SOLUBILITY OF PROTEINS IN SORGHUM GRAIN

by

MIR SHAMSUDDIN 265

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Osmania University Hyderabad, A. P. India

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TABLE OF CONTENTS

INTRODUCTION

Sorghum grain is an important feed and food crop. Improvements in sorghum grain by the addition of new genetic characters can make it more acceptable for human consumption in the United States. Sorghum grain with yellow endosperm and a vitamin A value has been investigated. Improvement in oil content and protein quality is possible (1). Quisenberry (2) reported that different sorghums do not appear to be of equal nutritional value. Sorghum breeders may also find it desirable to adopt nutritional values as a selection characteristic to be included in breeding programs.

Information on the solubility of protein in sorghum grain can be useful if nutritional value is related to the relative proportions of the different soluble fractions. Information on cereal proteins is also needed because physico-chemical properties have a significant effect on processing as well as on nutrition. Developments in milling and in food and feed formulation demand a thorough study of the effect of proteins and its constituents on products.

Deyoe (3) reported that protein content of sorghum grain has a range of 6 to 13% and Reid (4) showed ranges of 10.0 to 13%. Saba et al. (5) found sorghum grain varied in protein quality. Protein extraction and fractination studies showed these proteins are not strictly group specific.

The discovery of high lysine corn attracted the interest of many workers. It also indicates new varieties of sorghum grain with a high nutritive value are possible. Thus if the nature of protein present in sorghum is properly

studied and characterized, it would be possible to evaluate new sources of sorghum with improved nutritive value.

This investigation was undertaken to study the characteristics of soluble proteins and their relation to protein content and amino acid composition of the grain.

REVIEW OF LITERATURE

Protein Fractions

Sorghum grain (<u>Sorghum</u> <u>vulgare</u>) has world wide importance and dates back to ancient civilization in Africa and Asia. It now includes types that are major agriculture crops in many parts of the world. The economically important crop is known by such locally used names as "milo-maize," "Kafir corn," and gyp corn in the United States and Jawar in India. It is grown in areas where rainfall is limited to uncertain and where <u>Zea</u> <u>maize</u> (corn) and other cereals fail. The sorghum plant is more efficient than corn in absorbing and retaining moisture and seems to have the ability to remain dormant during dry periods and grows again when watered (6).

Sorghum grain is used primarily as a livestock feed in the United States. In Africa, China, and central India it is a basic food. Studies have indicated the nutritive value of sorghum grain varies considerably. Eng (7) reported increasing quantities of sorghum grain being used in all phases of the livestock industry, but its nutritional value is variable and ill-defined. Perhaps the variation in quality is an indirect result of the agronomic versatility of grain sorghum. Miller <u>et</u> <u>al</u>. (8) reported the result of analysis of large numbers of samples of milo sorghum grain for protein content. During 1962 a total of 231 analyzed samples showed a mean protein content of 9.0% with a range of 5.9% to 12.1% and in 1963 the mean protein content of 300 samples was 10.6% with a range of 7.2% to 13.5%. Further, Deyoe and Shellenberger (9) showed variations in amino acid composition. These results point to the value of evaluating the sorghum grain before use. Studies carried out

by Hubbard et al. (10) and Adrain and Sayerse (11) have shown that grain
sorghum is similar to maize in its chemical and anatomical composition.
Oke (12) pointed out the similarity in chemical composition of maize and
sorghum grain and showed sorghum could replace maize (or vice versa) without
significantly changing the nutritive value of the diet.

Exhaustive work has been done by several research workers in the area
of proteins in the seed of corn, wheat, barley, rice and soybean. The chemical
and physical properties of the fractions can be compared in evaluating the
nutritive value of grain. Similar data on sorghum grain is limited. The
procedures and methods adapted for other seeds and cereals might be applied
to evaluate the nutritive value of sorghum grain.

Studies on the proteins of corn was first reported by Gorham (13) in
1833. He described the presence of alcohol-soluble protein to which he gave
the name "zein." Zein provided 3.3% of the weight of the kernel and 40% of
the total protein content in corn. Chittenden and Osborne (14) were first
to recognize the presence of water-soluble proteins in corn. They described
the maize kernel as containing several distinct proteins well characterized
by their reactions and composition; three globulins (salt soluble), one or
more albumins (water soluble) and an alcohol soluble protein. Hopkins et al.
(15) found zein to be a major portion of corn proteins soluble in alcohol
and insoluble in water. Osborne (16) reported the solubility of proteins of
different seeds (including corn) varies greatly. Some protein fractions were
found to be water soluble and the remaining proteins were soluble in dilute
solutions of acids or alkalies. A portion was also soluble in 70% to 90%

alcohol. Osborne and Mendel (17) found that 22% of the total corn protein was soluble in 10% sodium chloride, 41% soluble in 90% ethanol, and 31% soluble in 0.2% potassium hydroxide solution, with 6.0% insoluble or lost. Thus the soluble proteins in corn kernels can be differentiated into albumins, globulins, proteoses, zein and glutelin.

It was estimated that less than one-half of the total soluble protein substance in the entire maize kernel was zein while glutelin was the most abundant protein. The proportion of zein to glutelin in high nitrogen content corn was less. Spitzer et al. (18) found 5.25% of the total nitrogen present as amide, 21.61% as globulin (soluble in 10% sodium chloride solution), 29.41% as zein (soluble in 90% alcohol), and 42.85% as glutelin. Showalter and Carr (19) were first to show the relation of different fractions of the soluble protein to the total protein content in corn. They reported that both amides and albumins seem to form a considerably greater proportion of the total protein in corn of a low protein content. In high-protein corn the amides and albumins were less. The globulins were determined by extraction with a 10% sodium chloride solution. The proportion of zein (soluble in 90% alcohol) to total protein is greater when the total protein content of corn increases. This increase in the proportion of the zein seems to be accompanied by a corresponding decrease in the proportion of the glutelin. It was concluded total nitrogen content affects the proportions of various proteins.

By selective breeding, corn of exceptionally high and low protein content has been developed (20). Soil, season and degree of maturity have a marked influence upon the chemical composition of corn (21, 22, 23). Considerable

effect upon the protein content of corn can result from the amount and type of fertilizer used and from the amount of available moisture but these factors are minor in the overall picture of corn production (24). Not only amounts but the composition of corn protein has been found to change as the grain matures. Even after mature grain has been harvested its composition may change as shown by the results of degradation of grain protein and oil during storage (25). Zeleny (26) studied the relative proportion of soluble protein in the corn kernel at different stages of maturity and found that zein is nearly absent in immature corn but sythesized at a rapid rate as corn approaches maturity. The rapid increase in ratio of zein nitrogen to total nitrogen was closely paralleled by decreased water-soluble nitrogen. Hansen et al. (27) also studied the relationship of alcohol-soluble protein to the total protein content of the corn and reported the alcohol-soluble protein (zein) in 18 samples ranging from 6.3% to 14.7% protein was linearly related to total protein content. Doty et al. (28) found the protein solubility characteristics of two hybrids of corn were distinctly different. Frey (29) reported that genetic factors are responsible for the proportion of zein (alcohol-soluble protein) to total protein content of corn. Hamilton et al. (30) conducted extensive studies on the chemical and physical composition of the corn kernel. They concluded the increase in protein as a result of fertilization was due to an increase in alcohol-soluble protein while total nitrogen insoluble in alcohol (glutelin nitrogen) was depressed and there was little change in albumin and globulin fractions. The combination of fertilization and crop rotation increased the protein content, but the proportion of

of total nitrogen as zein was increased by 12.5% and the proportion of albumin and globulin decreased by 3.1 and 9.3% respectively. Mertz et al. (31) conducted experiments on corn proteins. Electrophoretic analysis of protein extracts of germ and endosperm revealed alkaline copper extracts of the endosperm from the United States and Guatemalan samples of corn gave three fractions of an acid-soluble fraction, an alkali-alcohol fraction (zein) and an alkali-soluble and alcohol-insoluble fraction (glutelin). These three fractions accounted for 23.3, 49.7 and 21.6% respectively of the endosperm nitrogen in Guatemalan corn.

A considerable range of values has been reported for the amount of glutelin as well as other protein fractions in corn. The diversity of results undoubtedly reflects not only differences in samples of corn but also variability in the extraction procedure.

Results of protein fractionation studies of dent corn were reported by Mertz and Bressani (32) with a modified Osborne procedure and with an alkaline copper sulfite fractionation method. Values for the fractions obtained by the two methods were respectively: albumins plus globulins 12 and 23%, prolamine 34 and 47%, glutelin 37 and 24% and undissolved 17 and 6%. While these data were for the endosperm portion of the kernel, somewhat similar results would be obtained for the whole kernel since the endosperm accounts for 80 to 85% of the weight of the kernel and contains about 75 to 80% of the total protein (based on nitrogen analysis). The glutelin fraction in corn represents a significant proportion of the total protein, ranging up to 40 or 50%. Differences between the two fractionation procedures may arise from the effect of a reductive change of disulfide bonds by alkaline copper sulfite,

in particular, the increase in prolamine fraction accompanied by a decrease
of glutelins (33). Mitchell et al. (34) and Sauberlich et al. (35) showed a
significant increase in the zein fraction caused a decrease in the percentages
of other fractions. Results of Flynn et al. (36) indicated an increase in
the percentage of crude protein which might be due to either genetic differences
or nitrogen fertilization paralleled by an increase in the percentage of zein
with a decrease in the ratio of non-zein protein to zein. Bressani and
Mertz (37) separated into three fractions the soluble proteins from endosperm
and germ of corn from the United States and Guatemalan. The results indicated
that acid-soluble proteins in the endosperm varied from 17 to 26%, alkali-
and alcohol-soluble zein from 41.0 to 60.0% and alkali-soluble glutelin from
17.0 to 31.0% of the total nitrogen. Similar treatment of corn germ gave
30.0 to 40% acid-soluble, 5 to 15% alkali-alcohol-soluble and 49 to 54%
alkali-soluble proteins. This revealed that alcohol-soluble zein nitrogen
varied from 41.2% in Guatemalan to 59.7% in the United States high-protein
samples. The results also showed the alcohol-soluble nitrogen of endosperm
increased with increasing crude protein content of the whole grain.

Protein fractionation studies have also been carried out with other
cereals to study the proteins present in these grains. Cagampang et al.
(38) extracted protein from rice, and reported that soluble protein fractions
of milled rice, bran and rice polish of high and low protein samples of three
varieties indicated glutelin was the predominant fraction in the whole grain,
milled rice and rice polish. Albumin and globulin were concentrated in bran
and polish. Prolamine was evenly distributed in all three fractions.
Differences in total protein content of the whole grain was due mainly to
differences in glutelin content.

Results of studies on 32 samples of wheat flour reported by Pence
et al. (39) indicated a range of 13 to 22% of total soluble protein ex-
tracted. Albumin fractions were slightly higher than globulin fractions.
Albumin and globulin fractions were present in the range of 10 to 22%. Both
the albumin and globulin content as well as the ratio of albumin to globulin
varied significantly among the flours. The amount of soluble protein increased
directly with total flour protein. The relationship was reversed when expressed
as a percentage of the total protein. The ratio of albumin to globulin con-
tent was significantly correlated with protein quality. Meas (40) extracted
soluble protein from wheat flour by column percolation and reported a close
correlation between the total protein content and the quantities of protein
soluble in isopropyl alcohol. A less significant correlation was found for
proteins soluble in sodium hydroxide solution. Baking quality was highly
correlated with percentages of total protein soluble in isopropyl alcohol.
A negative correlation was found between baking quality and percentage of
protein soluble in distilled water.

Solubility and Extraction of Protein

Among the cereals, soluble proteins of corn, wheat and rice have been
studied extensively, but such literature is scanty for sorghum grain.

Different proteins have been classified according to their solubility in
various solvents. The albumins are soluble in water, globulins in saline
solution, prolamines in alcohol and glutelin in alkali solution. The amount
of each fraction obtained from a given source depends upon the method of
extraction and the strength of reagent used (41).

The classical approach to the isolation of glutelin from cereal grains is that of Osborne (1895-1914) involving a series of solvents. Water and saline solutions were used to remove albumins and globulins, after which alcohol is used to remove the prolamine fraction. The residue is treated with alkali to dissolve glutelin.

An alternative approach to fractionation of the proteins is that developed by Mertz and co-workers (32, 31). Initially, essentially all protein is brought into solution in an alkaline copper sulfite reagent. This dissolved protein is then fractionated on the basis of solubility in acid, alcohol and alkali. In the course of the extraction of different proteins, it is impossible to avoid partial mixing of different fractions. Further, even a careful technique can hardly remove every non-protein substance and result in obtaining a pure amino acid copolymer. Prolamines are almost exclusively located in seeds of the botanical family, _Gramineae._ Prolamines have been extracted (and more or less completely studied) from ten different species. Approximately half of the protein of _Gramineae_ seeds is prolamines except in the seeds of oats and rice which contain proportionally higher amounts of albumin or globulins and glutelins (42).

Both glutelins and prolamines are storage proteins located in seed endosperm inside "protein bodies" (43, 44). These fractions are extracted by alcohol-water mixtures, by organic solvent mixtures, by dilute acids like acetic acid or lactic acid, or by alkaline aqueous solutions, and sometimes in the presence of reducing substances as suggested by Mertz and Bressani (32).

Osborne (45) reported barley proteins were soluble in water and 5% sodium chloride. Bishop (46) reported that about 10% more protein could be

extracted from barley when it had been finely ground. Hofman-Bang (47)
also worked on barley and stated that to obtain uniform results the meal
should be ground in a ball mill until 95% passed a 100-mesh sieve. John
and Jones (48) noted there was no appreciable difference in the amount of
protein extracted from peanut meal by sodium chloride solutions at 40° to
50° C and at room temperature. On the other hand O'Hara and Sanders (49)
showed that variation in temperature affects the amount of protein extracted
from orange seed meal by 4N sodium chloride solution. Within reasonable
limits, time was not an important factor in extraction of flax seed proteins.
Jones and Gersdorff (50) extracted albumins, globulins and an alcohol-soluble
protein from wheat bran. Removal of 86.61% of the total soluble protein
from finely ground bran was done by successive extraction with distilled water,
4% sodium chloride, 70% alcohol and 0.5% sodium hydroxide solution. Johns
and Chernoff (51) reported that distilled water at room temperature extracted
2.5% of proteins from buckwheat flour, while 5 or 10% sodium chloride solu-
tions extracted 4.5%. A 5% solution of magnesium sulfate extracted 3.8% of
protein. Gortner et al. (52) reported the amount of protein extracted from
seeds by neutral salts depended upon the kind and concentration of salt used.
Smith et al. (53) observed that neutral salts disperse less of the nitrogenous
constituents from the soybean meal than are dispersed by sodium chloride
solution. Okano and Ninomiya (54) investigating soybean meal stated that when
an alcohol extraction was made first the amount of protein soluble in sub-
sequent extractions with both water and sodium chloride solutions was
decreased. Nagel et al. (55, 56) studied the ratio of water to meal,
temperature, time of extraction and particle size on the extractibility of

soybean proteins and concluded that meal must pass through a 100-mesh screen to be fine enough for extraction of maximum protein. Ratios of water to meal and shaking time were not critical. McIntyre and Kymal (57) reported that a common method of extracting rice protein employing a series of extractions with water, 5% sodium chloride, 70% ethanol and 0.2% sodium hydroxide leaves about 25% protein undispersed. The majority of rice protein can be dissolved by use of a detergent solution; however, Cagampang et al. (38) extracted 64-86% rice protein from rice flour passing a 100-mesh sieve by using a percolation method. The assessment of extraction of protein from milled rice by 18 solvents indicated that alkaline solutions are better extractants than acidic ones. Krishnamurti (58) found no significant difference between alkali and cuprammonium hydroxide 0.05 M sodium sulfite reagent as a protein solvent for some plant seeds, including wheat. Pence et al. (39) reported sodium chloride to be the best solvent for simultaneous extraction of albumins and globulins from wheat flour, while Meredith (59) reported that precipitation and solubility of wheat proteins depends on concentration of salt in the solution and not on the combination of salt and proteins. The salt affects the inter-and/or intra-molecular bonding system. Various extractants and conditions were evaluated by Fellers et al. (60) for effective solubilization of proteins of common mill feed fractions of wheat. They reported grinding of the feed significantly increased the solubilization of protein by alkali. Increasing the ratio of solvent to fine bran did not affect the quantity of sodium hydroxide necessary to maintain the pH of the solvent. Staker and Gortner (61) reported that corn proteins are much more sharply differentiated in their solubility than are those of most of the other cereals. They reported different globulin and albumin ratios

were found when extractions were made with distilled water followed by 0.5 M
potassium chloride solution or when only 0.5 M potassium chloride solution
was used. They also reported that flour passing 100 mesh was satisfactory
to extract maximum amounts of soluble proteins. Nagy et al. (62) studied
different factors affecting the solubility of corn proteins. Four grams of
defatted ground (to pass 100-mesh sieve) sample was rotated in a 200 ml
centrifuge tube for 2 to 24 hours successively with each of the following
solvents: water, a salt solution containing 5% sodium chloride and 0.05 M
Na_2HPO_4 and 0.01 M NaH_2PO_4 (pH about 7.5), alcohol (85%) and 0.2 sodium
hydroxide in series. The nitrogen content of each fraction was determined by
the Kjeldahl method. Salt and alkali solutions extracted proteins rapidly
(1 to 4 hours) whereas alcohol took longer and required buffering with sodium
acetate to obtain satisfactory extraction. The amount of salt-soluble protein
extracted was independent of salt concentration (5 to 10%) but was increased
about 2% by grinding the sample from 60 to 100 mesh. A volume of 200 ml of
each solvent was sufficient to remove the protein fraction. Prior contact
with alcohol decreased the amount of protein peptized by the salt and the
effect increased with the time. Likewise short contact with dilute alkali
depressed the solubility during the salt and alcohol extraction but to a lesser
extent. Foster et al. (63) extracted about 11% of corn protein with water,
both at room temperature and at cold-room temperature. These authors stated
that extraction with water did not have adverse affects upon subsequent ex-
tractions with other solvnets. Extraction with a salt solution (NaCl solution)
yielded about 22% protein (following water extraction of about 11%). The
same workers stated that extraction could be raised up to 90% by using detergent

reagents. Doty et al. (28) extracted protein with distilled water, 5% sodium chloride and 80% ethanol as successive solvents from corn previously dried, defatted and ground to pass an 80-mesh sieve. The 2.5-gram sample was shaken in a centrifuge tube first with 100 ml water and then twice with 75 ml portions. With the alcohol extraction 100 ml of 80% ethanol was mixed and kept over night, centrifuged and extracted twice using one-hour periods. Meas (64) developed a method to extract soluble protein by combining column chromotography and the lyrimetric determination of soil analysis. The proteins were successively extracted by means of increasing concentrations of the solvent in water or by a series of different solvents with increasing ability to dissolve protein. The extraction took 10 to 60 hours depending upon the particle size of the flour and the type of solvent used. The solvent separated proteins into sharply defined fractions. The amount of protein extracted was between 99 to 101% of the total protein content of flour.

Sastry and Verupaksha (65) used polyacrylamide gels to characterize sorghum grain proteins. These workers extracted sorghum grain endosperm protein with aqueous solvents. A water extract was prepared by shaking the flour with distilled water for 1.5 hours in a wrist action shaker. A flour to solvent ratio of 1:10 (w/v) was used. The suspension was centrifuged, and designated as an albumin fraction of sorghum protein. Globulin fractions were extracted with 1% sodium chloride solutions. An alcohol fraction (prolamine) was prepared by intermittently shaking 100 grams of defatted flour with 1 liter of 60% aqueous ethanol at 60° C for two hours. Only 10 to 12% of the total protein present in sorghum endosperm flour was extracted with distilled water or 1% sodium chloride solution under the conditions employed in the experiment, whereas nearly 45% of the total protein was extracted with 60% alcohol at 60° C.

A large quantity of material classified as non-protein matter was present in the water and salt extracts, but the alcohol extracts contained mostly protein (11.6% nitrogen). It was also concluded that prolamine was the major protein fraction and albumin and globulin fractions constituted smaller portions of the total soluble proteins of the defatted, dehulled sorghum flour.

Virupaksha and Sastry (66) studied the protein content and amino acid composition of five varieties of Indian sorghum. They used the same solvent system and procedure for extraction of protein as described in their earlier work (65) except 0.4% sodium hydroxide solution was used for extraction of glutelin. Further, the extracts and washings were combined and nitrogen was determined by the Kjeldahl method.

The results on fractionation of endosperm proteins indicated that pro-lamine and glutelin are the principal protein fractions and albumin and globulin account for less than 12% of the total endosperm protein of sorghum.

The same workers also reported the efficiency of extraction varied from 80.7 to 103.4% in five samples of sorghum grain. All three high-protein samples had increased prolamine content; therefore, it was concluded that the increased protein content in sorghum varieties may be attributed mainly to an increase in the prolamine fraction of the grain.

Soluble Protein and Amino Acid Relationships

Extensive work has been done on amino acid composition of protein in corn and other cereals to evaluate the nutritive value of the proteins. Osborne and Clapp (67) reported that rats died prematurely when corn was the

only source of protein in their diet. These authors also reported that the alcohol-soluble protein of corn contained no tryptophan or lysine. Willcock and Hopkins (68) reported that addition of tryptophan and lysine to a ration containing zein permitted young rats to grow slowly. Osborne and Mendel (17) demonstrated that the alcohol-soluble protein of corn, or zein, was nutritionally incomplete since animals rapidly lost weight on a diet containing zein as the only source of protein. Normal growth was reported following addition of lysine and tryptophan to the diet. Zein was also demonstrated to be absent from corn germ although it constituted the major protein of the endosperm. The rest of the endosperm protein, largely glutelin, was shown to contain the amino acids that zein lacks.

Hamilton et al. (69) reported that amide nitrogen made up 11.94% of the total nitrogen of corn grain. Cystine nitrogen made up 1.07% while arginine nitrogen was 8.73%, histidine nitrogen 4.83% and lysine nitrogen 2.2%. Jones and Csonka (70) and Csonka (41) found that alpha-glutelin from corn contained more lysine, tryptophan, arginine and histidine than zein. Doty et al. (28) reported that the physico-chemical nature of the protein in the grain from two single cross hybrids of corn was distinctly different. The samples that contained larger amounts of cystine, arginine, histidine, tryptophan and tyrosine also contained a larger percentage of alkali-soluble nitrogen and a smaller proportion of alcohol-soluble nitrogen. Mitchell et al. (34) studied the relationship between the protein content of corn and the nutritional value of protein. The proportion of tryptophan and lysine in the total protein of corn decreased with increasing protein content. Sauberlich et al. (35) found nitrogen fertilization and the variety grown could considerably

influence the protein and amino acid content of the samples. The amount of all amino acids increased with an increase in the protein content of corn, with considerable difference in the rate of increase among the individual amino acids. Miller et al. (71) reported that the amount of tryptophan, lysine and methionine in samples of corn varied directly with the crude protein content. The distribution of the amino acids was the same in both low-protein and high-protein corn. These workers found the same distribution of amino acids in corn of from 8.5% to 14.1% protein content. These workers pointed out that an increase in the yield resulting from nitrogen fertilization results largely from a marked increase in zein, known to be deficient in tryptophan and lysine.

Flynn et al. (36) worked out the relation between protein content of corn and concentration of amino acids and nicotinic acid. Microbiological assays were conducted of 13 samples of low-protein corn and 15 samples of high-protein corn for certain nutritionally essential amino acids and nicotinic acid. The values obtained for low- and high-protein corn were respectively (1) tryptophan 87 and 99 mg% (2) lysine 314 and 380 mg% (3) methionine 199 and 239 mg% (4) nicotinic acid 2.53 and 2.40 mg%. It was noted that high-protein corn contained more tryptophan, lysine, methionine and cystine than low-protein corn.

Mertz et al. (72) reported that endosperm of maize seed homozygous for the opaque-2 gene had a higher lysine content that that of normal kernels. Opaque-2 endosperm had a different amino acid pattern and 69% more lysine than normal seeds. The major reason for these changes was in the synthesis

of protein with a greater content of basic amino acids in the acid-soluble
fraction of the mutant endosperm. This was accompanied by a reduction in
the ratio of zein to glutelin.

Hepburn and Bradley (73) reported that samples of commercial wheats
containing different amounts of total nitrogen differed in the proportion of
certain amino acids. Comparisons of amino acid composition were made between
different varieties at a single level of nitrogen and between samples of
varieties which differed widely in nitrogen content. The proportion of amino
acids was markedly constant for all varieties of wheat with similar nitrogen
contents. Samples of highest and lowest nitrogen content exhibited small
differences in proportion of most amino acids. Values of glutamic acid,
phenylalanine and proline tended to be higher in high-protein samples whereas
the remaining amino acids showed the opposite trend.

Jimenez (74) studied the changes in amino acid composition as a result
of single gene substitution in corn. Amino acid analysis of protein fractions
of normal, opaque and floury endosperm revealed that in general, globulins,
zein and glutelins have different amino acid compositions, but no variations
were observed for most of the amino acids among all genotypes. The albumins
from opaque and floury varieties, however, had concentrations of several
amino acids that differed from normal. The lysine content of albumin was
high in all three genotypes. The globulins, smallest of the protein fractions,
were the richest of all four fractions in lysine. Glutelins, the major
protein fraction present in opaque and floury endosperms, also were relatively
rich in lysine content. The zein fraction had negligible lysine content in

opaque and floury endosperm, but was higher in normal corn than in the mutants mostly due to large amounts of this fraction. Jimenez (74) concluded that most of the lysine present in the endosperm was contributed by the glutelins in the three genotypes, as this fraction was higher in opaque and floury endosperm than in normal endosperm.

Hopkins et al. (75) originally demonstrated that changes in the protein content of corn induced by selective breeding were related to changes in proportions existing among the anatomical parts of the kernel. The most prominant change in the physical composition accompanying increasing protein content is a greatly increased proportion of horny over starchy endosperm. Anatomical changes were observed by Hamilton et al. (30) to accompany increased protein content of corn induced by changes in soil treatment and crop management. It was shown that horny endosperm contained a higher concentration of protein than the floury endosperm. Alcohol-soluble protein, zein, for all practical purposes, was confined to the horny endosperm. Frey (76) studied the inter-relationship of proteins and amino acids in corn samples. Samples of the F_2 generation and back crosses of two maize crosses were analyzed for total protein, zein, tryptophan, valine, leucine, and isoleucine. Statistical analysis showed that valine, leucine and isoleucine were more closely related to each other than to tryptophan in corn. In the samples studied zein became an increasingly greater proportion of the total protein as the percentage of total protein increased. Tryptophan and valine became a decreasing and leucine an increasing proportion of the total protein as the amount of protein increased in the corn grain. No trend was apparent for isoleucine. Frey (29) concluded that protein of low-protein corn samples were more nearly balanced nutritionally than that of high protein samples.

Bressani and Rios (77) studied the chemical and essential amino acid composition of 25 selections of sorghum grain. They found significant differences in essential amino acid composition among the genetically varied samples. The same workers also reported sorghum grain to be slightly higher than maize in its content of arginine, histidine, isoleucine, tryptophan and valine whereas levels of leucine, lysine, methionine, phenylalanine, tyrosine and threonine are very similar with those reported by other workers for corn. Adrain and Sayerse (11) also showed the relationship of corn to sorghum grain and indicated lysine to be the limiting amino acid in sorghum grain and millet. Mangay et al. (78) suggested that millet was deficient in lysine but contained a higher percentage of tryptophan than is found in sorghum.

Miller et al. (8), Burleson et al. (79), and Byrid et al. (80) reported that the nitrogen fertilization increased the protein content of sorghum grain. Miller et al. (8) analyzed samples of sorghum grain collected in 1958, 1961 and 1962 from different demonstration plots and found wide variations in protein content, which ranged from 5.9 to 12.1% in 1962.

Waggle et al. (81) studied the effect of three levels of nitrogen fertilization on amino-acid composition and distribution in sorghum. A direct relationship was found between increasing amounts of nitrogen fertilization and the level of protein and amino acids in the grain. Nitrogen fertilization increased the quantity of nutritionally essential amino acids in the grain. Not all amino acids increased proportionally as the protein increased. Lysine, histidine, arginine, threonine and glycine were the highest in grains of high level of nitrogen fertilization. Aspartic acid, serine, cystine, valine, methionine and tyrosine were not affected by nitrogen fertilization. Non-essential amino acids were higher in grain after nitrogen fertilization.

Baptist (82) reported that sorghum (tropical variation) was deficient not only in lysine but also in methionine and tryptophan. Vavich et al. (83) and Waggle et al. (84) reported lysine, arginine and threonine to be limiting amino acids for rats in sorghum grain. The latter investigators found that sorghum grain containing 10.5% protein was superior to that containing 15.3% protein. Deyoe and Shellenberger (9) studied the variation in levels of protein and amino acid composition and found significant differences in methionine, lysine and protein content due to hybridization and location.

According to Waggle and Deyoe (85) the amino acid composition is related to the protein content of sorghum grain. Glutamic acid, proline, alanine, isoleucine, leucine and phenylalanine were higher in concentration in the protein of grain of higher protein content. The concentration of lysine, histidine, arginine, threonine and glycine in the protein tended to decrease as the protein content increased. Waggle et al. (86), in experiments to determine the nutritive value of sorghum grain as measured by chick performance, found methionine, lysine and arginine to be limiting amino acids in the diets.

Virapaksha and Sastry (66) reported lysine as the most limiting amino acid in sorghum protein in grains in India. When lysine content of different varieties of sorghum was determined, lysine as percentage of protein was negatively correlated with the protein content of the seed. Composition of nine samples of high- and low-protein varieties of sorghum grain indicated that amino acid composition of varieties differs considerably. Variation of amino acid composition of the seed could be due to changes in the various protein fractions without any changes in chemical composition or it might be due to changes in chemical composition of one or more protein fractions (42).

MATERIALS & METHODS

Sorghum Grain Samples: Sorghum grain samples were composited by mixing hybrids from the 1966 crop. Samples of Dekalb's E-57 grown in Brown, Finney and Colby counties were collected from the Agricultural Experiment Station. Also obtained were Dekalb's F-64 from Brown, Greely and Finney counties; Lindsey Funk's 555 from Brown, Finney and Riley counties; R-S 625 from Brown, Finney and Riley counties; R-S 610 from Brown and Finney counties; Pioneer's 846 from Colby and Riley counties; and T. E. 66 from Colby and Riley counties.

Kjeldahl Protein Determination

All samples were cleaned, then ground with a Mikro-Sampl Mill[1] using a 0.025 inch screen. The material was ground to pass through a 100 mesh sieve. The nitrogen content of ground grain was determined by the micro Kjeldahl method, according to AOAC methods, and crude protein content of the 18 samples was estimated from Kjeldahl nitrogen by multiplying by the factor 6.25. (87)

Fractionation Procedure

Before grinding, all samples were cleaned of dirt and foreign material. The samples were then finely ground with a micro pulverizer mill using a 0.025 inch screen. The material was ground to pass through a Tyler 100-mesh sieve. After grinding, the samples were thoroughly mixed and uniform aliqots were obtained. The nitrogen content was determined by micro Kjeldahl assay (AOAC methods, 87) and crude protein content was established.

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[1] Mikro-Sampl Mill, Pulverizing Machinery Company, Summit, New Jersey.

To obtain different fractions of soluble proteins the modified Osborne-Mendel method as employed by Jimenez (74) was used with minor modifications. Two grams of sorghum grain flour (100 mesh) was subjected to four consecutive extractions using the following solvents: distilled water, 5% sodium chloride, 80% ethanol plus 0.2% sodium acetate and 0.2% sodium hydroxide solution.

<u>Water Extraction</u>: To extract water-soluble proteins (albumins) two grams of sample was transferred to a Reacter-R-Mill[2] and 50 ml of distilled water was added. The sample was then shaken vigorously for 9 minutes at room temperature. The resulting suspension was transferred to heavy walled centrifuge tubes and centrifuged at 10,000 rpm for 15 minutes. The supernatant was decanted into a flask. Filtration was avoided since successive extractions of one sample with different solvents could be accomplished and because filter paper is known to absorb proteins (88). After loosening the residue in the centrifuge tube by means of a glass stirring rod, 50 ml of fresh distilled water was added in small portions to transfer the flour residue from the centrifuge tube to the Reacter-R-Mill. The second extraction was made in the same manner as the first. The resulting suspension was again centrifuged and the clear supernatant liquid was decanted into a separate flask. The third water extraction was made in a similar manner.

<u>Sodium Chloride Extraction</u>: The flour remaining after three water extracts was treated with 5% sodium chloride solution to extract salt-soluble proteins (globulins). Fifty ml of sodium chloride solution was used to carefully transfer the residue from a centrifuge tube to the Reacter-R-Mill. The sample

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[2]Reacter-R-Mill, Equipment supplied by Udy Analyzer Company, Pullman, Washington.

was again shaken, centrifuged and the supernatant was collected in flasks as described previously. This procedure was repeated twice using 50 ml of sodium chloride solution each time. All salt fractions were collected separately and analyzed.

Alcohol Extraction: Following the water and salt extractions the flour residue was extracted with 150 ml of 80% ethanol plus 0.2% sodium acetate solution using the modified procedure by Nagy et al. (62). The extraction procedure was the same as already described except a buffered alcohol solution was used as the solvent.

Alkali Extraction: Following extraction with water, 5% sodium chloride and 80% ethanol plus 0.2% sodium acetate, 150 ml 0.2% sodium hydroxide solution was used to extract alkali-soluble proteins (glutelin) from the flour residue.

Protein Determination: Protein content of recovered fractions was estimated using the micro Kjeldahl procedure described in AOAC methods (87). Total nitrogen content of each sample collected was determined in duplicate and values obtained were combined for the fractions. Two ml aliquots were used for digestion using mercuric oxide as a catalyst.

Amino Acid Analysis

For amino acid analysis ground grain samples were hydrolyzed with acid. Thirty-five to fifty mg of sample was placed in a narrowed test tube. The 6 N hydrochloric acid was added at the rate of 1 ml per mg of protein in the sample. The test tubes were placed in a dry ice-alcohol bath, frozen, and attached to an aspirator. The test tubes were sealed under a reduced

atmosphere of 27 inches of mercury. Contents of tubes were hydrolyzed at 110 C for 22 hours. Humin was removed by filtering the hydrolyzed samples through a fritted disc funnel. The filtrate was evaporated to dryness three times under reduced pressure and then diluted to 10 ml with 0.2 N sodium citrate buffer pH 2.2.

Amino acid analyses of the hydrolyzed samples were made by ion exchange chromotography using a Beckman model 120B amino acid auto analyzer (89).

Correlation coefficients were calculated between (1) the percentages of four fractions of soluble proteins extracted and protein content of grain, (2) the soluble protein fractions and the distribution of five essential amino acids lysine, histidine, arginine, threonine, and glycine, (3) and between these amino acids and protein content of the grain according to the methods of Snedecor (90).

RESULTS AND DISCUSSION

Protein Content of Samples

Results of protein determinations (Tables 1 and 2) show differences in
protein content in 18 samples of different hybrid sorghum grains. The pro-
tein content ranged between 8.0 and 14.8%. Variations within the same
hybrid grown at different locations were observed; this agrees with the
results obtained by others working with corn and sorghum. Doty et al. (21)
and Fraps (91) showed that environmental factors such as soil type, fertiliza-
tion and moisture may affect the protein content of grain. Doty et al. (21)
indicated the protein content of corn may be related to genetic constituent.
Preliminary work of Miller et al. (8) and more recent work of Deyoe and
Shellenberger (9) and Waggle and Deyoe (85) also indicates variation in pro-
tein content and amino acid composition due to hybrid, location and fertiliza-
tion. Virupaksha and Sastry (66) working on Indian varieties also reported
large variation in protein content among varieties as well as in hybrids.

Characteristics of Protein Fractions

In preliminary studies, values obtained on four protein fractions from
sorghum grain revealed that several factors in the extraction procedure could
result in differences in values obtained. Results of these studies to develop
a method for extraction of soluble proteins in sorghum grain served to establish
the following conclusions.

The results of a percolation column method as employed by Meas (40) are
presented in Table 3. When ten grams of sample that passed through a 28-mesh
seive and either a 48- or 100-mesh sieve (Tyler sieve sizes) were used the values

Table 1 Summary of Grain Protein Level and Percentage
of Soluble Protein Fractions in Sorghum Grain

Code Number[1]	Protein Content %[2]	Soluble Proteins Fractions			
		Albumin %	Globulin %	Prolamine %	Glutelin%
CO – 88	8.0	27.98	14.51	21.36	36.05
MO – 88	14.7	22.78	11.51	30.08	34.96
GC – 15	8.4	28.21	13.50	26.70	31.31
BC – 15	9.2	24.83	11.86	29.47	33.80
GCD – 15	10.9	23.00	12.00	32.45	31.13
COC – 66	8.7	20.09	14.60	24.50	31.62
MO – 66	12.1	24.50	12.75	31.06	33.06
BC – 83	9.7	24.51	13.60	25.62	36.21
GCD – 83	10.9	23.80	13.10	27.41	35.64
BC – 38	10.9	25.80	9.70	27.99	37.56
GCD – 38	12.1	23.40	11.17	30.00	35.26
MO – 38	14.8	20.78	10.51	33.08	34.96
BC – 81	10.6	24.00	12.78	28.77	35.43
GCD – 81	11.9	21.16	11.68	31.72	33.65
MO – 81	13.0	23.06	11.25	32.64	33.04
CO – 14	8.5	26.00	13.15	29.05	31.60
BC – 14	9.1	24.30	13.12	30.90	31.92
GCD – 14	10.9	22.43	9.94	32.14	35.00
Mean	10.80	24.423	12.262	29.163	33.790
Range	8.0–14.8	20.78–29.09	8.70–14.60	21.36–33.08	31.15–37.56
Standard Deviation	2.039	2.293	1.433	3.141	2.415

[1]Hybrid Varieties

CO-88 – TE – 66
MO-88 – TE – 66
GC-15 – Dekalb's F-64
BC-15 – Dekalb's F-64
GCD-15 – Dekalb's F-64
CO-66 – Pioneer's 846
MO-66 – Pioneer's 846
BC-83 – RS 610
GCD-83 – RS 610

BC-38 – Lindsey Funk 555
GCD-38 – Lindsey Funk 555
MO-38 – Lindsey Funk 555
BC-81 – RS 625
GCD-81 – RS 625
MO-81 – RS 625
CO-14 – Dekalb's E-57
BC-14 – Dekalb's E-57
GCD-14 – Dekalb's E-57

[2]Kjeldahl Protein N x 6.25

Table 2   Solubility Fractionation of Protein of Sorghum Grain

Sample[1]	Protein Content[2] %	Albumin %	Globulin %	Prolamine %	Glutelin %	Total Extracted %
CO-88	8.0	26.222	13.87	20.32	34.30	95.13
MO-88	14.7	20.98	10.60	27.71	32.21	92.14
GC-15	8.4	21.83	10.47	20.67	24.28	77.40
BC-15	9.2	22.52	10.46	26.74	31.66	91.68
GCD-15	10.9	20.78	10.84	29.32	28.15	89.09
CO-66	8.7	26.46	13.28	22.58	28.76	90.96
MO-66	12.1	17.90	9.29	22.64	24.49	74.32
BC-83	9.7	19.72	10.94	21.97	29.17	80.56
GCD-83	10.9	19.74	5.35	21.34	28.15	76.45
BC-38	10.9	19.44	6.56	21.03	28.8	75.88
GCD-38	12.2	19.25	8.82	23.67	27.37	79.11
MO-38	14.8	18.76	9.48	25.40	28.79	82.34
BC-81	10.6	18.63	8.67	22.35	27.51	76.39
GCD-81	11.9	17.86	9.08	24.63	24.58	76.15
MO-81	13.0	17.79	12.06	28.42	28.69	87.96
CO-14	8.5	23.94	12.11	25.83	29.10	91.98
BC-14	9.1	23.14	12.49	29.43	30.40	95.26
CCD-14	10.9	20.16	8.93	28.88	31.5	89.88

[1]Hybrid Variety

CO-88 - T.E. - 66
MO-88 - T.E. - 66
GC-15 - Dekalb's F-64
BC-15 - Dekalb's F-64
GCD-15 - Dekalb's F-64
CO-66 - Pioneer's 846
MO-66 - Pioneer's 846
BC-83 - RS 610
GCD-83 - RS 610

BC-38 - Lindsey Funk 555
GCD-38 - Lindsey Funk 555
MO-38 - Lindsey Funk 555
BC-81 - RS 625
GCD-81 - RS 625
MO-81 - RS 625
CO-14 - Dekalb's E-57
BC-14 - Dekalb's E-57
GCD-14 - Dekalb's E-57

[2]Kjeldahl Protein N x 6.25.

Table 3 Solubility Fractionation of Protein of Sorghum Grain Passing a
100 Mesh (Using Two Different Methods)

	Column Method[1]	Shaking in Reactor-R-Mill[2]
	Sample CO-88[3] Protein 8.1%[4]	Sample CO-88 Protein 8.1
Albumin	19.0	26.622
Globulin	10.1	13.87
Prolamine	8.46	20.32
Glutelin	25.9	34.30
Total % Extracted	63.46	95.13

[1]Column percolation method as employed by Meas (1966). Using 250 ml of each solvent.

[2]Shaking the flour in Reactor-R-Mill.

[3]Hybrid Variety CO-88 - (T.E. 66).

[4]%, N x 6.25.

for total extracted soluble protein were 39, 51 and 63.4%. Meas (40) in studies with wheat and Cogampang et al. (38) in work with rice extracted 64 to 85% of the soluble protein using percolation columns.

In studies made by shaking the flour with the solvent in a Reacter-R-Mill from 75 to 95% of the proteins were extracted in a comparatively short time. The reason for lower values in the column percolation method may be due to protein differences or artifacts resulting in incomplete contact between solvent and protein. Vigorous shaking of flour with the solvent in the Reacter-R-Mill may have disintegrated the particles and brought protein materials into more intimate contact with the solvent as described by Staker and Gortner (61). The tendency for the sorghum to swell and reduce the rate of solvent flow also created problems with the percolation method.

Effect of Particle Size: Three different samples of flour made from sorghum grain classified as through 28, 48 and 100 mesh (Tyler sieve size) respectively were prepared by grinding samples BC-38, CO-88, and sorghum grits free of germ and bran in a Mikro-Sampl Mill and sieving. Results of protein determinations and extraction of soluble proteins are presented in Table 4. The data show larger amounts of soluble nitrogen can be extracted from flour passing through a 100-mesh sieve. Reduction in particle size increased the protein extractibility. This observation was also made by Nagel et al. (55) while extracting protein from soybean meal by Smith (53) and Nagy et al. (62) in work with corn and by Cogampang et al. (38) in studies with rice.

Solvent Meal Ratio: Results of extractions of soluble proteins of sorghum grain using different volumes of solvents are summarized in Table 5.

Table 4 Extraction of Sorghum Protein
(Using Flours of Different Particle Size)

Sample[1]	Protein %[2]	Protein Fractions				% Extracted
		Albumin	Globulin	Prolamine	Glutelin	
		28 mesh[3]				
BC - 38	10.4	9.23	2.89	9.12	12.60	33.84
CO - 88	7.8	7.08	4.32	11.34	14.02	36.75
grits	9.75	3.24	1.95	15.15	10.00	30.34
		48 mesh				
BC - 38	10.9	16.34	3.56	17.14	21.31	58.35
CO - 88	8.0	18.74	9.87	17.64	25.27	71.52
grits	9.02	10.35	2.60	29.18	20.08	62.21
		100 mesh				
BC - 38	10.9	19.44	6.56	21.03	28.3	75.33
CO - 88	8.0	26.98	13.00	20.32	34.30	94.60
grits	8.65	16.42	8.58	22.12	24.17	71.29

[1]Hybrid Varieties   BC - 38
                       CO - 88
                       grits - free of germ and bran

[2]Kjeldahl Protein N x 6.25.

[3]Tyler Sieve.

Table 5 Sorghum Grain Fractionation Using Different
Volumes of Each Solvent

Time	50 ml		100 ml		150 ml		200 ml	
Code Number[1]	CO-88	MO-66	CO-88	MO-66	CO-88	MO-66	CO-88	MO-66
Albumin %	15.52	14.31	21.52	16.13	26.64	18.01	26.00	17.81
Globulin %	6.00	6.20	10.90	8.61	13.87	9.97	18.87	7.87
Prolamine %	11.30	15.15	15.71	19.76	20.32	23.64	21.00	24.00
Glutelin %	18.60	18.01	26.25	22.07	34.30	27.51	34.00	20.00
TOTAL	51.42	50.67	74.40	66.57	95.13	79.13	94.87	69.68

[1]Hybrid Variety   CO – 88 – T.E. – 66
                      MO – 66 – T.E. – 66

Shaking time was 27 minutes in each case.

These results indicate that 150 ml of each solvent was sufficient to remove the maximum amount of soluble protein from 2 grams of flour. If 50 or 100 ml of each solvent was used to extract protein from 2 grams of sample only 51.42% and 74.40% of the total protein was obtained respectively. A volume of 150 ml of each solvent extracted 95.13% protein. A further increase of 50 ml of solvent did not increase the extraction of proteins soluble in that solvent. Reduction in extraction after increasing solvent volume from 150 ml to 200 ml may have been due to increase in temperature while shaking, resulted in denaturization of the protein. Temperature is a critical factor in extracting the soluble protein seeds.

Shaking time: Samples of sorghum flour were shaken with each solvent in a Reactor-R-Mill for 9, 18, 27 and 45 minutes (Table 6). Shaking for 27 minutes extracted the maximum amount of protein soluble in each solvent. Protein extraction at 9, 18, or 45 minutes was not as complete as at 27 minutes. Smaller protein extractions after 45 minutes may have been due to increased temperature during shaking and interaction of materials in the sorghum flour.

Fractionation Studies: The results of fractionating proteins of 18 samples of different protein contents and representing different hybrids are summarized in Table 1. The relative proportions of fractions are reported as percentages of the soluble protein extracted. The data indicate that all samples had a larger percentage of the glutelin fraction than of the three other soluble fractions; the percentage of glutelin varied between 31.15 and 37.56% (mean = 33.79, standard deviation ± 2.415) (Table 1). The globulin fraction was the smallest among the four fractions extracted and ranged from 8.70 to 14.60%

Table 6 Fractionation of Soluble Protein of Sorghum Grain

(Shaking for Different Times with Each Solvent)

Time	9 Minutes		18 Minutes		27 Minutes		45 Minutes	
Code #	CO-88[1]	grits[2]	CO-88	grits	CO-88	grits	CO-88	grits
Protein Content %	8.00	9.65	8.00	9.65	8.00	9.65	8.00	9.65
Albumin	10.00	6.54	23.52	10.34	26.00	14.76	22.32	13.99
Globulin	4.90	2.32	11.90	6.11	12.86	9.05	8.17	4.01
Prolamine	8.05	10.80	15.71	20.97	20.84	27.11	21.83	28.11
Glutelin	11.30	12.67	27.65	22.14	34.00	32.11	26.87	22.01
TOTAL	34.25	20.38	78.78	59.56	93.64	83.03	79.19	67.12

[1]Hybrid Variety CO-88 (T.E. - 66).

[2]Grits - free of germ and bran.

(mean = 12.202%, standard deviation ± 1.433). Albumin was higher in grain of low protein content whereas prolamine was higher in high-protein grains. Glutelin and prolamine are major protein fractions of corn endosperm protein (74, 37) and zein (prolamine) is the largest protein fraction in corn of high nitrogen concentration (19). Glutelin and prolamine are the principal proteins of sorghum endosperm (66).

The prolamine fraction (Table 1) of hybrid T. E. 66 with 14.7% protein was 30.08% of the total soluble protein whereas prolamine accounted for only 21.36% of the soluble protein in a sample of the same hybrid with 8.0% protein. In the same manner samples of Dekalb F-64, Pioneer's 846, R-S 610, Lindsey Funk 555, R-S 625 and Dekalb E-57 hybrids had the highest prolamine values in samples of high protein content. These data indicate that prolamine increases rapidly as the total protein content of the grain increases. Zeleny (26), Frey (29), and Hansen et al. (27) observed that zein protein increased markedly as total protein in corn increased. Therefore, the increase in protein content due to prolamine in sorghum hybrids appear similar to the increase in the zein fraction of corn. Similar changes in the proportion of prolamine in high-protein varieties were reported by Virupaksha and Sastry (66) in sorghum grain, by Bressani and Mertz (37) in corn, Cagampang et al. (38) in rice and McDermolt and Pace (92) in wheat.

These results also indicate that prolamine content of sorghum grain protein varies with the total protein content. This may be due to several factors related to protein content as observed by Hansen et al. (27) and Mertz et al. (72). Variation in prolamine protein of corn was regarded as due to varietal differences, selection, soil and fertilization by Frey (76).

The albumin fraction of sorghum grain samples decreased as the protein content of the grain increased. The percentage of the albumin fraction of T. E. 66 hybrid decreased from 27.98 to 22.78% as the protein content increased from 8.0 to 14.7%. In the same manner percentage of albumin of all samples studied decreased as the total protein content of the grain increased. Higher albumin values obtained in these studies than reported by Virupaksha and Sastry (66) might be due to the use of whole sorghum grain rather than endosperm.

These results indicate that albumin content decreased with an increase in protein content of the sorghum grain. An increase in prolamine content appears to parallel an increase in total protein content of the grain. Showwalter and Carr (19) observed that an increase in protein content of corn was due to an increase in zein fraction with a decrease in albumin, amide and glutelin fractions of the soluble protein. Zeleny (26) pointed that zein is nearly absent in the immature corn kernel but is synthesized at a very rapid rate as corn approaches maturity. Frey (29) stated protein in corn could be increased by selective breeding and fertilization, but the increase would be in the zein fraction.

Globulin content of all samples decreased as the total protein content of the grain increased (Table 1). The decrease in this fraction was smaller than the decrease in albumin. A variation from this general trend was observed in the hybrid Lindsey Funk 555. The globulin fraction was 9.7% in the sample having protein content of 10.9% whereas in the same hybrid the samples containing higher values of globulin, 11.17% and 10.51%, had protein contents of 12.1 and 14.8% respectively. This variation may have been due to hybrid differences.

The glutelin which accounted for most of the protein in all 18 samples in these studies (Tables 1 & 2), showed large variations. Glutelin fractions

were higher in samples of lower protein content within these hybrids and percentages present decreased as the protein content of the grain increased. Variation from this general trend was observed in samples of Pioneer's 846 hybrid grown at two different locations. These had glutelin values of 33.06% and 31.62% with respective protein contents of 12.1 and 8.7%. Similar changes were also observed in Dekalb F-64 hybrid grown at Brown County which had a glutelin content of 33.80% at a 9.2% protein level and 31.31% at 8.4% protein content.

The means and ranges for percentages of total protein and soluble protein fractions are given in Table 1. The correlation coefficients given in Table 7 between protein content and the soluble fractions are significant for albumin, globulin and prolamine. They also indicate negative correlation coefficients between protein content and albumin values. Positive relationships were found when albumin and prolamine fractions were compared to protein levels.

Relationship Between Protein Fractions and Amino Acid Content

Amino acid analyses were made on all 18 samples of hybrid grains. In these analyses whole seed hydrolyzates were used following procedures reported by Waggle et al. (84). Values were reported in grams of amino acid per 100 grams of protein (Tables 8 and 9). Data summarized in Table 8 show variations were found between different hybrids as well as between low- and high-protein content samples within hybrids. These results are similar to those reported by Deyoe and Shellenberger (9) for sorghum hybrids. Hybridization affected the protein content of corn and quality of protein as measured by amino acid

Table 7 Correlation Coefficients of the Relation between Protein Content and Soluble Protein Fractions in Sorghum Grain (18 Samples)

	1 Protein	2 Albumin	3 Globulin	4 Prolamine	5 Glutelin
1 Protein	1.000				
2 Albumin	-0.779	1.000			
3 Globulin	-0.656	0.672			
4 Prolamine	0.660	-0.8160	-0.704	1.000	
5 Glutelin	0.040	-0.2162	0.240	-0.099	1.000

Table 8 Amino Acid Composition of Sorghum Grain Hybrids
(Grams of Amino Acid per 100 Grams of Protein)[1]

Code Number[2]	CO-88	MO-88	GC-15	BC-15	GCD-15	CO-66	MO-66	BC-83	GCD-83
Protein[3]	8.000	14.700	8.400	9.200	10.900	8.700	12.100	9.700	10.900
Amino Acids									
Lysine	2.270	1.682	2.169	1.862	2.144	2.260	1.795	2.114	2.018
Histidine	2.049	1.946	2.388	2.560	2.118	2.405	2.025	2.256	2.103
Ammonia	3.024	3.191	2.935	3.521	3.013	3.390	3.003	3.033	3.146
Arginine	3.903	3.175	3.800	3.445	3.445	3.900	3.210	3.622	3.445
Aspartic Acid	7.475	7.129	6.848	7.082	6.763	7.927	6.731	6.696	6.860
Threonine	3.519	3.113	3.330	3.635	3.323	3.553	3.114	3.280	3.155
Serine	5.009	4.552	4.620	4.970	4.333	5.145	4.356	4.459	4.520
Glutamic Acid	24.853	24.957	22.967	25.440	21.687	26.389	23.144	22.567	23.721
Proline	9.123	8.965	8.955	9.958	8.635	9.773	8.270	8.321	9.063
Glycine	3.501	2.684	3.277	3.149	2.951	3.527	2.860	3.133	2.984
Alanine	11.227	10.784	9.682	10.793	9.486	11.937	9.909	9.463	10.740
Half Cystine	1.658	1.586	2.142	2.740	1.915	1.839	2.009	2.454	2.070
Valine	5.425	3.663	5.337	4.615	4.922	5.946	1.093	3.044	2.499
Methionine	1.209	1.132	1.094	1.410	1.067	1.212	1.059	1.114	1.124
Isoleucine	4.611	4.552	4.384	4.779	4.213	4.847	4.163	4.107	4.436
Leucine	15.211	14.626	14.138	15.819	13.397	16.280	14.029	13.460	14.923
Tyrosine	4.286	4.070	4.073	4.784	4.080	4.855	4.092	4.135	4.330
Phenylalanine	5.898	5.427	5.557	6.233	5.303	6.419	5.244	5.254	5.722

[1]Protein content was obtained by adding the grams of amino acid and ammonia accounted for by the amino acid analyzer.

[2]Hybrid Varieties:

CO-88 – TE-66
MO-88 – TE-66
GC-15 – DeKalb's F-64
BC-15 – DeKalb's F-64
GCD-15 – DeKalb's F-64

CO-66 – Pioneer's 846
MO-66 – Pioneer's 846
BC-83 – R-S 610
MO-83 – R-S 610

[3]%, Kjeldahl Protein N x 6.25.

Table 8 -- Continued

Code Number[1]	BC-38	GCD-38	MO-38	BC-81	GCD-81	MO-81	CO-14	BC-14	GCD-14
Protein[2]	10.900	12.100	14.800	10.600	11.900	13.000	8.500	9.100	10.900
Amino Acids									
Lysine	1.933	2.276	1.685	1.934	1.751	1.636	2.328	2.039	2.300
Histidine	2.120	2.315	2.020	2.088	1.292	1.789	2.713	2.429	2.249
Ammonia	3.066	3.631	3.201	2.987	2.985	2.750	3.519	3.124	2.863
Arginine	3.590	3.950	3.137	3.655	3.267	2.922	4.132	3.664	3.734
Aspartic Acid	7.107	8.526	7.491	7.086	6.952	6.442	8.139	6.433	7.329
Threonine	3.310	3.776	3.241	3.340	3.241	2.841	3.901	3.324	3.395
Serine	4.749	5.400	4.860	4.658	4.428	4.149	5.413	4.339	4.635
Glutamic Acid	24.408	28.915	27.057	23.984	23.872	22.057	28.181	21.353	23.176
Proline	9.167	10.116	9.576	9.150	8.768	8.261	10.121	8.507	8.619
Glycine	3.162	3.328	2.784	3.143	2.776	2.581	3.518	3.015	3.040
Alanine	10.666	12.359	11.742	10.187	10.471	9.890	11.883	9.220	9.950
Half Cystine	2.503	2.032	1.876	2.010	1.664	1.447	2.629	2.403	2.024
Valine	5.858	3.446	5.630	5.221	5.650	5.462	5.517	1.423	4.329
Methionine	1.554	0.562	1.057	1.020	1.238	1.175	1.044	1.181	1.082
Isoleucine	4.403	5.179	4.593	4.351	4.436	4.075	4.940	4.218	4.300
Leucine	15.012	17.680	16.591	14.612	14.968	14.124	16.914	13.337	13.996
Tyrosine	4.378	5.007	4.572	4.367	4.279	3.945	4.974	4.166	4.287
Phenylalanine	5.714	6.440	5.959	5.509	5.679	5.313	6.132	5.380	5.545

[1] Hybrid Varieties:

BC-38 - Lindsey Funk 555
GCD-38 - Lindsey Funk 555
MO-38 - Lindsey Funk 555
BC-81 - R-S 625
GCD-81 - R-S 625

MO-81 - R-S 625
CO-14 - DeKalb's E-57
BC-14 - DeKalb's E-57
GCD-14 - DeKalb's E-57

[2] %, Kjeldahl Protein N x 6.25.

Table 9 Summary of the Protein Level and Amino Acids Distribution
of Sorghum Grain (Grams of Amino Acid per 100 Grams of Protein)

	Mean %	Range %	Standard Deviation
Protein	10.80	8.0-14.8	2.039
Amino Acid			
Lysine	1.999	1.63-2.32	0.235
Histidine	2.159	1.29-2.75	0.315
Arginine	3.555	2.92-4.13	0.326
Aspartic Acid	7.167	6.433-8.52	0.568
Threonine	3.355	2.841-3.901	0.251
Serine	4.763	4.149-5.413	0.901
Glutamic Acid	24.371	21.353-28.915	2.386
Proline	9.073	8.267-10.121	0.615
Glycine	3.082	2.581-3.518	3.082
Alanine	10.559	9.463-12.359	1.529
Valine	4.392	1.093-5.946	0.928
Methionine	1.123	1.020-1.554	1.529
Isoleucine	4.474	4.075-5.179	0.304
Leucine	14.955	13.397-16.914	1.426
Tyrosine	4.204	4.070-5.07	1.276
Phenylalanine	5.707	5.244-6.440	0.394

analysis (28). The values obtained in these studies for arginine, aspartic acid, serine, glutamic acid, proline, alanine, leucine, isoleucine, and phenylalanine are slightly higher than those reported by Deyoe and Shellenberger (9) and Waggle et al. (84). The higher values may be due to a tendency of prolamine to increase with increased protein content or may be an effect of different samples and hybrids.

The data presented in Table 8 also reveal that lysine, histidine, arginine, glycine and cystine were higher in the protein of samples with lower protein content. These results followed trends observed in the values for soluble proteins (Table 1). The values for percentage of protein for the amino acids lysine, arginine, histidine, glycine, and threonine were also higher in samples which ocntained higher amounts of albumin and globulin fractions. Percentages of these amino acids in the protein decreased as the prolamine (alcohol-soluble) fraction of the sorghum grain protein increased. Thus the effects of the prolamine fraction appeared similar to those of zein protein as both lacked or contained lower levels of these amino acids. Lysine concentration was reported to be high in the albumin and globulin fractions of corn (74). Lysine, arginine, tryptophan and histidine concentrations are higher in low protein content corn which has higher amounts of albumin and globulin (29, 31, and 71).

The hybrid Lindsey Funk 555 (GCD 38) with 12.1% protein had higher concentrations of lysine (2.276), histidine (2.315), arginine (3.950), and glycine (3.328) than other samples of the same hybrid which had respectively 10.9 and 14.8% protein and percentage distribution for lysine of 1.933 and 1.685, histidine, 2.120 and 2.020, arginine, 3.590 and 3.137 and glycine 3.162 and 2.784 respectively. The amino acids aspartic acid, glutamic acid, proline,

alanine, leucine, isoleucine, tyrosine and phenylalanine were also higher
than in the other two samples of this hybrid. The protein solubility
obtained for this sample (GCD 38) indicated that data on the globulin frac-
tion was higher (11.17%) than in the other two samples of this hybrid.
Samples (GCD 38 and MO 38) both had higher values for other amino acids.
The amino acids with highest values in these samples were aspartic acid,
glutamic acid, proline, alanine, isoleucine, leucine, tyrosine and phenylalanine.
These data indicate the globulin fraction may have been richer in basic amino
acids. Globulins had a better distribution of all the essential amino acids
when compared to endosperm meal and the prolamine fraction. The prolamine
fraction was higher in concentrations of glutamic acid, aspartic acid,
proline, leucine and isoleucine (66).

The results of determination of coefficient variance between the distribu-
tion of five essential amino acids--lysine, histidine, arginine, threonine,
and glycine--versus the percentages of the four fractions extracted and protein
content of whole grain are summarized in Table 10. The data represented in
this table reveal significant negative correlations between lysine, histidine,
arginine, threonine and glycine and protein content of grain and between
arginine and glycine and prolamine fractions. These data indicate the con-
centration of lysine, histidine, arginine and glycine decreased as the pro-
tain content of the grain increased; this was similar to the effect noted by
Waggle and Deyoe (85). A high positive significant correlation was found
between the albumin fraction and lysine, histidine, arginine and glycine. A
positive correlation was also found between lysine, arginine and glycine and
the globulin fraction. A negative trend was observed between glutelin fraction

and these essential amino acids. This indicates the concentration of lysine, histidine, arginine and glycine increased as the albumin and globulin fraction increased and as prolamine decreased. Changes in amino acid make-up of opaque-2-mutant maize when compared to normal seed was due to a reduction in the amount of zein relative to other proteins (31). The results obtained with the present study also indicate relationships between the amino acid composition and relative protein fractions of sorghum grain are apparently due to differences in the proteins of sorghum grain as was indicated by Deyoe and Shellenberger (9). Virupaksha and Sastry (66) working with sorghum grain (Indian varieties) showed the globulin fraction had a better distribution of all the essential amino acids when compared to endosperm meal and the prolamine fraction. Concentrations of lysine, arginine, glycine, cystine and methionine in globulin fraction were nearly twice those of the endosperm meal and several times higher than those of prolamine fraction.

Table 10 Correlation Coefficients between Soluble Protein
Fractions and Essential Amino Acid in Sorghum Grain
(18 Samples)

	Protein	Albumin	Globulin	Prolamine	Glutelin
Lysine	-0.718**	0.611**	0.529*	-0.460	-0.284
Histidine	-0.581*	0.558*	0.341	-0.262	-0.317
Arginine	-0.753*	0.694**	0.565*	-0.530*	-0.231
Threonine	-0.552*	0.466	0.380	-0.270	-0.291
Glycine	-0.803**	0.827*	0.624**	-0.689**	-0.170

*Significant at the 5% level.

**Significant at the 1% level.

SUMMARY AND DISCUSSION

Eighteen samples of sorghum grain representing different hybrids and locations in Kansas were selected for protein determinations, fractionation of soluble proteins and amino acid analysis. Sorghum grain proteins were fractionated into four fractions on the basis of their solubility. Solvents used were distilled water, 5% sodium chloride, 80% ethanol + 0.2% sodium acetate and 0.2% sodium hydroxide. Shaking the flour with the solvent in a Reactor-R-Mill was used to extract maximum percentages of protein in a short time.

The preliminary studies conducted indicated (1) a shaking period of 27 minutes with each solvent was adequate to extract maximum soluble proteins, (2) a solvent to flour ratio of 150 ml for 2 grams of flour appeared to remove proteins soluble in each solvent, and (3) particle size was an important factor in extraction of soluble proteins. A particle size which passed through a 100-mesh Tyler sieve yielded good extractions of proteins.

Protein extraction studies indicated that glutelin was the largest protein fraction in low and high protein content sorghum grain. Globulins were smallest of the four fractions studied. Percentage of albumin was higher in low protein content sorghum grain and decreased as the protein content of the grain increased. Prolamine increased with increases in the total protein content of the grain. The increase in prolamine was accompanied by a decrease in albumin content. Globulin decreased with an increase in total protein content of grain, but the decrease was small. A large variation was observed in percentage of glutelin with increases or decreases in total protein content of the grain.

Soluble proteins of sorghum grain were correlated with protein content of the grain. Significant negative correlations ($P < 0.01$) were observed between protein content and percentage of albumin and globulin fractions. Prolamine was positively correlated with protein content. Significant ($P < 0.01$) negative correlations were observed between percentage of prolamine and percentages of albumin and globulin.

Protein content of sorghum grain affected the amino acid distribution in the protein. Variations in amino acid distribution were related to changes in soluble protein fractions. Lysine, histidine, arginine and glycine values were higher (percentage of protein) in low protein content samples. The distribution of these amino acids in sorghum grain was negatively correlated with the protein content of the grain.

Comparison of amino acid data with those of protein content of grain and percentages of soluble protein fractiosn indicated a negative relation between lysine, histidine, arginine, glycine and protein content of the grain. Positive correlation was found between these amino acids and albumin content and between lysine, histidine, arginine, and glycine and the globulin fraction. A negative trend was observed between these amino acids and the prolamine fraction.

ACKNOWLEDGEMENT

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SOLUBILITY OF PROTEINS IN SORGHUM GRAIN

by

MIR SHAMSUDDIN

B. V. Sc. and A. H., 1962

Osmania University Hyderabad, A. P. India

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AN ABSTRACT OF A MASTER'S THESIS

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KANSAS STATE UNIVERSITY
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Eighteen samples of sorghum hybrids were obtained from different locations in Kansas. Crude protein content of these samples was estimated by a micro Kjeldahl procedure. Protein content of these samples ranged from 8.0 to 14.8%. Four fractions of soluble proteins were extracted at room temperature. Albumin, globulin, prolamine and glutelin were extracted with distilled water, 5% sodium chloride, 80% ethanol + 0.2% sodium acetate and 0.2% sodium hydroxide solutions, respectively. Protein recovered in these four fractions was determined by a micro Kjeldahl procedure. The amino acid composition of the whole grain was estimated with an amino acid autoanalyzer. The amino acids determined were lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, tyrosine, half cystine, valine, methionine, isoleucine, leucine and phenylalanine. The amino acid values were reported as grams of amino acid per hundred grams of protein.

Preliminary studies conducted while developing an extraction method served to establish (1) a shaking time of 27 minutes as best for extraction of soluble proteins in each solvent and (2) a solvent to sample ratio of 150 ml for 2 grams of flour for removal of proteins soluble in each solvent. Particle size was found to be an important factor in extraction of soluble proteins, and a reduction of particle size to a finer form increased the solubilization. Passing through a 100-mesh Tyler sieve appeared to be a satisfactory particle size from which to remove maximum amounts of soluble proteins.

Studies on the four soluble fractions showed that glutelin was the largest fraction of the four soluble fractions in the sorghum grain. Globulin was the

smallest fraction. The albumin fractions were high in samples of low pro-
tein content and decreased with increases in the protein content of the
grain. Prolamine increased with increases in protein content of the grain
and the increased prolamine was accompanied by a decrease in percentage of
albumin. The globulin fraction decreased as the protein content of the
grain increased. Large varieties were observed in glutelin content with an
increase or decrease in protein content.

Variations were also observed in the amino acid composition of these
samples. Comparison of amino acid values and protein content of grain and
soluble protein fractions indicated lysine, histidine, arginine and glycine
values were higher in sorghum grain of low protein content and in sorghum
varieties having higher percentages of albumin and globulin. These amino
acids seemed to decrease in percentage with the increase in percentage of
the prolamine fraction.