19.07	18.73
Proline	10.13	9.56	9.28	9.46	9.47	8.29	9.21
Glycine	5.45	5.02	5.38	5.20	5.21	5.05	5.17
Alanine	6.68	8.02	7.52	7.96	7.21	7.20	7.58
Cystine[c]	0.72	0.69	---	1.14	---	---	---
Valine	4.59	4.83	5.14	3.93	5.37	6.08	5.07
Methionine[c]	1.51	1.20	---	1.52	---	---	---
Isoleucine	3.88	4.76	3.69	4.52	4.31	4.82	4.42
Leucine	10.56	11.39	10.01	11.39	9.36	9.46	10.32
Tyrosine	4.67	4.28	4.08	4.44	4.10	4.08	4.20
Phenylalanine	5.09	5.05	4.96	5.22	4.70	4.87	4.96
Recovery of amino acids, %	67.26	60.38	81.25	87.81	95.00	96.09	84.11
Crude protein in grain, %	9.8	8.3	8.4	12.8	13.5	14.8	11.6

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[b]Average of duplicate amino acid analyses.
[c]Cystine and methionine values estimated by performic acid oxidation.
[d]Amino acids with missing values were not determined.

TABLE VII

Amino acid composition of insoluble residue of sorghum grain and opaque-2 corn (grams of amino acid per 100 grams of protein).

Amino Acids[a]	Opaque-2[b] Corn	CO-6	CO-88	MO-6[b]	MO-81	MO-88	Sorghum Average
Lysine	3.58	0.76	0.69	0.46	0.33	0.31	0.50
Histidine	3.50	1.74	1.62	1.64	1.54	1.40	1.60
Ammonia	2.01	2.96	3.44	3.10	3.18	3.02	3.13
Arginine	4.97	1.78	1.88	1.80	1.19	1.38	1.69
Aspartic acid	7.82	6.19	6.62	5.98	5.87	5.72	6.06
Threonine	5.37	3.17	3.03	2.60	2.64	2.44	2.75
Serine	4.70	4.58	4.78	4.40	4.30	4.16	4.44
Glutamic acid	15.04	22.72	24.47	24.22	26.92	27.33	24.98
Proline	9.25	8.10	8.43	8.38	8.53	8.01	8.31
Glycine	5.87	2.74	2.68	2.08	1.99	1.68	2.21
Alanine	6.99	9.70	10.83	11.15	11.12	11.23	10.86
Cystine[c]	0.80	0.41	0.43	0.32	0.32	0.26	0.35
Valine	6.57	4.66	2.02	3.00	4.55	2.66	3.18
Methionine[c]	1.23	1.24	1.43	1.28	1.43	1.28	1.33
Isoleucine	3.99	4.36	4.56	4.18	4.10	3.98	4.23
Leucine	10.45	15.12	15.43	16.29	16.76	16.32	16.04
Tyrosine	2.95	4.37	4.16	4.00	4.25	4.06	4.14
Phenylalanine	5.00	5.80	5.84	5.71	5.74	5.51	5.72
Recovery of amino acids, %	62.42	69.17	105.72	133.68	115.88	136.78	112.25
Crude protein in grain, %	9.8	8.3	8.4	12.8	13.5	14.8	11.6

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[b]Average of duplicate amino acid analyses.
[c]Cystine and methionine values estimated by performic acid oxidation.

Average Amino Acid Composition of the Protein Fractions

The average amino acid compositions of the albumins, globulins, prolamines, glutelins, and scleroproteins are given in Table VIII for the five sorghum grain samples in comparison with those for opaque-2 corn. Due to the highly variable protein content of sorghum grain hybrids, the average amino acid composition for the five sorghum samples of this study ranging in protein content from 8.3 to 14.8% should be representative of that expected in other sorghum grains.

Albumin Fraction. The concentration of lysine in the albumin and globulin fractions of sorghum grain was nearly twice that of the whole grain protein and glutelin fraction, but several times higher than in the prolamine and insoluble protein fractions. The albumin and globulin fractions of opaque-2 corn had a lysine content similar to that of the entire protein of corn and the same fractions of sorghum grain, whereas the glutelin and insoluble fractions were about two-thirds that of the albumin fraction, and several times higher than that of the prolamine fraction.

Concentrations of lysine, aspartic acid, threonine, and glycine of the albumin fraction of sorghum grain were nearly double that of the whole kernel protein, whereas glutamic acid, phenylalanine, and leucine were about one-third less than the whole kernel protein. As compared to the entire protein of opaque-2 corn, the albumin fraction of opaque-2 corn had generally one-third more histidine, glutamic acid, leucine, and phenylalanine; and one-third less proline and glycine.

Globulin Fraction. The amino acid compositions of the globulin fractions for both opaque-2 corn and the average of the five sorghum grain samples were quite similar. The globulin fraction had a more desirable distribution of the essential and related amino acids as was noted by Virupaksha and Sastry (14). Exceptions noted in the globulin fraction of sorghum grain were that the concentrations of lysine, arginine, and glycine were nearly double that of the entire protein of sorghum grain which was in agreement with work on corn by Boundy et al. (51). The concentrations of aspartic acid and threonine were one-third higher and glutamic acid, proline, alanine, and leucine were one-third lower than in the entire protein of sorghum grain. In contrast, the globulin fraction of opaque-2 corn had approximately one-third more lysine and arginine than the entire protein of opaque-2, but was similar to sorghum grain in that glutamic acid and proline were both one-third lower in the globulin fraction than in the whole grain protein.

The globulin fraction of both opaque-2 corn and sorghum grain had a lower content of glutamic acid, proline, alanine, and leucine than zein, which agreed with the results of Boundy et al. (51).

Prolamine Fraction. Basically the same trends were found in the amino acid composition of the prolamine fraction of both opaque-2 corn and the average of the five sorghum grain samples as compared to the respective whole grain protein. The amino acid composition of the prolamine fraction from opaque-2 corn of this study compared closely with the prolamine fraction of opaque-2 corn endosperm reported by Jimenez (5), Table IX. One of the more notable exceptions was the aspartic acid content of 5.61% for the prolamine fraction as compared to 9.00% in the entire protein of opaque-2 corn, whereas little difference was observed in a similar comparison of sorghum grain.

The concentration of lysine in the prolamine fraction of both opaque-2 corn and the average of the five sorghum grain samples was several times lower than the respective whole grain protein, albumin, globulin, and glutelin fractions. Though the lysine content of the insoluble protein of sorghum grain was also low, 0.50% of the protein, the insoluble protein of opaque-2 was materially higher in lysine, 3.58%.

Concentrations of histidine, 0.82%, and arginine, 1.83%, in the average of the prolamine fractions of sorghum grain were approximately half those of the entire protein of sorghum grain, 2.07, 3.32% respectively, while histidine and arginino contents, 1.18 and 1.95% respectively of the prolamine fraction of opaque-2 corn were approximately one-third those of the whole corn protein, 3.52 and 6.56% respectively. The glycine content of the prolamine fractions of both opaque-2 corn, 1.82%, and the avorage of the five sorghum grain samples, 1.79%, tended to be higher in the respective whole grain protein, 4.93 and 2.81%. An increase of aspartic acid and leucine in the prolamine fraction of opaque-2 corn was observed over the entire protein of opaque-2 corn, whereas little difference was observed in a similar comparison with the average of the five sorghum grains.

Amino acids of the prolamine fraction of the average sorghum grain that were comparable in concentrations to the entire protein of sorghum grain included aspartic acid, threonine, serine, alanine, valine, isoleucine, tyrosine, and phenylalanine, whereas a similar comparison with the opaque-2 prolamine fraction included serine, threonine, proline, valine, glutamic acid, alanine, tyrosine, isoleucine, and phenylalanine as compared to the entire protein of opaque-2 corn.

Glutelin Fraction. The average amino acid composition of the glutelin fractions of the five samples of sorghum grain was in close agreement with that for the glutelin fraction of CSH-1-Bijapur reported by Virupaksha and Sastry (14) with the exception of a much lower content of glutamic acid and proline in the present study.

In comparing the amino acid composition of the prolamine and glutelin fractions of sorghum grain, the amounts of lysine, histidine, arginine, threonine, serine, and glycine in the glutelin were several times higher than the levels of those amino acids in the prolamine fraction, which agrees with Virupaksha and Sastry (14). Amounts of glutamic acid, alanine, and leucine were lower in the glutelin fraction than in the prolamine fraction.

The amino acid content of the glutelin fractions averaged for the five sorghum grain samples and compared with those for the entire protein of sorghum grain indicated a slightly higher amino acid content of lysine, histidine, arginine, aspartic acid, threonine, and proline in the glutelin fraction, whereas glutamic acid, alanine, and leucine were slightly lower. The concentration of glycine in the glutelin fraction was nearly double that of the whole grain protein, while serine, isoleucine, tyrosine, and phenylalanine were about the same in both the glutelin and whole grain protein.

The amino acid composition of the glutelin fraction of opaque-2 corn as compared with the average of the glutelin fractions for the five sorghum grain samples was similar for both cereal grains.

The glutelin fraction of opaque-2 corn had an amino acid composition similar to that of the entire protein of opaque-2 corn except for lysine, aspartic acid, serine, proline, leucine, and phenylalanine which tended to be lower. Amounts of histidine, arginine, threonine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, and tyrosine were about the same in both the glutelin fraction and entire protein of opaque-2 corn.

Glutelins of opaque-2 corn contained more aspartic acid, arginine, and valine than zein, whereas glutamic acid and leucine were less than in zein which was in agreement with the results reported by Lloyd and Mertz (47).

The amino acid contents of both the sorghum grain and opaque-2 corn glutelins tended to be midway between those of the globulins and the zeins, except for the higher content of histidine and proline in the glutelin fraction. This agrees with work on corn by Boundy et al. (51).

Insoluble Residue. The insoluble protein of sorghum grain was considerably lower in lysine, cystine, and arginine content than that of the entire protein of sorghum grain, whereas leucine and phenylalanine were slightly higher. The following amino acids of the insoluble protein of sorghum grain were found to be in close agreement quantity-wise with that of the entire protein of sorghum grain: histidine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, and tyrosine. Thus the amino acid composition of the insoluble protein of sorghum grain conformed very closely to that of the entire protein of sorghum grain.

Similar comparisons with the insoluble protein of opaque-2 corn as it compared to the entire protein of opaque-2 corn indicated a much lower content of cystine, whereas lysine, arginine, aspartic acid, glutamic acid, and tyrosine were slightly lower in the protein of the insoluble residue. Amino acids slightly higher in the soluble protein of opaque-2 corn included threonine, proline, glycine, valine, and phenylalanine, whereas histidine, serine, alanine, methionine, isoleucine, and leucine were not materially different. The amino acid composition of the insoluble opaque-2 protein compared closely with that of entire protein of opaque-2 corn even though 86.1% of the total protein was solubilized.

The insoluble protein of opaque-2 corn and the average of the five sorghum grain samples differed substantially more in amino acid composition than did any of the four soluble fractions of the two grains. However, the differences in insoluble proteins of the two grains tended to follow the differences noted in the respective whole grain protein, regardless of the percent of protein solubilized. Approximately 86% of the total protein of opaque-2 corn had been solubilized with the four solvents whereas only 30 to 40% of the total protein of sorghum grain was solubilized by the same procedure.

TABLE VIII

Average amino acid composition of protein fractions and whole grain (grams of amino acid per 100 grams protein).

Amino Acids[a]	Whole Grain Corn[b] %	Whole Grain Sorghum Gr.[c] %	Whole Grain Sorghum Gr.[c] ± Error[d]	Albumin Corn[b] %	Albumin Sorghum Gr.[c] %	Albumin Sorghum Gr.[c] ± Error[d]	Globulin Corn[b] %	Globulin Sorghum Gr.[c] %	Globulin Sorghum Gr.[c] ± Error[d]
Lysine	4.14	1.88	0.14	5.41	4.47	0.44	5.98	4.61	0.20
Histidine	3.52	2.07	0.08	2.55	1.86	0.08	3.12	2.11	0.04
Ammonia	2.12	2.98	0.06	2.23	2.48	0.48	1.76	1.94	0.08
Arginine	6.56	3.32	0.12	6.38	5.29	0.49	9.05	9.17	0.25
Aspartic acid	9.00	6.38	0.09	9.79	11.45	0.41	9.16	9.78	0.21
Threonine	3.97	3.06	0.06	4.95	5.74	0.21	4.37	5.01	0.04
Serine	4.71	4.31	0.09	4.59	5.23	0.19	5.10	5.30	0.09
Glutamic acid	17.38	22.29	0.51	14.79	14.59	0.97	14.60	13.72	0.23
Proline	8.44	7.84	0.06	10.70	6.48	0.70	4.31	5.52	0.42
Glycine	4.93	2.81	0.13	7.74	7.68	0.29	5.89	5.89	0.36
Alanine	6.50	9.65	0.13	8.14	9.04	0.24	6.81	6.44	0.49
Cystine	2.50	1.63	0.10	1.64	0.90	0.18	8.64	2.11	0.20
Valine	4.77	4.14	0.35	4.95	4.90	1.04	5.80	5.41	0.25
Methionine	1.85	1.54	0.08	1.12			6.42	1.34	0.12
Isoleucine	3.32	3.90	0.04	3.51	4.04	0.26	4.03	4.28	0.12
Leucine	9.28	13.62	0.22	5.30	6.83	0.39	7.37	7.71	0.13
Tyrosine	3.82	3.98	0.04	2.77	3.48	0.14	3.62	3.47	0.06
Phenylalanine	4.15	5.07	0.04	2.60	3.62	0.23	4.60	4.24	0.06

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[b]Average of duplicate amino acid analyses.
[c]Average value of the five samples of sorghum grain in Tables III-VII.
[d]Standard deviation of the distribution of sample means or commonly called the "standard error" of the mean (57).

TABLE VIII (Contd.)

Average amino acid composition of protein fractions and whole grain (grams of amino acid per 100 grams protein).

Amino Acids[a]	Prolamine Corn %	Prolamine Sorghum Gr.[c] %	Prolamine Sorghum Gr.[c] ± Error[d]	Glutelin Corn %	Glutelin Sorghum Gr.[c] %	Glutelin Sorghum Gr.[c] ± Error[d]	Insoluble Residue Corn[b] %	Insoluble Residue Sorghum Gr.[c] %	Insoluble Residue Sorghum Gr.[c] ± Error[d]
Lysine	0.53	0.45	0.08	2.93	2.73	0.14	3.58	0.50	0.09
Histidine	1.18	0.82	0.02	3.28	3.14	0.11	3.50	1.60	0.06
Ammonia	2.47	2.98	0.12	1.84	2.44	0.14	2.01	3.13	0.09
Arginine	1.95	1.83	0.22	6.54	5.29	0.32	4.97	1.69	0.10
Aspartic acid	5.64	6.86	0.20	6.99	7.88	0.12	7.82	6.06	0.16
Threonine	2.81	2.58	0.04	3.78	4.14	0.08	5.37	2.75	0.14
Serine	4.98	3.93	0.09	4.00	4.78	0.13	4.70	4.44	0.11
Glutamic acid	22.81	25.09	0.66	19.39	18.73	0.17	15.04	24.98	0.86
Proline	9.67	8.45	0.72	10.13	9.21	0.24	9.25	8.31	0.10
Glycine	1.82	1.79	0.10	5.45	5.17	0.06	5.87	2.21	0.20
Alanine	8.34	10.07	0.21	6.68	7.58	0.16	6.99	10.86	0.28
Cystine				0.72			0.80	0.35	0.04
Valine	3.62	4.07	0.53	4.59	5.07	0.35	6.57	3.18	0.67
Methionine				1.51			1.23	1.33	0.04
Isoleucine	3.92	3.75	0.20	3.88	4.42	0.20	3.99	4.23	0.10
Leucine	17.21	15.89	0.17	10.56	10.32	0.45	10.45	16.04	0.31
Tyrosine	4.77	4.55	0.11	4.67	4.20	0.06	2.95	4.14	0.06
Phenylalanine	6.54	5.62	0.17	5.09	4.96	0.09	5.00	5.72	0.06

[a]Amino acid values were corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[b]Average of duplicate amino acid analyses.
[c]Average value of the five samples of sorghum grain in Tables III-VII.
[d]Standard deviation of the distribution of sample means or commonly called the "standard error" of the mean (57).

TABLE IX

Amino acid composition of soluble protein fractions of opaque-2 whole corn as compared to opaque-2 corn endosperm of Jimenez (5) (grams of amino acid per 100 grams protein).

Amino Acids[a]	Albumin S[b]	Albumin J[c]	Globulin S[b]	Globulin J[c]	Prolamine S[b]	Prolamine J[c]	Glutelin S[b]	Glutelin J[c]
Lysine	5.41	4.05	5.98	5.16	0.53	0.07	2.93	4.73
Histidine	2.55	1.81	3.12	3.24	1.18	1.04	3.28	3.46
Arginine	6.38	4.35	9.05	9.31	1.95	1.64	6.54	7.76
Aspartic acid	9.79	42.39	9.16	9.34	5.64	5.41	6.99	8.53
Threonine	4.95	2.59	4.37	4.32	2.81	2.75	3.78	4.13
Serine	4.59	2.51	5.10	8.68	4.98	5.37	4.00	4.97
Glutamic acid	14.79	14.97	14.60	17.68	22.81	24.24	19.39	15.92
Proline	10.70	6.82	4.31	5.57	9.67	9.78	10.13	6.54
Glycine	7.74	3.74	5.89	8.36	1.82	1.39	5.45	4.67
Alanine	8.14	4.23	6.81	6.50	8.34	9.59	6.68	6.38
Cystine	1.64	0.78	8.64	---	1.13	0.67	0.72	0.88
Valine	4.95	2.36	5.80	5.24	3.62	2.85	4.59	7.42
Methionine	1.12	---	6.42	0.90	0.61	0.56	1.51	2.33
Isoleucine	3.51	2.31	4.03	3.27	3.92	4.38	3.88	4.54
Leucine	5.30	3.29	7.37	5.97	17.21	20.23	10.56	10.28
Tyrosine	2.77	1.89	3.62	1.95	4.77	2.58	4.67	2.17
Phenylalanine	2.60	1.90	4.60	4.52	6.54	7.46	5.09	5.30
Protein extracted %	9.0	13.2	11.2	3.9	9.5	22.8	56.4	50.0
Actual % recovery of amino acids	81.44	62.50	87.79	74.25	122.34	88.32	67.26	88.44

[a]Amino acid values were corrected to 100% recovery.
[b]Based on values from this work.
[c]As reported by Jimenez (5).

Percentages of Amino Acids Recovered by the Fractionation Technique

The extraction procedure was run in duplicate while the amino acid analysis was not repeated thus raising the question of the reliability of these analyses. To check these results, the amount (grams) of each amino acid recovered in each protein fraction was summed, and the summation compared with those obtained from the amino acid analysis of the entire protein of the respective grain, Tables X-XV.

Tables X-XV give the amino acid fraction, %, calculated for each protein fraction by multiplying the percentage of the total protein extracted from the respective fraction (or percentage of protein remaining in the case of insoluble material) times the grams of amino acid per 100 grams of protein in the respective protein fraction. The calculations were made for each amino acid of all samples of sorghum grain and opaque-2 corn used in the experiment.

The range of the efficiency of recovery for a particular amino acid is given in Table XVI for the five sorghum grain samples. Amino acids with the largest range listed first include: valine, glycine, threonine, tyrosine and isoleucine, serine, aspartic acid, proline, arginine, phenylalanine, glutamic acid, leucine, alanine, histidine, and lysine.

Lysine had the lowest efficiency of recovery of any amino acid determined in both opaque-2 corn and sorghum grain samples. However, the range on the efficiency of recovery for lysine was narrower than any of the other amino acids determined.

In using average values for efficiency of recovery for specific amino acids (Table XVI), the following amino acids were $100 \pm 5\%$ for opaque-2 corn: threonine, glutamic acid, proline, glycine, alanine, valine, and tyrosine. A similar comparison on the average efficiency of recovery for the five sorghum grain samples had the following amino acids between $100 \pm 5\%$: aspartic acid, threonine, proline, glycine, isoleucine, leucine, and phenylalanine. Using the same criteria, amino acids of opaque-2 corn outside of a range of 100$\pm$ 15% included lysine, histidine, and aspartic acid, whereas amino acids of the five sorghum grain samples included lysine, histidine, arginine, and valine. Thus it appears the efficiency of recovery for basic amino acids was generally lower than for either the acidic or neutral amino acids.

For a particular amino acid, the data reveals a close agreement between the grams of amino acid per 100 grams of protein added together for each of the five protein fractions and compared to the amino acid content of the entire protein of the respective grain. This close agreement indicated that major errors have been minimized in the experiment.

TABLE X

Summation of the individual contents of amino acids from five protein fractions as they compare to the amino acid content in the entire protein of opaque-2 corn.

Amino Acids	Whole[bf] Corn	Amino Acid Fraction, %[a] Albumin	Globulin	Prolamine	Glutelin	Residue	Total Grams[c] Recovered	Efficiency[d] of Recovery
Lysine	4.14	0.46	0.67	0.05	1.64	0.34	3.16	76.3
Histidine	3.52	0.22	0.35	0.11	1.84	0.34	2.86	81.2
Ammonia	2.12	0.19	0.20	0.22	1.03	0.19	1.83	86.3
Arginine	6.56	0.54	1.01	0.18	3.66	0.48	5.87	89.5
Aspartic acid	9.00	0.83	1.02	0.51	3.91	0.75	7.02	78.0
Threonine	3.97	0.42	0.49	0.26	2.12	0.52	3.81	96.0
Serine	4.71	0.39	0.57	0.45	2.24	0.45	4.10	87.0
Glutamic acid	17.38	1.26	1.64	2.08	10.86	1.44	17.28	99.4
Proline	8.44	0.91	0.48	0.88	5.67	0.89	8.83	104.6
Glycine	4.93	0.66	0.66	0.16	3.05	0.56	5.09	103.2
Alanine	6.50	0.69	0.76	0.76	3.74	0.67	6.62	101.8
Cystine	2.50	0.14	0.97	---	0.40	0.08		
Valine	4.77	0.42	0.65	0.33	2.57	0.63	4.60	96.4
Methionine	1.85	0.10	0.72		0.84	0.12		
Isoleucine	3.32	0.30	0.45	0.36	2.17	0.38	3.66	110.2
Leucine	9.28	0.45	0.82	1.57	5.91	1.00	9.75	105.1
Tyrosine	3.82	0.24	0.40	0.43	2.62	0.28	3.97	103.9
Phenylalanine	4.15	0.22	0.52	0.60	2.85	0.48	4.67	112.5
Protein Extracted %		8.5	11.2	9.1	56.0	9.6	94.4	
Actual % Recovery on Amino Acids[e]	99.55	81.44	87.79	122.34	67.26	62.42		

[a]Amino acid fraction = percentage of the total protein extracted multiplied by the grams of amino acid per 100 grams protein in the respective protein fraction.

[b]Grams of amino acid per 100 grams of entire protein in the grain corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.

[c]Total grams recovered = summation of the grams of amino acid per 100 grams of protein in the respective protein fraction for the five protein fractions.

[d]Efficiency of recovery = c divided by b and multiplied by 100.

[e]Actual % recovery of amino acids = grams of amino acid recovered per 100 grams of Kjeldahl protein.

[f]Average of duplicate amino acid analyses.

TABLE XI

Summation of the individual contents of amino acids from five protein fractions as they compare to the amino acid content in the entire protein of sorghum grain (CO-6).

Amino Acids	Whole[b] Sorghum	Amino Acid Fraction, %[a] Albumin	Globulin	Prolamine	Glutelin	Residue	Total Grams[c] Recovered	Efficiency[d] of Recovery
Lysine	2.32	0.26	0.34	0.03	0.51	0.43	1.57	67.7
Histidine	2.32	0.08	0.13	0.07	0.62	0.99	1.89	81.5
Ammonia	2.83	0.05	0.12	0.28	0.58	1.69	2.72	96.1
Arginine	3.76	0.30	0.52	0.16	0.93	1.01	2.92	77.7
Aspartic acid	6.48	0.63	0.58	0.59	1.69	3.53	7.02	108.3
Threonine	3.26	0.29	0.31	0.21	0.86	1.81	3.48	106.7
Serine	4.49	0.26	0.32	0.34	0.97	2.61	4.50	100.2
Glutamic acid	20.87	0.53	0.85	2.10	4.09	12.95	20.52	98.3
Proline	7.82	0.24	0.24	0.83	2.11	4.62	8.04	102.8
Glycine	3.14	0.36	0.38	0.15	1.20	1.56	3.65	116.2
Alanine	9.19	0.45	0.43	0.85	1.77	5.53	9.03	98.2
Cystine	1.82	0.03	0.13		0.15	0.23		
Valine	4.61	0.37	0.38	0.39	1.07	2.66	4.87	105.6
Methionine	1.61	0.03	0.09		0.26	0.71		
Isoleucine	3.90	0.23	0.27	0.26	1.05	2.48	4.29	110.0
Leucine	13.12	0.38	0.48	1.29	2.52	8.62	13.29	101.3
Tyrosine	3.98	0.16	0.23	0.36	0.95	2.49	4.19	105.3
Phenylalanine	5.06	0.19	0.27	0.43	1.12	3.31	5.32	105.1
Protein Extracted, %		4.9	6.3	8.4	22.1	57.0	98.7	
Actual % Recovery on Amino Acids[e]	103.10	66.31	78.63	68.21	60.38	69.17		

[a]Amino acid fraction = percentage of the total protein extracted multiplied by the grams of amino acid per 100 grams protein in the respective protein fraction.

[b]Grams of amino acid per 100 grams of entire protein in the grain corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.

[c]Total grams recovered = summation of the grams of amino acid per 100 grams of protein in the respective protein fraction for the five protein fractions.

[d]Efficiency of recovery = c divided by b and multiplied by 100.

[e]Actual % recovery of amino acids = grams of amino acid recovered per 100 grams of Kjeldahl protein.

TABLE XII

Summation of the individual contents of amino acids from five protein fractions as they compare to the amino acid content in the entire protein of sorghum grain (MO-6).

Amino Acids	Whole[b] Sorghum	Amino Acid Fraction, %[a] Albumin	Globulin	Prolamine	Glutelin	Residue	Total Grams[c] Recovered	Efficiency[d] of Recovery
Lysine	1.81	0.28	0.21	0.02	0.38	0.25	1.14	63.0
Histidine	1.97	0.09	0.10	0.06	0.49	0.90	1.64	83.2
Ammonia	3.05	0.08	0.09	0.21	0.44	1.71	2.53	82.9
Arginine	3.30	0.19	0.42	0.11	0.77	0.99	2.48	75.1
Aspartic acid	6.41	0.47	0.43	0.47	1.16	3.30	5.83	90.9
Threonine	3.11	0.23	0.23	0.18	0.61	1.43	2.68	86.2
Serine	4.47	0.21	0.24	0.28	0.71	2.43	3.87	86.6
Glutamic acid	22.74	0.67	0.62	1.91	2.91	13.37	19.48	85.7
Proline	7.97	0.32	0.23	0.40	1.44	4.62	7.01	87.9
Glycine	2.76	0.33	0.27	0.11	0.79	1.15	2.65	96.0
Alanine	9.70	0.40	0.30	0.75	1.21	6.15	8.81	90.8
Cystine	1.60	0.06	0.12	0.02	0.17	0.18	0.55	34.4
Valine	2.80	0.32	0.22	0.13	0.60	1.66	2.93	104.6
Methionine	1.53	0.06	0.07		0.23	0.71		
Isoleucine	3.99	0.16	0.19	0.28	0.69	2.31	3.63	91.0
Leucine	14.04	0.31	0.34	1.13	1.73	8.99	12.50	89.0
Tyrosine	4.10	0.18	0.15	0.33	0.67	2.21	3.54	86.3
Phenylalanine	5.09	0.16	0.19	0.42	0.79	3.15	4.71	92.5
Protein Extracted,%		4.5	4.5	6.9	15.2	55.2	86.3	
Actual % Recovery on Amino Acids[e]	103.75	63.69	86.81	92.31	87.81	133.68		

[a]Amino acid fraction = percentage of the total protein extracted multiplied by the grams of amino acid per 100 grams protein in the respective protein fraction.

[b]Grams of amino acid per 100 grams of entire protein in the grain corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.

[c]Total grams recovered = summation of the grams of amino acid per 100 grams of protein in the respective protein fraction for the five protein fractions.

[d]Efficiency of recovery = c divided by b and multiplied by 100.

[e]Actual % recovery of amino acids = grams of amino acid recovered per 100 grams of Kjeldahl protein.

TABLE XIII

Summation of the individual contents of amino acids from five protein fractions as they compare to the amino acid content in the entire protein of sorghum grain (CO-88).

Amino Acids	Whole[b] Sorghum	Amino Acid Fraction, %[a] Albumin	Globulin	Prolamine	Glutelin	Residue	Total Grams[c] Recovered	Efficiency[d] of Recovery
Lysine	2.08	0.22	0.16	0.04	0.55	0.42	1.39	66.8
Histidine	1.99	0.10	0.07	0.04	0.54	0.99	1.74	87.4
Ammonia	3.04	0.13	0.06	0.15	0.43	2.11	2.88	94.7
Arginine	3.36	0.33	0.33	0.11	0.99	1.15	2.91	86.6
Aspartic acid	6.37	0.57	0.37	0.39	1.49	4.06	6.88	108.0
Threonine	3.11	0.26	0.18	0.14	0.76	1.86	3.20	102.9
Serine	4.34	0.28	0.18	0.21	0.93	2.93	4.53	104.4
Glutamic acid	21.70	0.74	0.46	1.25	3.29	15.02	20.76	95.7
Proline	7.58	0.26	0.19	0.44	1.67	5.18	7.74	102.1
Glycine	3.04	0.34	0.21	0.11	0.97	1.65	3.28	107.9
Alanine	9.60	0.40	0.25	0.51	1.35	6.65	9.16	95.4
Cystine	1.62	0.06	0.05			0.26		
Valine	4.54	0.13	0.21	0.24	0.93	1.24	2.75	60.6
Methionine	1.40	0.06	0.03			0.88		
Isoleucine	3.94	0.21	0.16	0.19	0.66	2.80	4.02	102.0
Leucine	13.25	0.36	0.28	0.82	1.80	9.47	12.73	96.1
Tyrosine	3.90	0.17	0.12	0.23	0.73	2.55	3.80	97.4
Phenylalanine	5.05	0.21	0.14	0.28	0.89	3.59	5.11	101.2
Protein Extracted,%		4.9	3.5	5.2	18.0	61.4	93.0	
Actual % Recovery on Amino Acids	106.79	61.57	77.12	70.20	81.25	105.72		

[a]Amino acid fraction = percentage of the total protein extracted multiplied by the grams of amino acid per 100 grams protein in the respective protein fraction.

[b]Grams of amino acid per 100 grams of entire protein in the grain corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.

[c]Total grams recovered = summation of the grams of amino acid per 100 grams of protein in the respective protein fraction for the five protein fractions.

[d]Efficiency of recovery = c divided by b and multiplied by 100.

[e]Actual % recovery of amino acids = grams of amino acid recovered per 100 grams of Kjeldahl protein.

TABLE XIV

Summation of the individual contents of amino acids from five protein fractions as they compare to the amino acid content in the entire protein of sorghum grain (MO-81).

Amino Acids	Whole[b] Sorghum	Amino Acid Fraction, %[a] Albumin	Globulin	Prolamine	Glutelin	Residue	Total Grams[c] Recovered	Efficiency[d] of Recovery
Lysine	1.61	0.08	0.16	0.02	0.50	0.21	0.97	60.2
Histidine	1.90	0.04	0.08	0.04	0.60	0.96	1.72	90.5
Ammonia	2.85	0.07	0.08	0.12	0.37	1.99	2.63	92.3
Arginine	3.12	0.11	0.33	0.08	1.07	0.93	2.52	80.8
Aspartic acid	6.04	0.26	0.36	0.27	1.38	3.67	5.94	98.3
Threonine	2.92	0.14	0.17	0.11	0.74	1.65	2.81	96.2
Serine	4.21	0.12	0.20	0.16	0.88	2.69	4.05	96.2
Glutamic acid	23.94	0.36	0.52	1.06	3.27	16.85	22.06	92.1
Proline	7.95	0.15	0.18	0.41	1.67	5.34	7.75	97.5
Glycine	2.57	0.19	0.22	0.08	0.92	1.24	2.65	103.1
Alanine	9.79	0.21	0.25	0.43	1.27	6.96	9.12	93.2
Cystine	1.58	0.01	0.08			0.20		
Valine	4.08	0.08	0.19	0.20	0.95	2.85	4.27	104.6
Methionine	1.56	0.01	0.05	0.09	0.03	0.90		
Isoleucine	3.74	0.10	0.14	0.17	0.76	2.57	3.74	100.0
Leucine	13.44	0.15	0.26	0.71	1.65	10.49	13.26	98.7
Tyrosine	3.89	0.08	0.12	0.21	0.72	2.66	3.79	97.4
Phenylalanine	4.95	0.08	0.15	0.26	0.83	3.59	4.91	99.2
Protein Extracted, %		2.3	3.6	4.4	17.6	62.6	90.5	
Actual % Recovery on Amino Acids[e]	108.88	55.04	79.37	92.64	95.00	115.88		

[a]Amino acid fraction = percentage of the total protein extracted multiplied by the grams of amino acid per 100 grams protein in the respective protein fraction.

[b]Grams of amino acid per 100 grams of entire protein in the grain corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.

[c]Total grams recovered = summation of the grams of amino acid per 100 grams of protein in the respective protein fraction for the five protein fractions.

[d]Efficiency of Recovery = c divided by b and multiplied by 100.

[e]Actual % recovery of amino acids = grams of amino acid recovered per 100 grams of Kjeldahl protein.

TABLE XV

Summation of the individual contents of amino acids from five protein fractions as they compare to the amino acid content in the entire protein of sorghum grain (MO-88).

Amino Acids	Whole[b] Sorghum	Amino Acid Fraction, %[a] Albumin	Globulin	Prolamine	Glutelin	Residue	Total Grams[c] Recovered	Efficiency[d] of Recovery
Lysine	1.61	0.10	0.11	0.01	0.52	0.18	0.92	57.1
Histidine	1.90	0.05	0.05	0.03	0.57	0.81	1.51	79.5
Ammonia	2.85	0.10	0.04	0.11	0.38	1.74	2.37	83.2
Arginine	3.12	0.12	0.24	0.06	0.98	0.79	2.19	70.2
Aspartic acid	6.04	0.30	0.24	0.27	1.40	3.29	5.50	91.1
Threonine	2.92	0.16	0.13	0.10	0.76	1.40	2.55	87.3
Serine	4.21	0.14	0.13	0.15	0.82	2.40	3.64	86.5
Glutamic acid	23.94	0.44	0.34	0.99	3.36	15.74	20.87	87.2
Proline	7.95	0.24	0.18	0.36	1.46	4.61	6.85	86.2
Glycine	2.57	0.23	0.11	0.06	0.89	0.97	2.26	87.9
Alanine	9.79	0.26	0.11	0.40	1.27	6.47	8.51	86.9
Cystine	1.53	0.03	0.06			0.15		
Valine	4.08	0.09	0.12	0.18	1.07	1.53	2.99	73.3
Methionine	1.61	0.01	0.04	0.06		0.74		
Isoleucine	3.74	0.09	0.11	0.17	0.85	2.29	3.51	93.8
Leucine	13.44	0.15	0.20	0.64	1.66	9.41	12.06	89.7
Tyrosine	3.89	0.08	0.09	0.18	0.72	2.34	3.41	87.7
Phenylalanine	4.95	0.08	0.11	0.23	0.86	3.17	4.45	89.9
Protein Extracted, %		2.7	2.5	4.0	17.6	57.6	84.4	
Actual % Recovery on Amino Acids	111.08	49.52	81.48	103.91	96.09	136.78		

[a]Amino acid fraction = percentage of the total protein extracted multiplied by the grams of amino acid per 100 grams protein in the respective protein fraction.
[b]Grams of amino acid per 100 grams of entire protein in the grain corrected to 100% recovery of Kjeldahl protein and to a moisture-free basis.
[c]Total grams recovered = summation of the grams of amino acid per 100 grams of protein in the respective protein fraction for the five protein fractions.
[d]Efficiency of recovery = c divided by b and multiplied by 100.
[e]Actual % recovery of amino acids = grams of amino acid recovered per 100 grams of Kjeldahl protein.

TABLE XVI

Efficiency with which each amino acid was recovered averaged over all sorghum grain samples and compared to opaque-2 corn.

Amino Acids	Efficiency of Recovery[a] of Amino Acids: Opaque-2 Corn	Efficiency of Recovery[a] of Amino Acids: Sorghum[b] Grain	Range of Recovery for Amino Acids in Sorghum Grain
Lysine	76.3	63.0	57.1--67.7
Histidine	81.2	84.4	79.5--90.5
Ammonia	86.3	89.8	82.9--96.1
Arginine	89.5	78.1	70.2--86.6
Aspartic acid	78.0	99.3	90.9-108.3
Threonine	96.0	95.9	86.2-106.7
Serine	87.0	94.8	86.5-104.4
Glutamic acid	99.4	91.8	85.7--98.3
Proline	104.6	95.3	86.2-102.8
Glycine	103.2	102.2	87.9-116.2
Alanine	101.8	92.9	86.9--98.2
Valine	96.4	90.8	60.6-105.6
Isoleucine	110.2	99.4	91.0-110.0
Leucine	105.1	95.0	89.0-101.3
Tyrosine	103.9	94.8	86.3-105.3
Phenylalanine	112.5	97.6	89.9-105.1

[a]Efficiency of recovery = ratio (in %) of the summation of the grams of an amino acid per 100 grams of protein in the respective protein fraction for the five protein fractions to the grams of amino acid per 100 grams of entire protein in the grain.

[b]Average of five samples (Table XI - XV).

SUMMARY AND DISCUSSION

Protein fractionation of five samples of sorghum grain and one sample of opaque-2 corn was performed using modifications of procedures of Osborne and Mendel (1), Nagy et al. (4), and Jimenez (5). The samples were subjected to successive extraction with each of the following solvents: distilled water, 5% sodium chloride, 80% ethanol plus 0.2% sodium acetate, and 0.2% sodium hydroxide. Insoluble material was retained for analysis.

The efficiency with which soluble protein was extracted from five samples of sorghum grain ranged from 26.4 to 40%, whereas the extraction efficiency for opaque-2 corn was 86.1%. To check these results, the protein content of the insoluble material was used to calculate the percent of total protein recovered. Total protein recovered ranged from 84 to 98.6% for the five sorghum grain samples, whereas 96.7% of the total protein was recovered from opaque-2 corn.

Glutelin and prolamine represented the two major soluble-protein fractions in sorghum grain which was in agreement with reports made by Jimenez (5) and Virupaksha and Sastry (14). Though glutelin was found to be the major soluble-protein fraction of the opaque-2 corn kernel, only small differences were observed between the quantities of globulin and prolamine.

The proline content of the albumin fraction tended to increase as the protein content of sorghum grain increased, whereas the leucine content of the albumin fraction tended to decrease with increased protein content. Concentrations of lysine and glycine in the insoluble residue of sorghum grain tended to decrease as protein content of the grain increased, while the amounts of leucine and glutamic acid tended to increase with increased protein content.

The concentrations of lysine, arginine, and glycine in the albumin and globulin fractions of sorghum grain were nearly double that of the entire protein of sorghum grain, whereas the globulin fraction of opaque-2 corn had approximately one-third more lysine and arginine than the entire protein of opaque-2 corn.

The same trends were found in the amino acid composition of the prolamine fractions of both opaque-2 corn and sorghum grain when compared to that of the respective whole grain protein. Lysine content of the prolamine fraction of both opaque-2 corn and sorghum grain was several times lower than that of the respective whole grain protein, albumin, globulin, and glutelin fractions. The aspartic acid content of the prolamine fraction of opaque-2 corn was about half that of the entire protein of opaque-2 corn, whereas little difference was observed in a similar comparison for sorghum grain.

In comparing the amino acid compositions of the prolamine and glutelin fractions of sorghum grain, the amounts of lysine, histidine, arginine, threonine, serine, and glycine were several times higher than the levels of these amino acids in the prolamine fraction.

The glutelin fraction of opaque-2 corn and sorghum grain had an amino acid composition which tended to be midway between those of the globulins and prolamine, except for the higher content of histidine and proline in the glutelin fraction.

The amino acid compositions of the glutelin fractions of both opaque-2 corn and sorghum grain were very similar.

Differences in the amino acid composition of the insoluble protein of both opaque-2 corn and sorghum grain tended to follow the differences observed in the respective whole grain protein, regardless of the percent of protein solubilized.

SUGGESTIONS FOR FUTURE RESEARCH

Results of this preliminary study with high-lysine corn and sorghum grain indicated this method of protein fractionation to be quantitatively and qualitatively satisfactory for studying the amino acid composition of soluble and insoluble protein fractions. However, more research must be done in an attempt to determine factors in sorghum grain which cause its protein to be more difficult to extract than the protein of high-lysine corn under similar conditions. Use of higher concentrations of sodium hydroxide (0.5% w/v) with this extraction procedure will solubilize greater quantities of protein and simultaneously cause considerable gelatinization of the starch.

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AMINO ACID COMPOSITION OF THE FRACTIONATED PROTEINS
OF SORGHUM GRAIN AND HIGH-LYSINE CORN

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Protein fractionation of five samples of sorghum grain of both low- and high-protein type has been compared to high-lysine (opaque-2) corn. Results indicated the protein of opaque-2 corn to be more easily extracted with a modified Mendel-Osborne technique than sorghum grain protein. The efficiency with which soluble protein was extracted from the sorghum grain ranged from 26.4 to 40.0%, whereas the extraction efficiency of opaque-2 corn was 86.1%. To check these results, the protein content of the insoluble residue was used to calculate the percent of total protein recovered. Total protein recovered ranged from 84 to 98.6% in the five sorghum samples, whereas 96.7% of the total protein was recovered from opaque-2 corn. The glutelin and prolamine fractions represented the two major soluble-protein fractions of sorghum grain, whereas glutelin was the major fraction of opaque-2 corn.

Amino acids were determined by ion exchange chromatography for the whole grain, four different soluble-protein fractions, and the insoluble residue. As the protein content of sorghum grain increased, the proline content of the albumin fraction tended to increase, whereas the leucine content of the albumin fraction tended to decrease with increased protein content. Concentrations of lysine and glycine in the insoluble protein of sorghum grain tended to decrease as protein content of the grain increased, while the amounts of leucine and glutamic acid tended to increase with increased protein content. Concentrations of lysine, arginine, and glycine in the albumin and globulin fractions of sorghum grain were nearly double that of the entire protein of sorghum grain.

Trends observed in the amino acid composition of the prolamine fraction of opaque-2 corn and sorghum grain were similar, whereas the glutelin fraction had an amino acid composition midway between that of the globulins and prolamines. In comparing the amino acid composition of the prolamine and glutelin fractions of sorghum grain, the contents of lysine, histidine, arginine, threonine, serine, and glycine were several times higher than the levels of those amino acids in the prolamine fraction.

Differences in the amino acid composition of the insoluble protein of both grains tended to follow differences observed in the protein of the respective whole grain.