

DISTILLERS DRIED GRAINS WITH SOLUBLES:
SEPARATION OF HIGH PROTEIN FRACTIONS AND
EVALUATION OF PROTEIN CHARACTERISTICS

by

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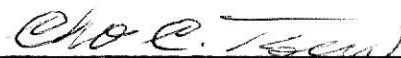
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This thesis is dedicated,
with love and gratitude,
to my Mother and Father.

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INTRODUCTION

Distillers feeds are by-products of the fermentation and distillation of cereal grains used in the production of ethanol for beverage or industrial purposes. Various grains and mixtures of grains, such as corn, wheat, milo, rye, barley and rice, are used as the carbohydrate source for yeast fermentation, with corn being used most extensively (Stark 1977). The distilling industry uses approximately one percent of all the corn produced in the U. S. (Anon 1970a). In 1981, 500,000 tons of distillers feeds were produced (Way 1982).

After fermentation and distillation are completed, the wet stillage is removed and further processed into distillers feeds. There are several different types of distillers feeds, depending on the method used to produce them. The grain portion of the stillage can be dried along with the thin stillage (liquid portion) yielding distillers dried grains with solubles (DDGS). Alternatively, the two portions can be processed and dried separately; the coarse grains and thin stillage producing distillers dried grains (DDG) and distillers dried solubles (DDS), respectively.

Distillers feeds are a rich source of protein and other nutrients. Since most of the starch has been converted to simple sugars and fermented to CO_2 and alcohol, the remaining constituents become concentrated by as much as three times (Tsen 1982b). Presently, essentially all distillers grains produced are used for animal feeding in either the wet or dry form. Many studies have been done on the feeding of distillery by-products to animals, such as beef cattle (Klopfenstein 1976), dairy cattle (Loosli and Warner 1957, Warner 1970), sheep (El Hag 1969), swine (Wahlstrom et al. 1970, Synold 1944), and poultry (Sloan 1941, Dixon and Couch 1970).

As yet, there has been no commercial usage of distillers grains in

human foods. But because of its high nutrient content, DDG is a possible source of protein and fiber for enrichment and/or fortification of human foods. Several studies have already been done with this concept in mind (Satterlee 1976, Wu and Stringfellow 1981, Tsen 1982, Tsen et al. 1982).

One of the problems associated with the protein in DDG is its relatively poor quality. Although protein content is usually 25 - 35%, it is not always of great value. Ranhotra (1982) analyzed several DDG samples and found that most had very low PER values of .50 - .65, compared to 2.50 of casein. He attributed the low PER to the unavailability of lysine.

Tsen (1982) reported PER and apparent digestibility values of three DDGS samples to be 1.30 and 80.0%, 0.60 and 64.8%, and 0.09 and 62.2%, respectively. When the first sample was heated at 140°C for 3 hours, the PER decreased to -0.33 and the digestibility fell to 50.2%. He concluded that nutrient quality was dependent on raw materials, as well as on the processing methods used, especially the drying step. The heat during drying rapidly destroys the nutritive value of the DDGS (Tsen 1982). It is believed that the heat process caused Maillard reactions to occur, severely decreasing the availability of lysine, the limiting amino acid.

This study was divided into two parts. The purpose of the first experiment was to try to obtain a high protein DDGS product by dry milling and fractionation methods. Modifications were made to the process of Wu and Stringfellow (1981) in an attempt to improve protein and yield percentages obtained. The product would be more concentrated in protein, thus of more value as a food supplement. In addition, less DDGS product would be required to get the desired protein increase; thus, the detrimental effects on the product, such as color and flavor changes, volume decreases, etc., would be minimized.

The second part of the study was directed at the causes of the poor quality

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protein found in the DDGS. The effects of the heating (drying) process on the proteins in DDGS was analyzed to better understand the reasons for the great decrease in nutritional quality due to heating. Several aspects were examined, including total lysine and available lysine, as well as protein solubility changes and protein structure changes.

The amino acid lysine is the limiting essential amino acid in corn DDGS, as well as in most grains; thus, protein quality is dependent upon the lysine available to the digestive process. Unfortunately, due to the epsilon amino group, lysine is very susceptible to loss due to Maillard browning reactions, as well as to crosslink formation with other amino acids if the heating is severe enough.

The initial Maillard reaction is a condensation between the epsilon amino group of the lysine with the carbonyl group of a reducing sugar (or aldehyde from oxidized fat breakdown). After a series of reactions, the deoxyketosyl, or Amadori compound, is formed. This compound then proceeds through a very complex system of reactions, which ultimately produce brown colored pigments and flavor compounds. Detailed descriptions of the Maillard reaction can be found in Hodge (1953), Dworschak (1980), and Mauron (1981). Possible crosslink formations of lysine and other amino acids are elucidated by Dworschak (1980).

The early Maillard reaction products, such as the Amadori compound will, upon acid hydrolysis, yield lysine. Thus, lysine will still be recoverable and determined as total lysine. But very little, if any, of the lysine in this form is actually available (Hurrell and Carpenter 1981). Thus, total lysine determination is not a good indication of protein damage. Therefore, a method of determining available lysine should be used. There are several methods in use for this purpose, and many accounts of their application (Pomeranz and Miller, 1963, Blom et al. 1967, Finley and Friedmann 1973, Hurrell

and Carpenter 1975, and Hurrel and Carpenter 1981). Most are based on the reactivity of the unbound epsilon amino group of lysine.

The DDGS samples in this study were analyzed for available lysine to get an indication of protein quality damage. The Udy-Carpenter dye binding method for available lysine was used because of its simplicity and short analysis time. The method is based on the complexing of the Acid Orange 12 dye with the basic amino acids, arginine, histidine, and lysine. Amount of dye bound to a known sample weight is determined before and after reaction with propionic anhydride, which blocks any available lysine groups. The difference between the two readings, then, is considered to be due to the amount of available lysine present. When using this method though, the term "reactive" lysine is preferred over "available" lysine, since some lysine with a free epsilon amino group is not actually available, yet will bind the dye. In addition, some lysine units with a bound epsilon amino group may still be available, (Hurrel and Carpenter 1981). Nevertheless, it is believed that this method gives a good indication of heat damage to food (Hurrel and Carpenter 1975).

The proteins in DDGS were subjected to the alkaline extraction procedure of Wu and Sexson (1976a) to obtain a protein concentrate. The purpose of this was two-fold. First, the amount of soluble protein is an indication of protein denaturation. The more severely a protein is heated and denatured, the less soluble a protein will be. Secondly, these proteins were put through a gel filtration column to separate the proteins into several peaks according to the molecular size. With increased heating of the DDGS, the effect on the protein separation was noted.

REVIEW OF LITERATURE

Processes of Ethanol Production

The following is a brief outline of ethanol production from grain sources. More detailed descriptions can be found in Stark (1954), Simpson (1977), and Lyons and Rose (1977).

Stark (1954) lists the main process steps in the production of ethanol from grain.

- 1) milling
- 2) cooking
- 3) conversion of starch
- 4) fermentation
- 5) distillation
- 6) recovery of grain residues

Most modern distilling operations utilize a continuous process.

1) Milling

The grain is first milled to reduce the particle size to assist in hydration (Stark 1954). Roller, hammer, or attrition mills can be used. A minimum amount of flour should be produced, since it will burn before the larger particles are gelatinized.

2) Cooking

The purpose of the cooking step is to thoroughly gelatinize the starch to permit enzymatic hydrolysis by microbial or barley malt enzymes (Lyons and Rose 1977). Most distilleries now utilize a continuous cooking process. The advantages of continuous cooking over a batch process are more uniform cooking,

automatic control, uniform power requirements, lower net steam consumption, and lower capital investments (Stark 1954). The continuous cooker consists of a wide bore stainless steel tube into which steam is injected. The mash is pumped through the tube under pressure. Simpson (1977) reports that in a continuous cooking system, a 10 minute holding time at 150°C and 65 p.s.i. is necessary for proper gelatinization of corn starch.

3) Conversion

After cooking, the mash is cooled to below 65°C , and enzymes are added to bring about saccharification of the starch to fermentable sugars. Industrially produced enzymes, alpha-amylase and amyloglucosidase may be used, or malted barley can be added as the source of enzymes (Simpson 1977). In the latter, alpha-amylase and beta-amylase hydrolyze the starch. The pH of the mash must be above 4.0 for maximum enzyme activity (Simpson 1977). The system reaches equilibrium when the maltose content approaches 60 - 70%, taking approximately two minutes. The remaining carbohydrates are limit dextrins. Limit dextrinase continues to act on the dextrins to produce simple sugars during fermentation.

4) Fermentation

After cooling to below 33°C and adjusting the pH to 4.8 - 5.0 (Stark 1954), an inoculum of Saccharomyces cerevisiae is added to give a concentration of 5 - 20 million cells/ml in the mash (Lyons and Rose 1977). It is very important to maintain the temperature below 33°C for optimum yeast growth. Maximum yeast activity occurs within 12 - 30 hrs., but fermentation continues due to action of limit dextrinase. Fermentation is complete within 40 - 60 hrs. By then, the specific gravity is below 1.000, and the alcohol content is 6 - 10% (Simpson 1977).

5) Distillation

Separation and concentration of alcohol from the mash mixture are possible because of the difference in volatility between alcohol and water. This process is known as rectification (Simpson 1977). In a modern continuous still, up to five columns may be utilized in a series to efficiently produce a highly purified and concentrated distillate. Fermented mash or beer is pumped into the upper section of the still and direct steam enters the bottom. The steam passing up the column strips the alcohol from the beer as it flows downward and across each plate. The product stream proceeds to a series of columns which are designed to remove impurities such as fusel oils, and aldehydes and esters. The final column further concentrates the alcohol to 85 - 86% (Stark 1954).

6) By-Product Recovery

There are essentially two incentives to encourage distilleries to recover the by-products (Simpson 1977). First, their recovery increases economic return. Distillers feeds are valuable feed supplements. Second, distillers must meet anti-pollution regulations.

Wet stillage is removed from the still at about 6 - 8% solids (Stark 1954). Thus, the recovery of usable distillers feeds requires removal of large amounts of water.

Cooley (1938) and Stark (1954) give detailed descriptions of processes used to recover distillers by-product. Wet stillage is first passed over a screen with 1.0 mm holes, producing thin stillage (3.6% solids) and thick stillage (20% solids). The thick stillage feeds into a rotary press which removes liquid (2.8 - 3.1% solids) producing solids at about 40% water.

The thin stillage may then be centrifuged to remove more solids, giving a

liquid at 3.2% solids. The liquid stillage is run through a series of multiple effect evaporators which produce a syrup of 25% (Cooley 1938) to 40 - 50% solids (Stark 1954).

At this point, the syrup may be recombined with the press cake and dried in rotary driers to 10 - 12% moisture, producing distillers dried grains with solubles (DDGS). Conversely, each component may be dried separately, yielding distillers dried grains (DDG), and distillers dried solubles (DDS). The distillers feeds must be dried to prevent spoilage by anaerobic and aerobic organisms during transport and storage. The yield of total by-product per bushel of grain mixture is usually at 16.5 - 17.0 lbs., of which 8 - 9 lbs. are dried grains, and 8.0 - 8.5 lbs. are dried solubles (Bauernfeind et al. 1944a).

Analysis of Nutrient Composition of Distillers Feeds

The first extensive work done to evaluate the nutritional composition of distillers grains was performed by a group of researchers with Hiram Walker and Sons, Inc. in the early and mid 1940's (Boruff et al. 1940, Bauerenfeind and Boruff 1944, Bauerenfeind et al. 1944a, Bauerenfeind et al. 1944b, Baumgarten et al. 1945, Baumgarten et al. 1946, Boruff 1947). Analysis was run primarily on corn based by-products, but distillers grains from wheat, wheat flour, rye, and barley malt were also investigated. Proximate analyses were determined, as well as amino acid, vitamin, and mineral contents of DDG, DDS, and DDGS.

Boruff (1940) determined vitamin A, D, B (U.I./g) and riboflavin (mg/g) contents of distillers feeds by chick feeding assay methods. Results showed that DDS and DDGS were good sources of the water soluble B complex, as well as riboflavin. Conversely, DDG, without solubles, were low in water soluble vitamins. Obviously, the water soluble vitamins become part of the thin stillage after the screening step of by-product recovery. All fractions were

found to be low in the fat soluble vitamins A and D.

Bauerenfeind et al. (1944a) compared analyses of the three types of by-product (DDG, DDS, and DDGS). Their results are given in Table 1. DDS and DDG varied considerably in fiber and ash contents due to insolubility of fiber and solubility of ash. As would be expected, DDGS was intermediate between DDG and DDS in all cases. It was also found that all three distillery by-products had appreciable mineral content. DDS, though, was highest in total ash.

In another study, Bauerenfeind et al. (1944b) compared the proximate analyses, mineral, and vitamin compositions of fermentation by-products of wheat, wheat flour, and rye with those of corn. Results after different cooking temperatures were also analyzed. Corn, because of a higher percent of insoluble proteins, gave DDS lower in protein and DDG higher in protein content than the other three grains tested. In all cases, with increased cooking temperature, more protein was found in the DDG, and less in the DDS. Once the protein is precipitated, it is retained on the coarse grain residues as they pass over the screen.

The authors also showed that there was some loss of thiamine during the heat processes. Conversely, a possible synthesis of pantothenic acid and riboflavin by yeast may have taken place.

The amino acid contents of grain fermentation by-products from corn, wheat, flour, rye, and barley malt were determined by Baumgarten et al. (1945) using microbiological assays. The values were compared to the amino acid composition of the respective raw ingredients. It was concluded that the protein of the raw material does not undergo any extensive changes with regard to amino acid composition. Thus, the amino acid composition of the distillers grain can be predicted with a fair degree of accuracy from the composition of the mash bill. Also, the distribution of amino acids in the different products (DDG, DDS, DDGS) was nearly the same. When products from the different mash bills were compared,

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Table 1
Proximate Analyses of Corn Distillers By-Products^a

By-Product	Protein (%)	Fat (%)	Fiber (%)	Ash (%)
DDS	27	7	1	8
DDG	30	10	12	2
DDGS	29	9	7	4

^aBaurenfeind et al 1944a

only a slight variation was observed in amino acid content.

In order to obtain reliable values for the nutrient composition of distillers grains, the Distillers Feed Research Council (DFRC) conducted a three-year survey of analytical assays performed by distillery companies and commercial laboratories (Carpenter 1970). Final results were derived from hundreds of individual values and are assumed to represent the typical composition of a product from a corn mash bill. The report lists values for moisture, protein, fat, fiber, and ash, as well as eighteen amino acids, linoleic and linolenic acids, eleven vitamins, and eighteen minerals. DDG, DDS and DDGS were all analyzed.

Ranhotra et al (1982) investigated five DDG samples for nutritional composition and protein efficiency ratio (PER). Four samples were from corn mash bills; one sample was from a 100% milo mash. Compositions of all five were essentially the same, except that the milo sample was higher in protein (34.9% vs approximately 27% for the corn samples), and slightly lower in dietary fiber (29.1% vs. 32.4 - 35.8% for corn samples). Overall, the five analyses were similar to the DFRC study (Anon 1970b).

Since the recovered amino acid nitrogen was appreciably lower than the Kjeldahl values, Ranhotra (1982) suggested that non-protein nitrogenous compounds, most likely from yeast, contributed substantially to the total nitrogen content. In addition, except for one sample, the PER values were very poor (.50, .53, .54, .65, and 1.66) in consideration of the protein and lysine values. The author theorizes that this may be a result of the overestimation of protein; or lysine availability may be inhibited by other DDG components, such as tannins or other phenolics.

PER values and digestibilities of DDGS samples were determined by Tsen (1982) (Table 2). It was found that these values can vary considerably between

Table 2
Protein Efficiency Ratios (PER) and Digestibilities of Diets
Containing Casein (Control), Distillers Dried
Grain Flours (F, E, and B) and Heated DDG-F Samples^a

<u>Treatment</u>	<u>PER^b</u>	<u>Digestibility^c</u>
Casein	2.50	91.7
DDGF	1.30	80.0
DDGE	0.60	64.8
DDGB	0.09	62.2
DDGF Δ 1.5 hr	0.02	60.6
DDGF Δ 3.0 hr.	-0.33	50.2

^aTsen (1982)

^badjusted

^capparent

different DDG samples. One sample was heated for 1.5 and 3 hours at 140° C, resulting in severely decreased PER values. The heat rapidly destroyed the protein quality and reduced the nutritive value. Thus, a major reason for the variance in nutritive quality of the different DDGS samples may be due to the amount of heat used to dry the final product.

Wu et al. (1981) broke down base of stillage material into five different fractions and characterized each. The base of still residue (solids) was extracted with a series of solvents to characterize the types of proteins in distillers grain. Base of still residue protein was found to be much less soluble in these solvents than unprocessed corn protein. This decrease is due to heat denaturation during distillation and drying. The authors also concluded that proteolysis of protein occurs during the distillation process, because the amount of low molecular weight proteins found in base of still supernatant (liquid) is greater than that of corn.

Factors Affecting Composition of Distillers Grains

DeBecze (1949) discusses in detail the factors that affect the final composition of DDGS, as well as DDG and DDS. He attempts to demonstrate the mechanism and magnitude of the major factors. All of these factors are brought about either by the raw materials or by the processing methods used in the distillery. Although there are variations in different distillers feeds, variations are minimized in any one plant because equipment, and processing conditions and procedures remain constant (Anon 1970b).

The protein of DDGS is a direct result of the protein content of the starting grains and of the yield of DDGS (lb. recovered/bushel mash) (DeBecze 1949). The final protein content can be predicted by knowing the values of the above factors. Protein content of raw ingredient varies with the type of grain,

variety, quality, and proportion of the different grains in the mash. A 1% variation in protein content of the starting grains can change the final protein content by 3%.

Increased yield of DDGS will bring about a decrease in final protein value due to the dilution effect of non-fermented carbohydrates. For example, if 2/3 of the initial solids are fermented and ultimately removed as alcohol and carbon dioxide, the protein percentage is tripled in the residual solids. Thus, the higher the content of fermentable starch, the greater will be the final protein content.

Of course, all of the fermentable starch is not completely removed. Thus, yield (and ultimately protein content) is greatly affected by what DeBecze (1949) calls "success of fermentation" of the distillery process. In his discussion, he lists the major factors influencing the success of fermentation:

- 1) fineness of grind
- 2) cooking temperature
- 3) converting power of the enzymes used
- 4) fermenting ability of the yeast
- 5) amount and composition of stillage used in mash making
- 6) initial temperature and pH, and their changes during fermentation
- 7) fermentation time

Of all of the above factors, the cooking temperature appears to have the greatest effect on amount of carbohydrate fermented. Higher temperatures result in a greater degree of starch gelatinization, leading to more complete starch conversion. Consequently, fermentation success is higher; thus, final protein content increases.

Protein content of DDG and DDS depends on the previously discussed parameters, as well as several other factors. Essentially, the protein content of

each depends on the protein distribution between the two fractions.

In an attempt to explain this, DeBecze (1949) divided wet stillage into three portions. The first is the quick settling, coarse, undissolved grain residue containing fiber and undissolved proteins (40 - 50% of total stillage). Its protein content of this first portion is greatly dependent on cooking temperature. When the temperature is increased, more protein is denatured and becomes insoluble, thereby increasing the protein content of the coarse portion and of the DDG.

The second portion is the slow separating, finely suspended solids made up of protein, dead yeast, and coagulum (25 - 30% of total). Particle size ranges from a few to 100 microns. The protein content is approximately 50%. The third portion is comprised of the dissolved substances (25 - 30% of total).

He concluded that the protein distribution between DDG and DDS partly depends on the amount of retention of the slow settling and dissolved substance portions on the coarse particles of the DDG. The amount of retention varies with the size, shape and amount of coarse particles, the dilution of the stillage, and the procedure used to separate the stillage. It is understandable then, that whenever protein (or any other component) is high in the DDG portion, it will be low in the DDS. It also appears that distillers feeds cannot be produced with uniform composition, considering the number of factors determining composition, and the intricate mechanisms of these factors.

Potential as a Human Food Source

There is presently no distillers by-product utilized commercially in human foods. But because the grains are rich in protein, fiber, and other nutrients, there has been interest in adding them to food, in either the whole grain form, or as a protein concentrate of the distillers grain.

Satterlee (1976) produced a distillers protein concentrate (DPC) from both

corn and wheat DDG using an alkaline extraction procedure (pH 12.2) to precipitate the protein. He analyzed the DPC for chemical and nutritional properties, as well as several functional characteristics. It was found that the lysine level of the concentrate increased slightly over the starting grains as a result of yeast protein. He compared PER and protein digestibilities of corn protein vs. DPC (1.40 and 78.32 for corn, 1.45 and 78.0 for DPC), and concluded that DPC and the starting corn grain were almost identical in protein quality.

Another approach to obtain a high protein concentrate was studied by Wu and Stringfellow (1981). DDG and DDGS were dry fractionated by first pin-milling, and then sifting the ground material through a series of flour sieves. Samples were run at varying moisture levels and pin-milled one to three times at varying r.p.m.s. Results showed that protein content increased with decreased particle size. Defatting the starting material did not increase protein shift, however increased water content did increase the protein shift. The best results were obtained with DDGS at 21% water and twice pin-milled at 14,000 rpm. After sieving on a 50 mesh screen, the high protein fraction contained 43% protein with a 41% yield.

Tsen (1982) and Tsen et al. (1982) suggest using distillers by-product as a protein and fiber supplement in bakery foods. They used a 15 - 25% replacement of wheat flour with DDG flour in several types of cookies, and also tried 10 - 20% replacement of flour in white bread.

The DDG in the cookies reduced both spread and thickness, although spread ratio increased. The cookies were darker in color; thus, the authors recommend using DDG in chocolate type cookies. The most acceptable product was a light colored DDG in a chocolate chip cookie.

DDG flour in the bread formulation had a weakening effect on dough structure, increasing flour absorption, and decreasing both development and stability times.

The 10% DDG level bread had acceptable volume and grain, but the 20% level loaf was greatly reduced in volume and grain score. Analytical results showed similarities between whole wheat bread and DDG bread, especially the 10% level product. Both were comparable in protein and fat content and in appearance, although the 10% DDG bread was slightly lighter in color. A sensory evaluation test showed that the acceptability of the 10% DDG bread was not significantly different than that of the whole wheat bread.

MATERIALS AND METHODS

Materials

DDGS

The six distillers grains used in the study were obtained from the Distillers Feed Research Council, Cincinnati, Ohio. The four DDGS samples varied in color and particle size characteristics, but all were obtained from original mashes predominately made up of corn. Two additional samples of DDGS-B and DDGS-F, called DDGS-B (1) and DDGS-F (a) were obtained and used in the first part of the study to augment the data. These two samples had similar, but not exact, proximate analyses and characteristics to DDGS-B and DDGS-F.

Proximate compositions are shown in Table 3. The protein contents ranged from 29.1% to 34.1% (dry basis). DDGS-B and DDGS-F were studied most extensively because they represented typical dark colored and light colored DDGS, respectively. DDGS-F and DDGS-F (a) were medium brown in color; DDGS-B and DDGS-B (a), though, were very dark brown, almost black, in appearance. It was assumed that the DDGS-B and DDGS-B (a) had been subjected to more intensive drying conditions during the recovery process than were DDGS-F and DDGS-F (a). DDGS-G and DDGS-E were intermediate in color between the B and F samples.

Corn Meal

The corn meal used was a degermed yellow corn meal, with the composition shown in Table 3.

ANALYTICAL METHODS

Moisture

Moisture of the grain samples was determined in a hot air oven by AACC method 44-15A.

Table 3
Composition of Distillers Dried Grains with
Solubles and Corn Meal^a

Sample	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Fiber (%)	Color (disc #24-#63)
DDGS-B	9.9	28.3	4.9	11.8	12.5	7.5
DDGS-B (a)	10.1	29.1	5.0	11.5	12.8	7.6
DDGS-F	9.8	28.1	4.6	7.4	11.2	62.0
DDGS-F (a)	10.3	29.6	4.7	8.5	10.9	63.5
DDGS-G	7.3	34.1	5.6	8.7	13.0	29.2
DDGS-E	6.5	29.0	5.2	9.6	11.1	16.1
Corn Meal	10.7	8.8	0.47	1.0	5.6	----

^aAll values except moisture are dry basis.

Protein

Crude protein was determined using AACC method 46 - 11. Nitrogen content was multiplied by the factor of 6.25.

Ash

Percent ash was determined using AACC method 08 - 01.

Crude Fat

Fat content was determined with AACC method 30 - 25.

Fiber

Crude fiber was determined by AACC method 34 - 15.

Color

Color, measured as relative spectral reflectance, was determined on an Agtron multi-chromatic reflectance spectrophotometer model M-500A. Standard discs, numbers 24 and 63, were used to standardize the instrument of 0 and 100% reflectance, respectively. The red mode was used for DDGS samples. A higher value indicated a lighter colored sample.

Amino Acid Analysis

Samples were analyzed for total amino acid content on a Dionex D-300 Kit single column amino acid analyzer. P-toluene-sulfonic acid hydrolysis was used in the process.

Moisture Content of Protein Concentrate

The moisture content of the protein concentrate precipitated in the alkaline

extraction method (Wu and Sexson 1976) was determined using an O'Haus infrared moisture determination balance¹. DDGS protein concentrates were dried at 5 watts for 3 minutes, and then at 4.5 watts for 14 minutes to get a constant weight. Corn protein concentrates were dried at 5 watts for 5 minutes, and 4.5 watts for 25 minutes.

EXPERIMENTAL METHODS

Dry Fractionation of DDGS

High protein fractions were obtained using a dry fractionation procedure involving air-separation, pin-milling, and sieving. Figure 1 shows the four processes that were used to separate the distillers grain fractions. One kilogram samples were used in each run. "As-is" moisture was approximately 10%. Moisture levels of some of the samples were adjusted before pin-milling to 15% and 20% levels. Moisture level was increased by adding sufficient water in a tumbling mixer, and tempering overnight in a large plastic bag.

In Process I, the samples were simply ground in a pin-mill² at 14,000 rpm. Milled samples were then sifted³ over 32W, 48W, 70GG, 9XX, and 10XX sieves for five minutes. Opening sizes of these sieves are shown in Table 4. In Process II, samples were first continuously defatted with hexane in a Soxhlet extraction apparatus for 24 hours. The grain was then processed as in I. Processes I and II are analogous to the method of Wu and Stringfellow (1981).

In Processes III and IV, attempts were made to increase yield and protein

¹model no. 6010

²Alpine model 160A

³Smico laboratory sifter model 3-487

Figure 1. Separation Processes of High Protein Fractions
From DDGS Samples

**THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH DIAGRAMS
THAT ARE CROOKED
COMPARED TO THE
REST OF THE
INFORMATION ON
THE PAGE.**

**THIS IS AS
RECEIVED FROM
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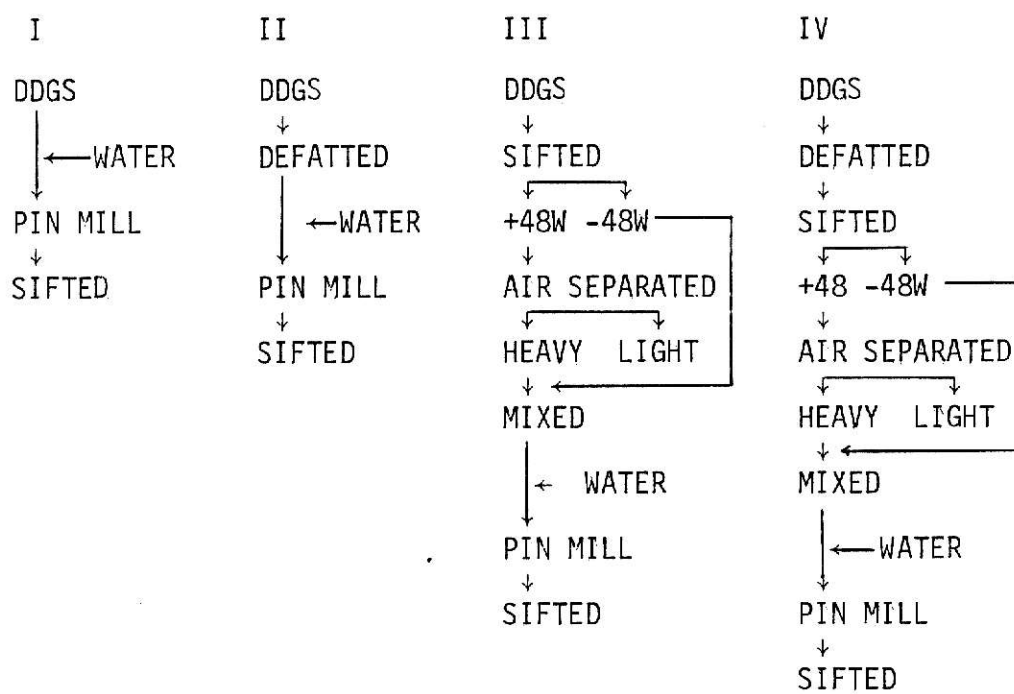


Table 4
Opening Sizes of Sieves Used in Separation
Processes of DDGS

<u>Sieve</u>	<u>Size of Openings (Microns)</u>
32W	566
48W	390
70GG	240
9XX	150
10XX	136

content over the procedure of Wu and Stringfellow (1981). Several modifications were made to the basic procedure. Process III and IV are the same, except for the defatting step in Process IV. DDGS samples were first sifted over a 48W sieve for two minutes. The over 48W fractions were then air-separated in a North Dakota Seed Blower (air flow 0.75 of maximum) to remove a low density, high fiber fraction which was discarded. The remaining over 48W heavy fraction was mixed back with the through 48W material. At this point, the moisture level was adjusted for the 15% and 20% moisture samples. The remaining pin-milling and sifting steps were the same as in Process I and II. Each fraction was collected off the final sieve cloths and weighed. Yield percent of starting material was calculated, and percent protein was determined.

PROTEIN INVESTIGATIONS

Preparation of samples:

DDGS-B and DDGS-F were placed in shallow aluminum pie pans in 100 g quantities, and heated for 30, 60, 120, 180, and 360 minutes at 120°C. Corn meal samples were heated in 100 g quantities for 60, 180, 360, and 720 minutes. Samples were then removed, cooled, and stored in polyethylene bags under refrigeration until needed.

Dye Binding of "Reactive Lysine"

Reactive lysine values were obtained using the Udy-Carpenter Method, available from the U.D. Corporation, Boulder, CO. This procedure measures the amount of chemically available lysine, or lysine that is not complexed due to a heat induced reaction, such as the Maillard reaction. It was used to give an indication of the extent of heat damage incurred by the corn proteins.

Specifically, the method measures the moles of Acid Orange 12 dye bound before and after the available lysine epsilon groups are blocked by propionic anhydride. Several modifications were made in the procedure to accommodate the use of laboratory equipment that was immediately available.

Reagents:

1. Acid Orange 12 (C. I. 15970)

The dye was obtained from Aldrich Chemical Co. (cat. #19,813-7). To obtain 100% purity, the dye was purified according to the procedure of Udy (1971). Dye solution was prepared at 1.32 g/L using acidic buffer.

2. Acidic buffer

10.0 g oxalic acid dihydrate (Fischer #A-218), 60 ml acetic acid (J.T. Baker 3-9507) and 1 ml propionic acid (Fischer #A-258) were dissolved in distilled water, then diluted to 1 liter.

3. Standard solution

1.20 g of purified Acid Orange 12 dye was dissolved in distilled water, then diluted to 1 liter. Proper amounts of this solution were further diluted in test tubes to give concentrations of .50, .55, .60, .65, .70, and .75 g/L as shown in Table 5.

All other reagents were prepared as in the Udy-Carpenter procedure.

Equipment

A laboratory horizontal shaker⁴ and Bausch and Lomb Spectronic 20 spectrophotometer were used in place of the U. D. Corp. React-R-Shaker, and U. D. Corp. Color Computer, respectively.

⁴Eberbach

Table 5
Preparation of Standard Curve Solutions
of Acid Orange 12 Dye

<u>Concentration (g/L)</u>	<u>ml 1.20 g/L Solution</u>	<u>ml Distilled Water</u>
.50	5.0	7.0
.55	5.5	6.5
.60	6.0	6.0
.65	6.5	5.5
.70	7.0	5.0
.75	7.5	4.5

Procedure

Samples were first ground in the Udy Cyclone Sample mill through a 0.5 mm screen. The reagent addition procedure and shaking times are the same as enumerated by Udy-Carpenter.

After reaction and equilibration, 30 drops of solution were filtered through the filter disc and dropper cap, and collected in a small test tube. This solution was then diluted 1.0 ml to 100 ml in a 100 ml flask. Diluted solutions were then read at 480 nm in a 1.0 cm cuvette. Readings were compared to standard solutions read at the same time from a standard curve. Amount of unbound dye remaining in solution could then be determined.

Calculations

Total mmols remaining was determined by converting remaining g/L of dye to mmols/L, and multiplying by total volume (.0453 L). By difference, mmols bound was calculated. Mmoles bound divided by grams of sample gave mmols bound/g sample. The 'B' value was subtracted from the 'A' value, and multiplied by 146.3 (molecular weight of lysine) to give mg reactive lysine/g sample. This was then converted to g reactive lysine/100 g protein for each sample.

Protein Extraction

A protein concentrate was obtained using an alkaline extraction method for corn proteins of Wu and Sexson (1976a). Again, several modifications were made in the procedure. Adequate sample was ground twice in the Udy Cyclone Sample Mill using a 1.0 mm screen, and to maximize percent yield, a 1:10 sample to solvent ratio was used, as opposed to a 1:6 ratio used by Wu and Sexson (1976a).

An 80 g sample of ground material was combined with approximately 600 ml of 0.1 N NaOH, and stirred magnetically. While monitoring with a pH meter,

0.1 N NaOH and 1.0 N NaOH were added in the necessary combination to bring the total volume added to 800 ml, and to a pH of 11.7. The mixture was stirred for 25 minutes, then centrifuged⁵ in 250 ml plastic centrifuge bottles at 3300 x G (7000 r.p.m.) for 15 minutes. The centrifuge was maintained at 5°C. The supernatant was adjusted to pH 4.7 with 6N HCl (1.0N HCl for corn samples), while being stirred magnetically, then centrifuged under the same conditions. The precipitate was collected as protein concentrate. The extraction procedure was repeated on the solids from the first centrifugation, and the precipitate obtained was combined with the precipitate from the first supernation. Protein and moisture contents of each concentrate were determined.

Gel Filtration

A gel filtration procedure was employed to analyze the protein concentrates of DDGS and corn, and to observe any effect of heat on protein components of the grain sample. It was decided to use a 40 cm column, after a 70 cm column was found to be unsatisfactory. It was difficult to obtain a properly poured 70 cm column with good separation capabilities; it also took too long to run each sample. The eluent buffer used was 0.1 M tris hydroxy amino methane (Fisher T-395), with 0.02% sodiumazide (Fisher 5-272) to prevent microbial growth. The pH of the buffer was 10.0 - 10.1.

Column Preparation

Approximately 7.0 - 7.5 g of Sephadex 200 was hydrated at 90°C for 5 hours in buffer solution, then allowed to cool at room temperature overnight. Fines were removed by allowing the gel to settle for several minutes in a 1000 ml

⁵Bechman Model J-21

graduated cylinder, and removing the supernatant solution and suspended fines by aspiration. This was repeated 3 - 4 times, until a rapidly sedimenting preparation was obtained. Gas bubbles were then removed by transferring the gel into a 1000 ml vacuum flask, and aspirating for 4 - 5 hours.

The column used was a 40 cm x 2.6 cm glass column from Pharmacia Fine Chemicals, Inc. It was mounted and checked with a small level, to assure that the column was vertical. A Haake⁶ water circulator pumped water through the outer water jacket at 20°C. A column flow adapter was assembled onto the bottom of the column before pouring the gel.

The columns were then poured all at once using a column extender, and the gel was allowed to settle for several hours. At this point, excess gel was removed by aspiration, and another flow adapter was fitted on the top of the column. The gel bed was permitted to equilibrate by allowing 500 - 600 ml of buffer to flow through the column. The buffer flow was then reversed to ascending, to prevent gel collapse.

An LKB 8300A Uvicord II ultraviolet flow through detector was used at 280 nm to analyze the concentration of peaks after elution. The LKB 6520 Chopper Bar Recorder was used to print out the protein peaks, based on percent transmission of U.V. light. A Buchler Polystaltic⁷ peristaltic pump controlled the flow rate of the buffer.

Approximate molecular weights of the protein fractions were determined using the proteins ferritin (m.w. 440,000), catalase (m.w. 232,000), albumin (m.w. 67,000), and chymotrypsinogen A (m.w. 25,000). The standard proteins were obtained from Pharmacia Fine Chemicals.

Sample Preparation

Two methods of sample preparation were used in this study. A 5 ml sample

⁶Model FK2

⁷Model #2-6100

was used on all runs.

Method I

This method was used for the DDGS concentrates. Samples was suspended in 20 ml buffer in an 8 dram vial by swirling on a vortex mixer for 2 - 3 minutes. After the appropriate amount of DDGS-F concentrate was found that gave adequate absorption peaks after elution from the column, the solution was then measured at 280 nm (1 - 50 dilution) in a Varian Techtron U.V. - Visible spectrophotometer⁸. For each succeeding sample, an amount was used that gave the same absorption after suspension in the buffer.

Method II

This technique was employed when it was found that the corn protein concentrate would not readily disperse in the buffer solution. Adequate sample (to give approximately .375 g protein) was weighed and combined with 100 ml buffer in a one pint blender jar. The blender was run at low speed for 15 minutes. The solution was then centrifuged⁹ in 40 ml plastic centrifuge tubes at 2000 rpm (480 x G) for 3 minutes. The supernatant was then used as the sample liquid. This method also gave an indication of change in solubility of the protein.

RESULTS AND DISCUSSION

Dry Fractionation of DDGS Samples

Table 6 shows the original particle size distributions and protein contents of the DDGS samples used. Particle sizes of DDGS-B and DDGS-B (a) were larger than those of DDGS-F and DDGS-F (a). In addition, DDGS-B (a) were

⁸Model 635

⁹Sorvall Model SS-3

Table 6
Particle-Size Distributions and Protein Contents
of DDGS^a

Sieve-Size	DDGS-B		DDGS-B (a)		DDGS-F		DDGS-F (a)	
	Yield (%)	Protein (%)	Yield (%)	Protein (%)	Yield (%)	Protein (%)	Yield (%)	Protein (%)
Over 20W	47.4	25.7	46.3	30.0	25.3	27.1	33.5	28.8
20W-32W	28.6	28.2	27.9	28.2	20.1	28.1	24.4	29.6
32W-48W	18.5	29.9	19.0	28.1	30.1	27.9	28.4	30.0
48W-70GG	3.4	31.7	5.4	34.8	19.2	30.2	8.6	32.3
70GG-10XX	2.1	35.9	1.4	40.9	5.3	33.5	5.1	33.1

^aDry Basis

similar, but DDGS-F was slightly larger than DDGS-F (a) in particle size distribution. Overall, as particle size decreased, protein percentage increased.

DDGS samples were processed and sifted by the four different methods for the purpose of obtaining fractions that were more concentrated in protein, and therefore more valuable as a protein source. In addition, for practicality reasons, it was desirable to obtain as high a yield as possible. Unfortunately, one of the two usually must be sacrificed to attain the other. Thus, besides analyzing protein percent or yield percent alone, in order to assess the worth of each process or fraction, percent protein was multiplied by percent yield of starting material to obtain what was designated as the "protein - yield index". This simply gives total protein content of each fraction. When graphed it gives an indication of the efficiency of the process compared to simply milling and sifting (Process I), in producing high protein (HP) fractions at high yields. In the following discussion of results, high protein, or HP fractions, will refer to the 70GG - 9XX, 9XX - 10XX, and <10XX fractions, the three smallest particle size fractions obtained. This is an arbitrary selection, used only for comparison of the different processes and moisture levels. Some processes did result in relatively high protein values in fractions of larger particle size than those designated as HP fractions; these would also be valuable as a protein source.

DDGS-B

Results of DDGS-B fractionation are shown in Table 7 and Figures 2-7. At "as is" moisture, protein content at 38.2% was achieved by Process I in the <10XX fractions. The yields of the HP fractions were rather low. Improvements in yield and/or protein percent were obtained by utilizing Processes II, III, and IV, and water addition. As can be seen in Table 7 and Figures 2 and 3,

Table 7
Yields^a and Protein Contents^b of Sifted Fractions
From DDGS-B by Each Process

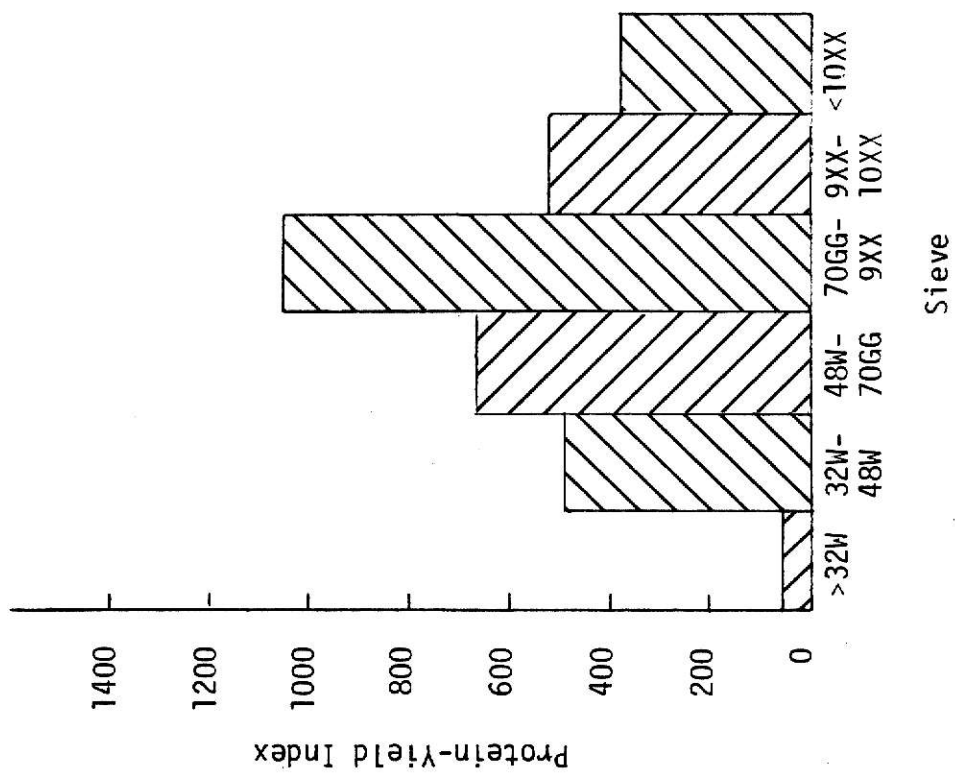
Process	Sieve-Size	Moisture Levels (%)					
		"as is"		15.0		20.0	
		Yield (%)	Protein (%)	Yield (%)	Protein (%)	Yield (%)	Protein (%)
I	Over 32W	5.2	18.5	12.7	12.8	24.2	15.5
	32W-48W	23.2	22.3	25.4	20.0	26.3	27.6
	48W-70GG	22.5	35.5	24.0	35.0	24.2	38.0
	70GG-9XX	33.2	36.8	28.3	40.5	23.8	41.9
	9XX-10XX	13.9	37.8	9.1	39.4	1.5	43.2
	Thru 10XX	2.0	38.2	0.5	41.2	0	----
II	Over 32W	3.7	15.8	8.1	16.2	25.4	18.1
	32W-48W	24.0	20.5	25.9	22.4	29.9	32.5
	48W-70GG	20.1	33.3	21.1	34.6	16.7	42.9
	70GG-9XX	24.4	43.0	23.1	44.5	11.8	46.4
	9XX-10XX	11.7	44.2	10.1	46.7	6.5	47.8
	Thru 10XX	8.1	47.3	3.7	47.1	1.7	49.7
III	Over 32W	2.5	17.1	7.6	15.1	17.6	15.2
	32W-48W	11.3	23.4	12.2	22.7	18.1	26.3
	48W-70GG	20.7	34.8	22.2	35.1	18.8	38.2
	70GG-9XX	29.8	37.2	25.8	40.8	22.5	42.7
	9XX-10XX	12.9	39.9	11.8	41.2	3.0	43.5
	Thru 10XX	2.8	40.1	0.8	42.5	0	----
IV	Over 32W	1.0	19.5	7.2	18.3	9.3	20.5
	32W-48W	9.2	25.1	27.8	21.8	25.0	34.2
	48W-70GG	14.1	35.8	17.5	39.5	21.2	43.5
	70GG-9XX	24.0	42.6	12.4	43.5	12.1	46.3
	9XX-10XX	14.6	46.1	7.7	47.3	6.0	47.9
	Thru 10XX	11.6	48.3	1.9	48.2	0.9	50.3

^aYields indicate percent against starting material.

^bData are on a dry basis.

Figure 2
Protein-Yield Index of DDGS-B at "As-is"
Moisture. Processes I and II

II



I

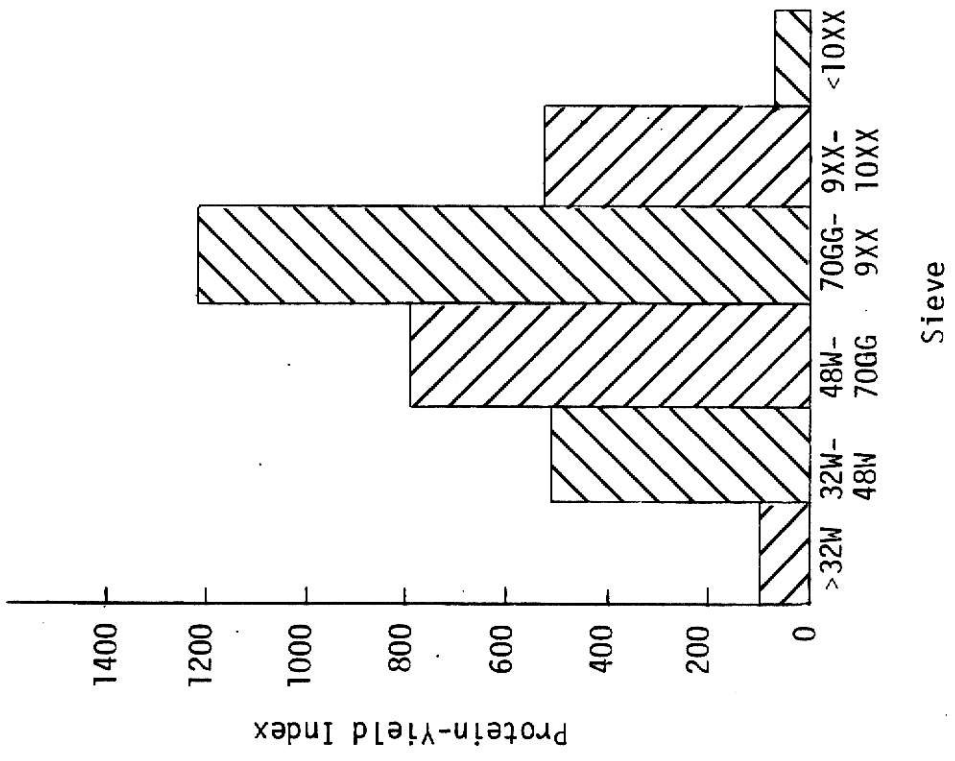


Figure 3
Protein-Yield Index of DDGS-B "As is"
Moisture. Processes III and IV

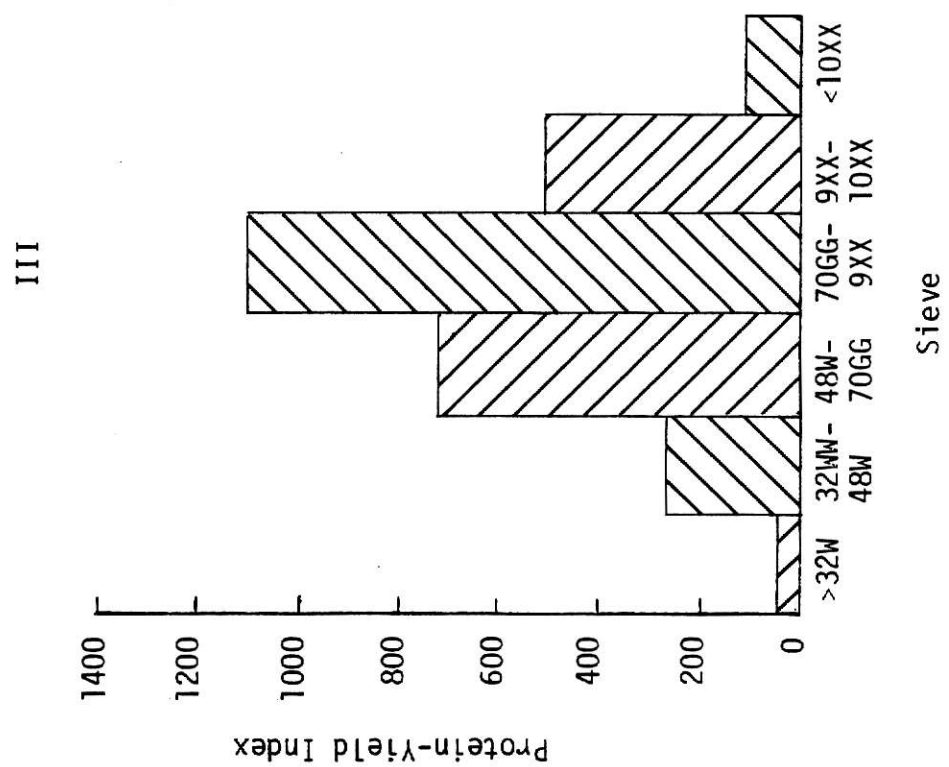
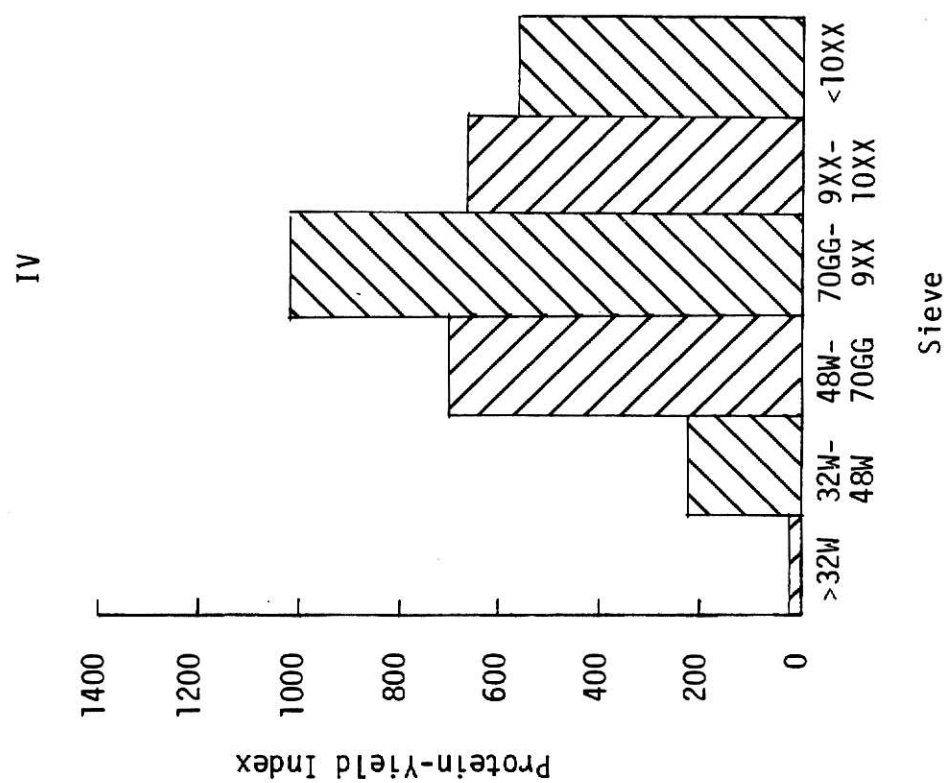


Figure 4
Protein-Yield Index of DDGS-B at 15% Moisture
Processes I and II

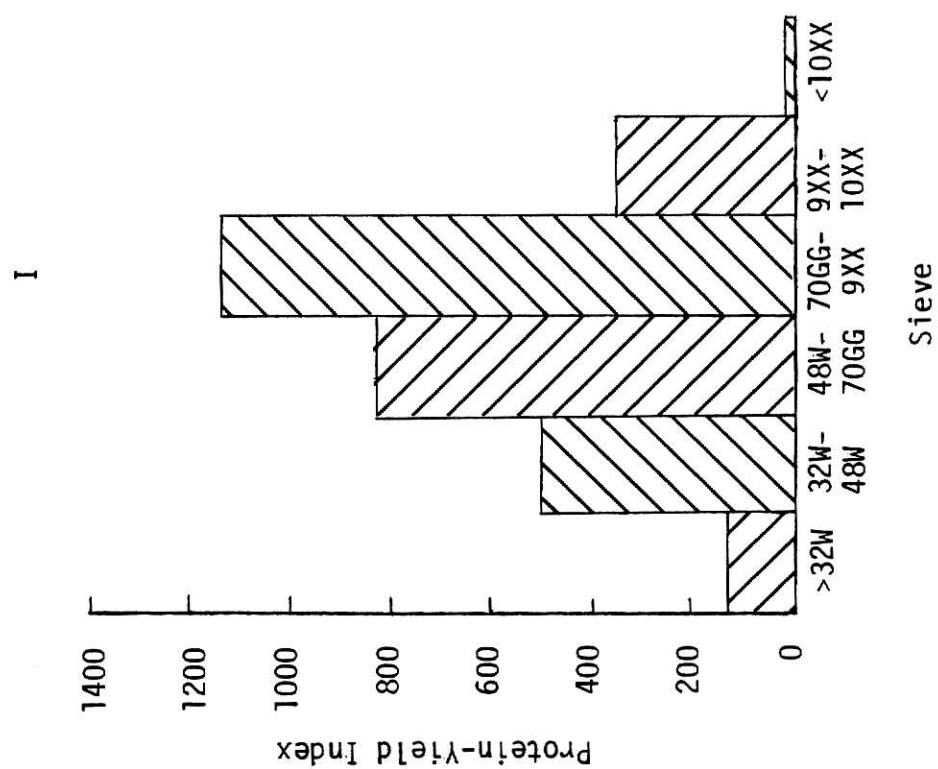
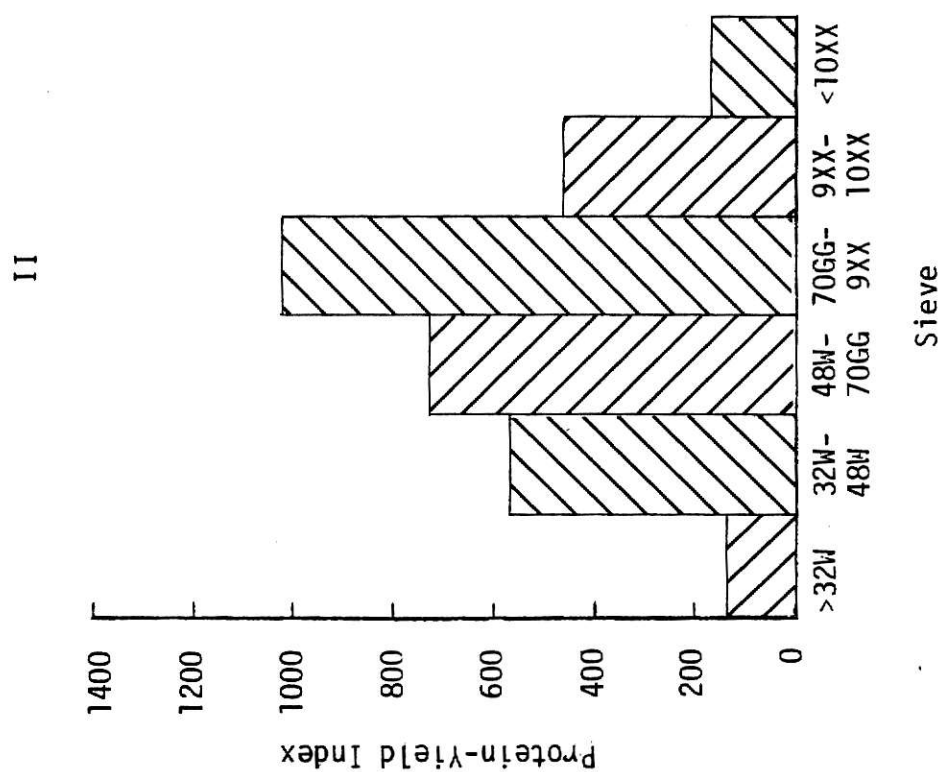


Figure 5
Protein-Yield Index of DDGS-B at 15% Moisture
Processes III and IV

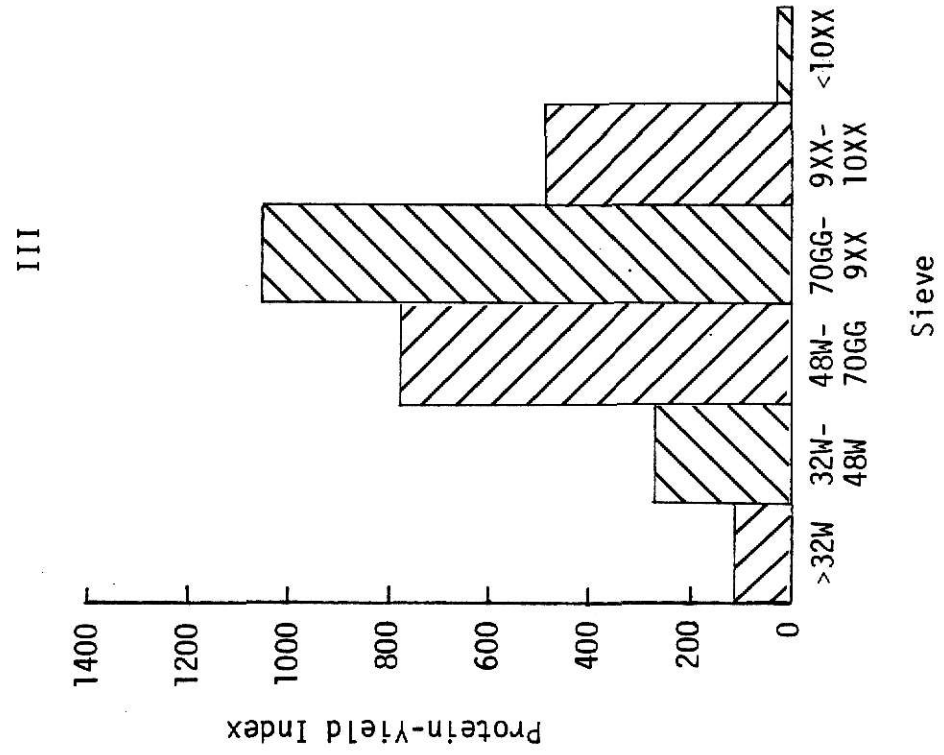
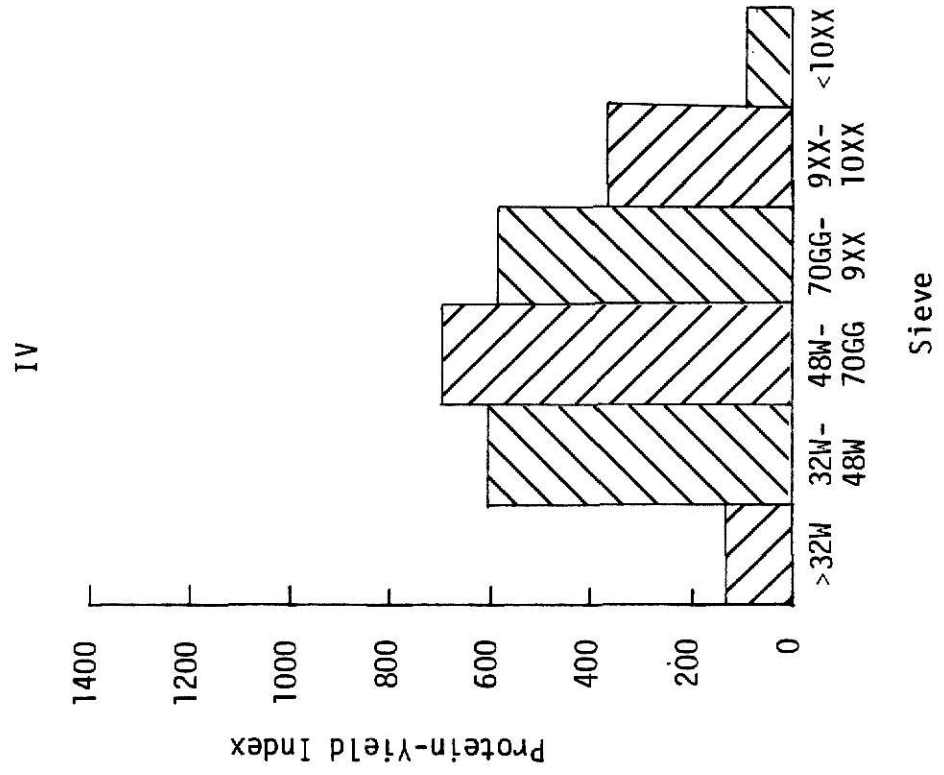


Figure 6
Protein-Yield Index of DDGS-B at 20% Moisture
Processes I and II

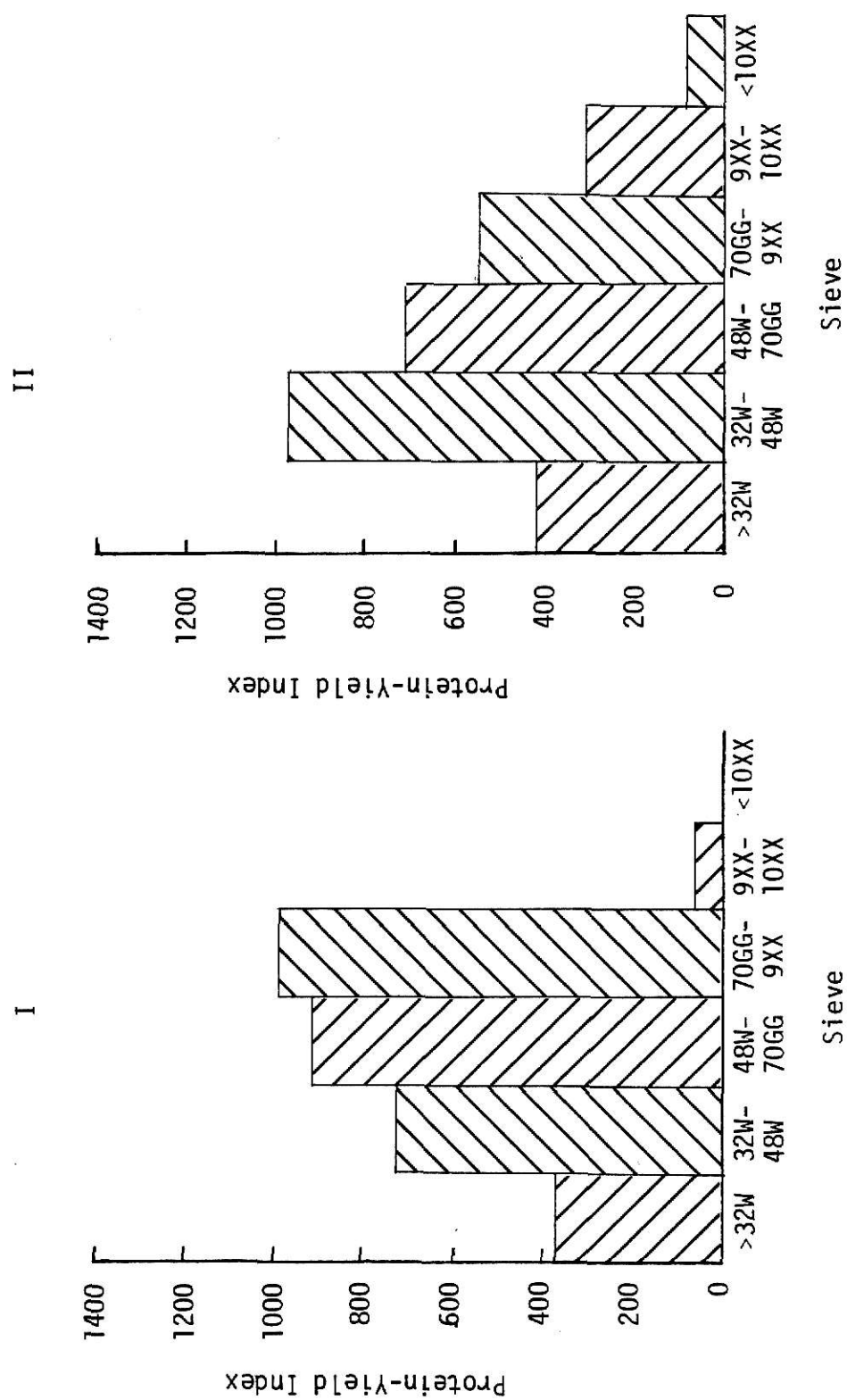
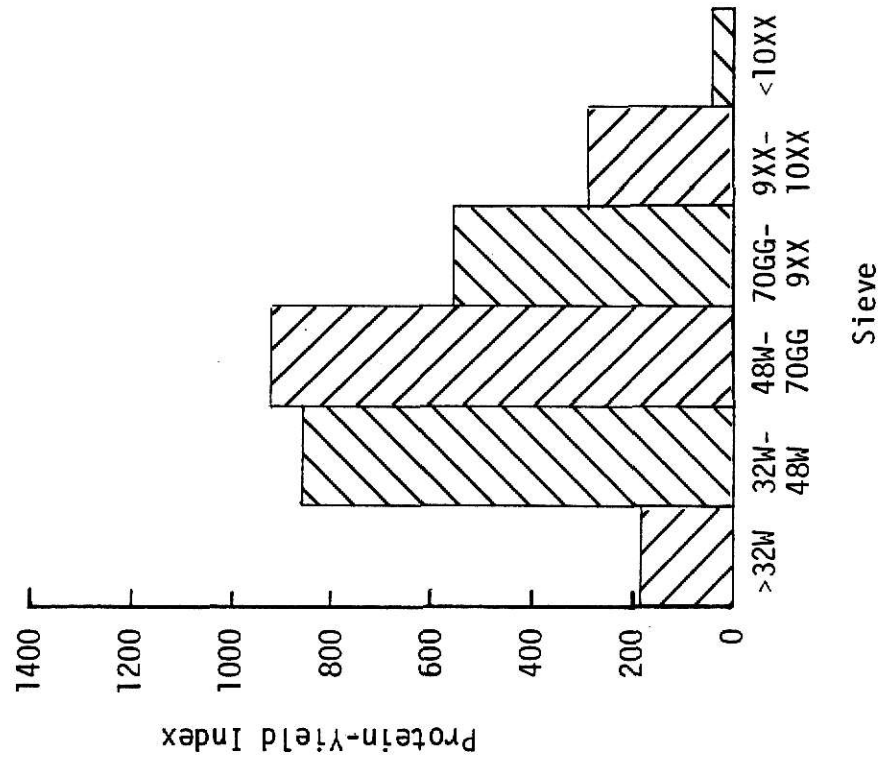
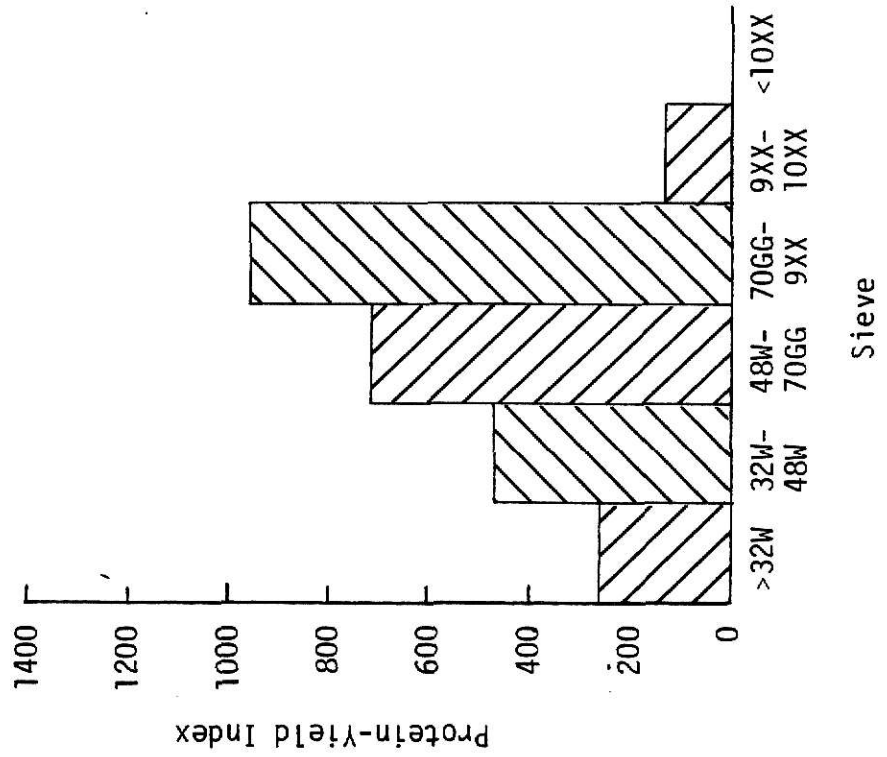


Figure 7
Protein-Yield Index of DDGS-B at 20% Moisture
Processes III and IV

IV



III



the most successful method at "as is" moisture as indicated by protein-yield index was achieved with Process IV. The graphs clearly show a shift, from Process I to IV, toward the two smaller fractions, indicating a favorable concentration of protein at a relatively high yield. Yield and protein values for the HP fractions of Process IV were 24.0% and 42.6%, 14.6% and 46.1%, and 11.6% and 48.3%, respectively. It is apparent from Table 7 that the increase in protein-yield index is a result of increases appear more significant.

Water addition to 15% and 20% moisture contents before pin-milling increased protein contents of the HP fractions, but greatly decreased their yield values. For example, Process IV at 20% moisture gave a 50.3% protein product in the <10XX fraction, but yield was only 0.9%. It is also evident when comparing "as is" moisture with the 15% and 20% levels (Figures 2 and 3 vs 4, 5, 6, and 7), that the defatting and air-separation procedures had much less effect on increasing protein or yield percent when moisture was increased. In fact, at the 15% and 20% levels of DDGS-B, Process IV is not superior to Process II, as it was at "as is" moisture.

DDGS-F

DDGS-F separation results are shown in Table 8 and in Figures 8-13. Overall, the protein values of the HP fractions were lower than the corresponding DDGS-B fractions, even though both products initially had nearly equal contents. In each case at "as is" and 15% moisture, protein content was less than 40%, while several values at 20% water reached just over 40%. These lower protein results are thought to be due to the smaller initial particle size of DDGS-F compared to DDGS-B. For this reason, air separation of particles by density was not as effective because density differences were less. Also, DDGS-F contained less fat than DDGS-B (7.4 vs 11.8); thus removal of fat from DDGS-F

Table 8
Yields^a and Protein Contents^b of Sifted Fractions
From DDGS-F by Each Process

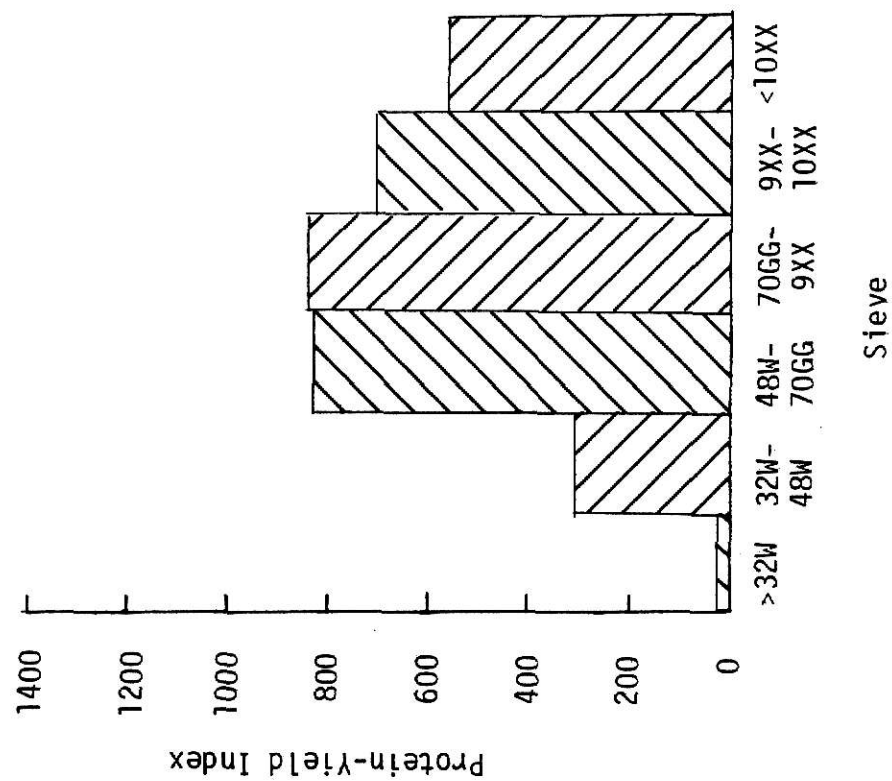
Process	Sieve-Size	Moisture Levels (%)					
		"as is"		15.0		20.0	
		Yield (%)	Protein (%)	Yield (%)	Protein (%)	Yield (%)	Protein (%)
I	Over 32W	2.0	22.7	3.0	19.6	8.5	21.5
	32W-48W	19.4	24.3	25.6	25.1	34.2	28.9
	48W-70GG	32.7	31.1	34.0	33.1	30.2	35.1
	70GG-9XX	20.1	34.2	26.1	37.0	23.4	36.3
	9XX-10XX	17.1	34.5	8.9	36.0	3.7	35.8
	Thru 10XX	8.7	33.4	2.4	36.5	0	----
II	Over 32W	1.1	24.1	1.8	26.2	6.4	24.5
	32W-48W	12.0	25.9	21.8	27.2	37.7	30.5
	48W-70GG	26.9	31.1	32.1	34.8	27.9	37.9
	70GG-9XX	22.3	37.7	19.7	39.3	14.7	40.5
	9XX-10XX	18.3	38.8	11.4	40.5	6.4	40.5
	Thru 10XX	14.3	39.2	8.3	39.2	1.9	4.1
III	Over 32W	0.4	20.1	2.0	19.4	6.5	22.6
	32W-48W	17.6	28.9	18.5	29.5	24.7	33.7
	48W-70GG	30.2	34.4	28.1	35.6	27.9	35.5
	70GG-9XX	25.0	35.1	25.8	36.8	18.4	36.4
	9XX-10XX	4.1	33.2	3.1	35.8	1.5	35.4
	Thru 10XX	1.7	33.6	1.5	35.5	0	----
IV	Over 32W	0.3	28.5	1.6	29.1	2.3	28.6
	32W-48W	6.2	29.9	16.9	32.1	21.1	33.8
	48W-70GG	8.9	30.6	25.3	36.7	24.6	36.9
	70GG-9XX	13.5	37.8	17.3	40.4	14.5	39.9
	9XX-10XX	23.8	38.6	10.1	40.3	8.2	40.0
	Thru 10XX	22.3	38.3	4.2	39.6	4.4	40.1

^aYields indicate percent against starting material.

^bData are on a dry basis.

Figure 8
Protein-Yield Index of DDGS-F at "As-is"
Moisture. Processes I and II

II



I

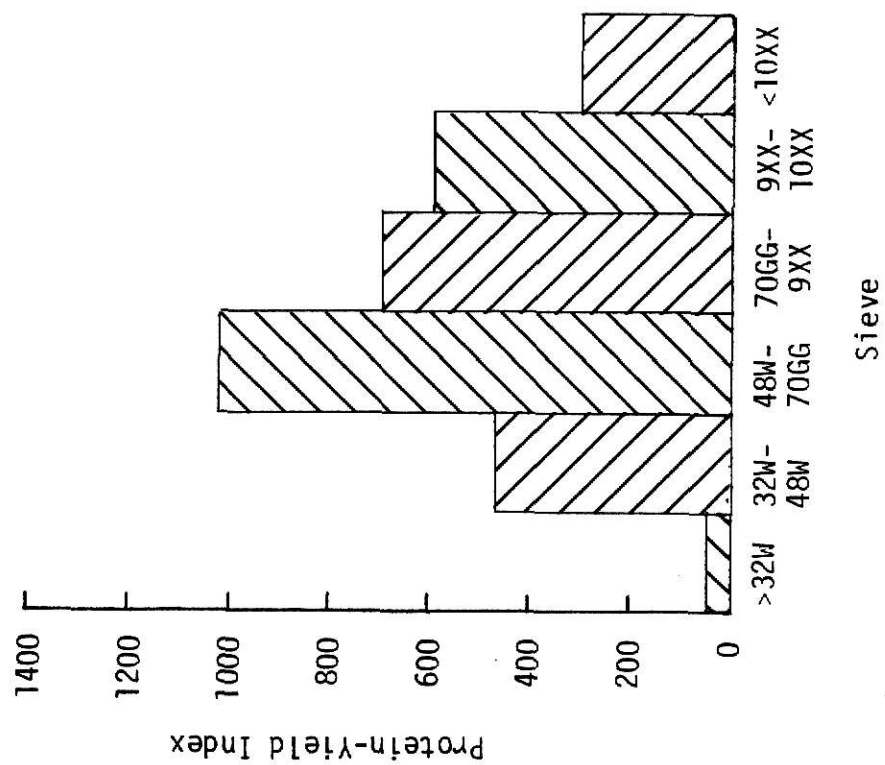
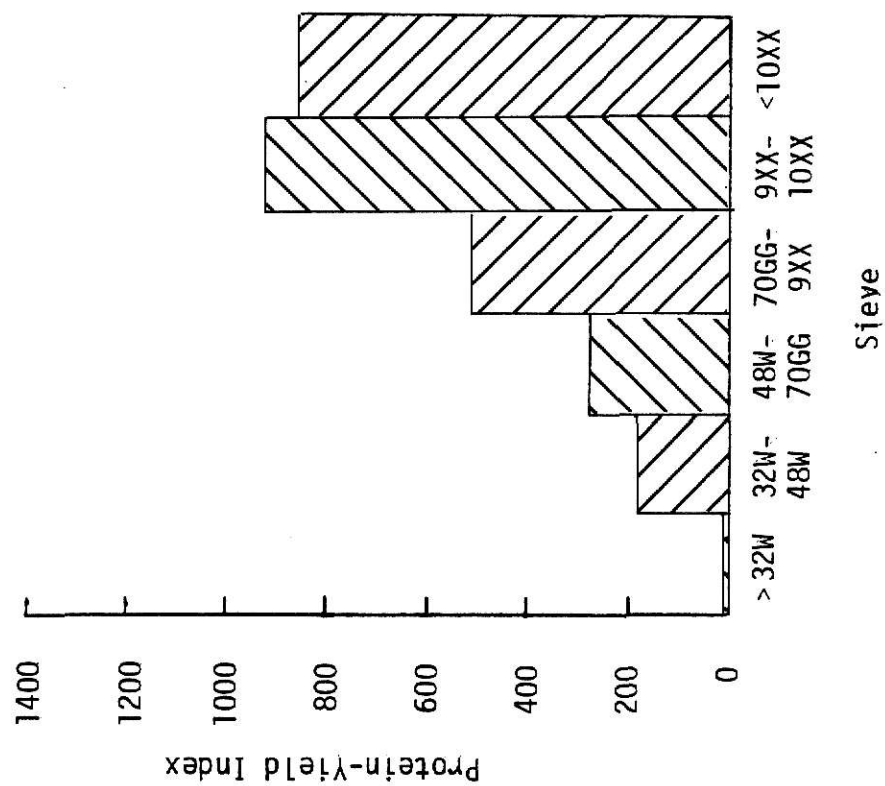


Figure 9
Protein-Yield Index of DDGS-F at "As-is"
Moisture. Processes III and IV

IV



III

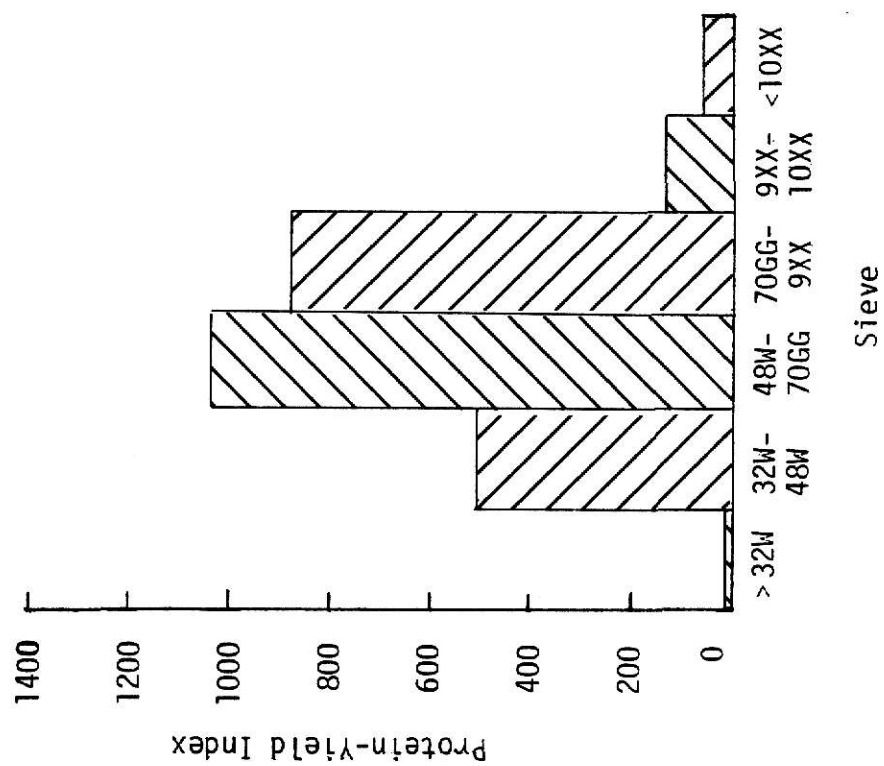
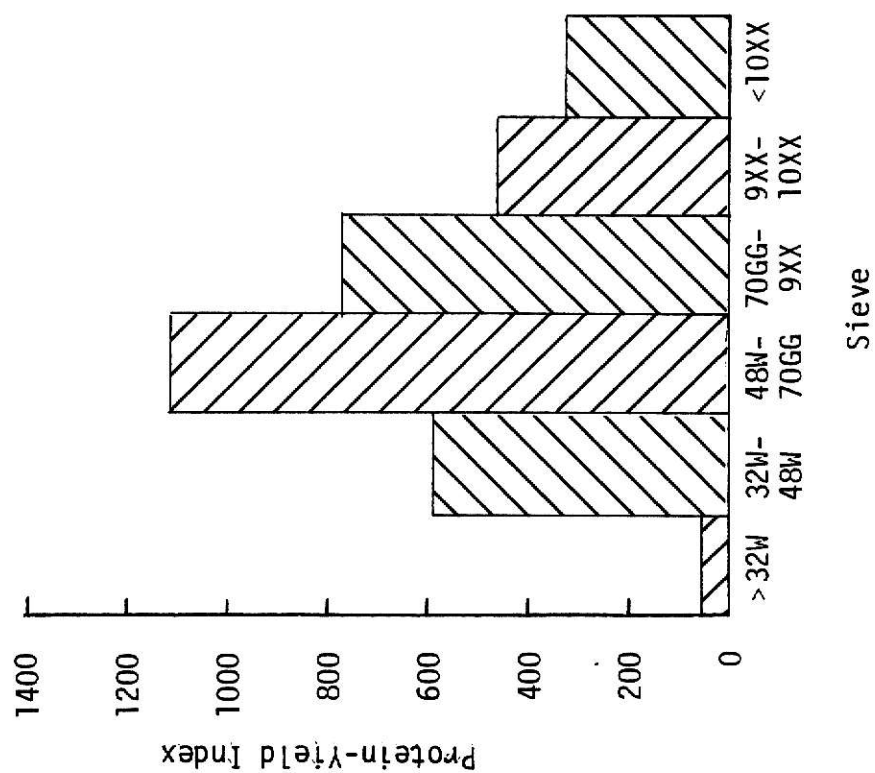


Figure 10
Protein-Yield Index of DDGS-F at 15% Moisture
Processes I and II

II



I

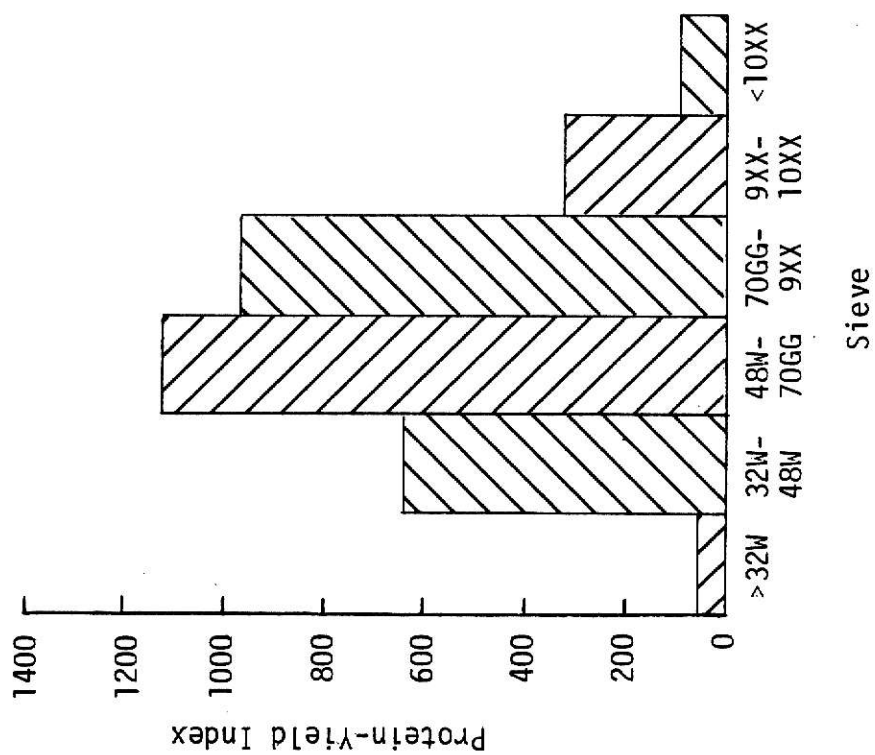


Figure 11
Protein-Yield Index of DDGS-F at 15% Moisture
Processes III and IV

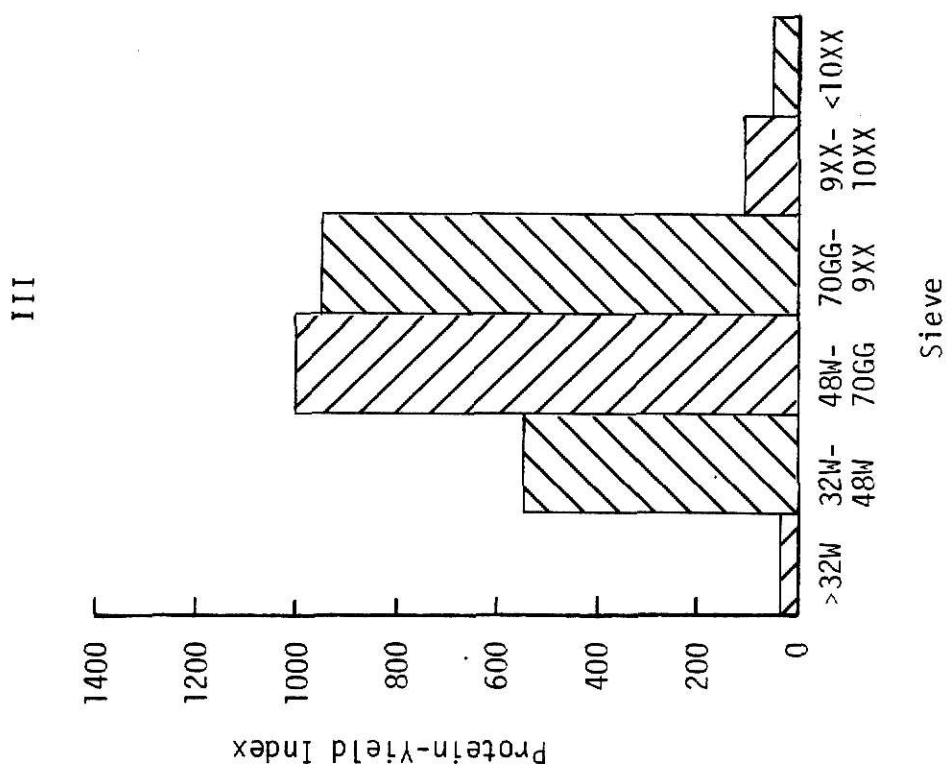
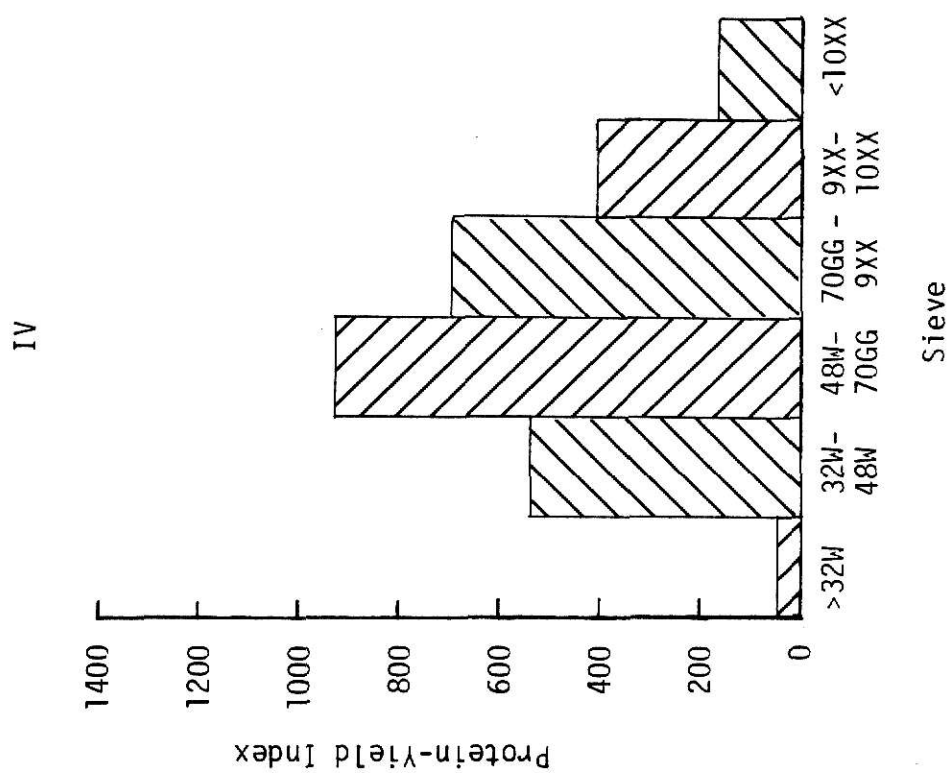


Figure 12
Protein-Yield Index of DDGS-F at 20% Moisture
Processes I and II

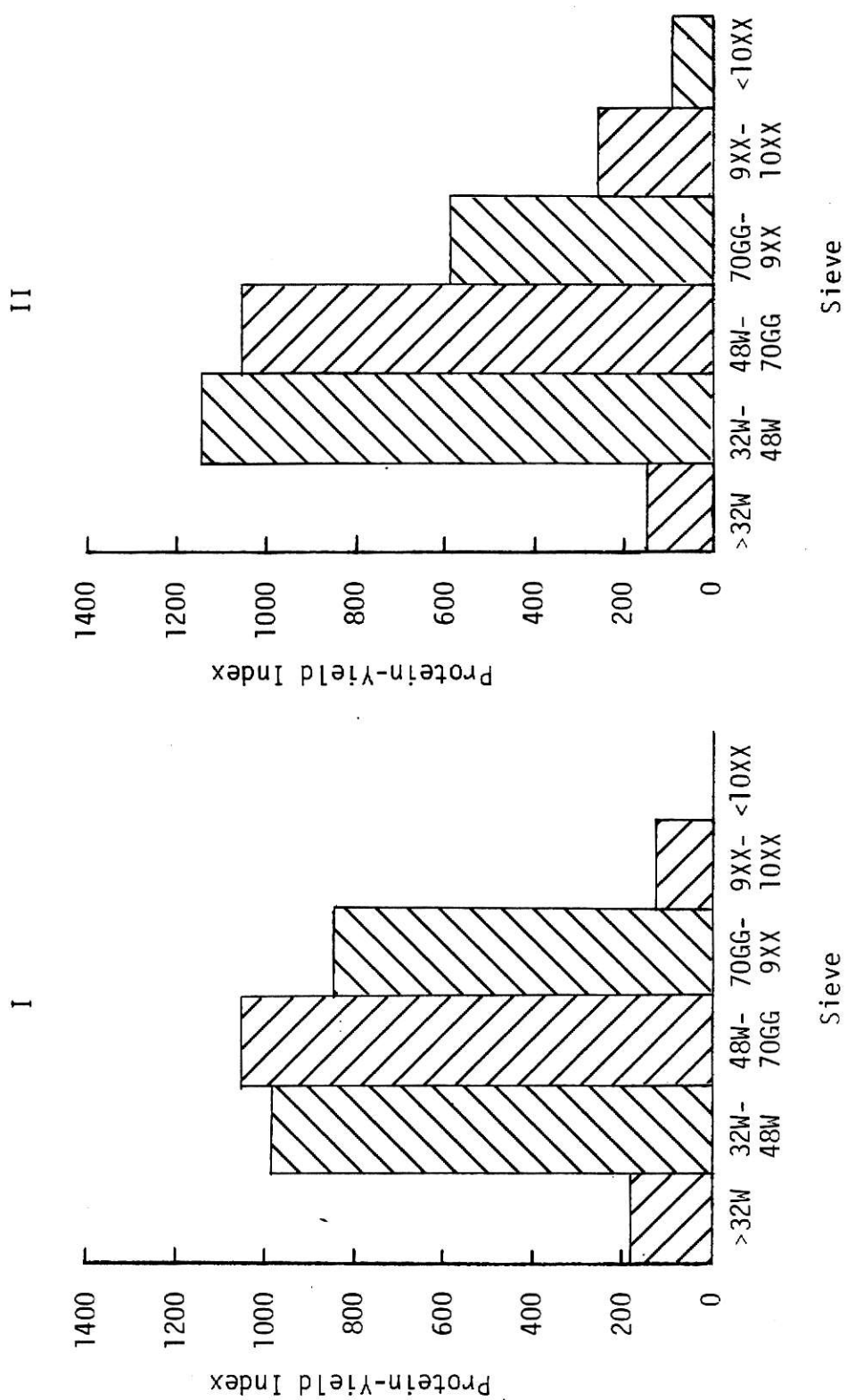
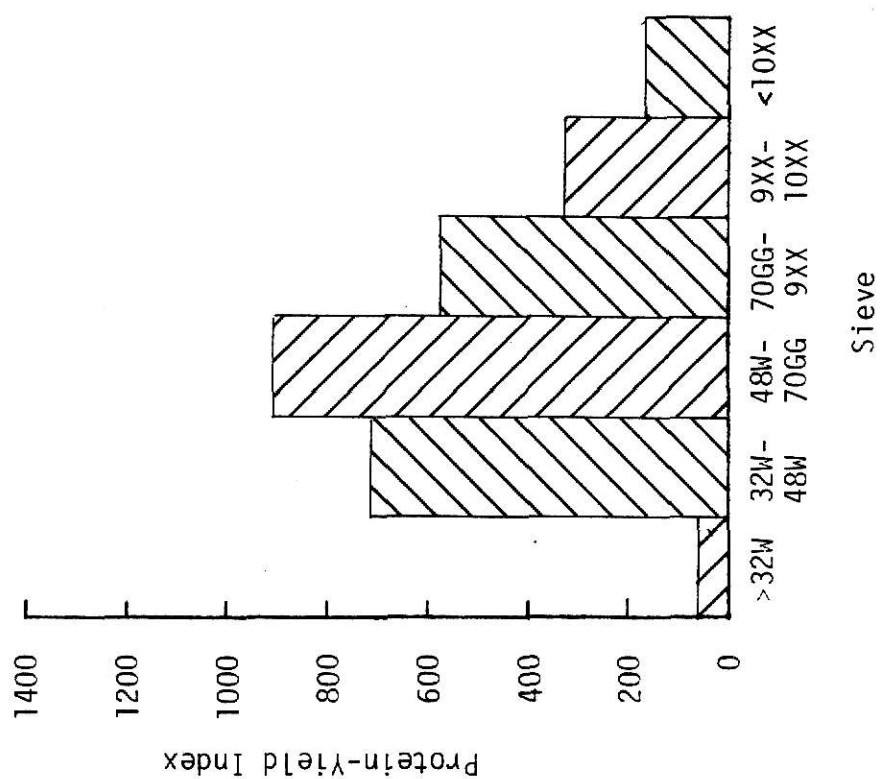
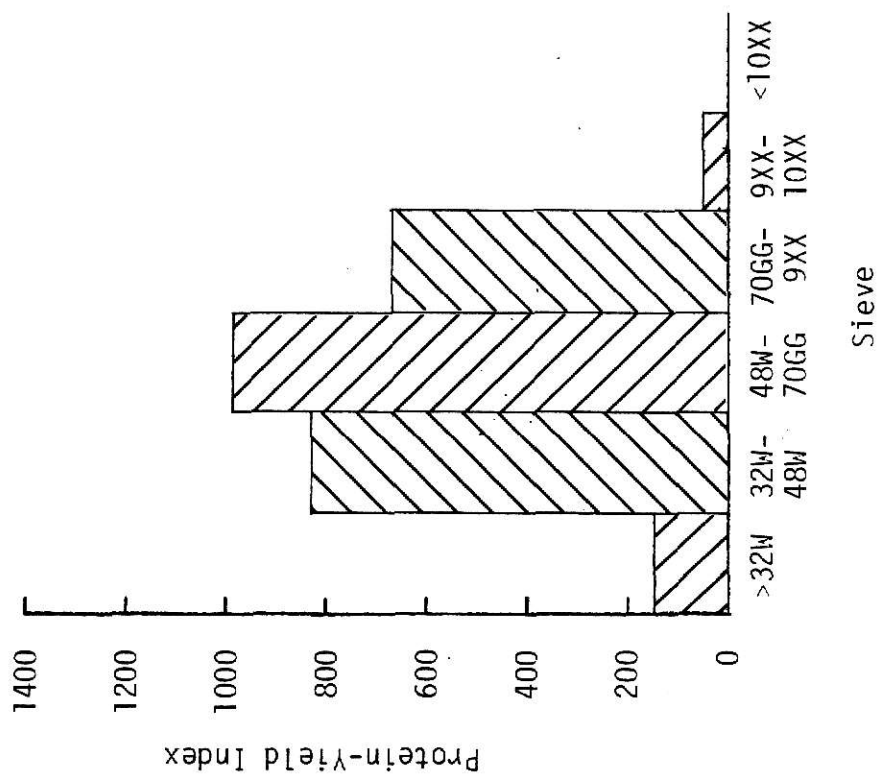


Figure 13
Protein-Yield Index of DDGS-F at 20% Moisture
Processes III and IV

IV



III



would not result in as great an increase in protein percent as in DDGS-B. Conversely, in essentially every case of DDGS-F separation, 9XX - 10XX, and <10XX fraction yields were greater than DDGS-B, again due to the smaller initial particle size of DDGS-F.

As with DDGS-B, Process IV at "as is" moisture had the most desirable combination of high yield and high protein in the HP fractions. Both yield and protein were improved over Process I. The increase in protein-yield index though, was mainly due to the increase in yield percent of the fractions, and less to the increase in protein percent.

Water addition to DDGS-F before pin-milling increased protein percent, but greatly decreased yield of the HP fractions. And as before, defatting and air-separation had less improving effect on yield and protein content on the HP fractions. Also, it appears again that Process II resulted in higher protein-yield index values of the HP fractions than Process IV after water addition.

It can be seen in the preceding results that defatting alone (Process II) was more effective in increasing yield and protein percent (and protein-yield index) of the HP fractions than air separation alone (Process III). In fact, especially in the case of DDGS-F, Process III gave little if any further improvements over Process I. This may be due to loss of material (especially protein rich material) in the low density fraction that is discarded, reducing yield results. DDGS-F was more affected because of the smaller and more uniform particle size, which made air separation more difficult. When both operations were performed in Process IV at "as is" moisture, very significant improvements were made in both yield and protein percent. Their combined effect on protein-yield index is more than additive of the increases resulting from using each one singly. At 15% and 20% moisture contents though, this does not occur. The increases brought about by defatting and air separation steps are not nearly

as significant.

Fractionation results of DDGS-B (a) and DDGS-F (a) are given in Tables 9 and 10 and in Figures 14-16, and 17-19, respectively. For simplicity, Processes I and IV only were analyzed.

Final protein contents of the HP fractions of DDGS-B (a) were similar to those of DDGS-B, and the protein contents of DDGS-F (a) were similar to DDGS-F, although yield values varied. Again, yield and protein percents significantly increased due to the defatting and air-separation steps, but as before, the increase was much less apparent at 15% and 20% moisture levels (Figures 14-19).

In DDGS-F (a), increase in protein yield index in the two smallest fractions were as before, due mainly to increases in yield percent and not to increases in protein content. This is a reflection of the smaller initial particle size of the DDGS-F (a) versus DDGS-B (a).

Table II lists total yields and calculated protein contents¹ of the HP fractions of the four samples used at the three moisture levels. The highest yields, 59.6%, was obtained with DDGS-F at "as is" moisture by Process IV (38.3% protein). The highest protein value, 49.8%, resulted with DDGS-B (a) at 20% moisture by Process IV, but the yield was only 21.4%. Overall, Process IV appeared superior at "as is" moisture levels, depending on the sample and moisture level. It is very evident from these results that when water is added, protein percent increases, but with major sacrifices in yield of the HP fractions. Usually, the increase in protein did not seem worth the loss in the total amount obtained.

¹calculated by adding the protein yield index values of the three HP fractions, and dividing by the total yield of the HP fractions.

Table 9
Yields^a and Protein Contents^b of Sifted Fractions
From DDGS-B (a) by Processes I and IV

Process	Sieve-Size	Moisture Levels (%)					
		"as is"		15.0		20.0	
		Yield (%)	Protein (%)	Yield (%)	Protein (%)	Yield (%)	Protein (%)
I	Over 32W	0.5	15.9	16.8	16.4	22.5	15.3
	32W-48W	8.2	20.9	24.6	21.3	22.1	25.6
	48W-70GG	54.0	31.5	26.4	34.0	25.9	38.5
	70GG-9XX	29.9	36.1	26.2	38.2	24.7	41.9
	9XX-10XX	7.2	37.9	5.9	39.1	4.7	42.2
	Thru 10XX	0.2	37.5	0.1	39.5	0.1	42.8
IV	Over 32W	0.2	15.4	11.4	16.1	17.1	15.2
	32W-48W	3.5	18.9	22.1	26.3	21.1	27.8
	48W-70GG	16.0	22.9	18.5	38.8	15.5	42.3
	70GG-9XX	34.5	34.2	14.0	47.9	16.4	49.9
	9XX-10XX	15.5	43.1	7.7	47.5	4.3	49.5
	Thru 10XX	5.4	44.6	1.4	47.2	0.7	49.6

^aYields indicate percent against starting material.

^bData are on a dry basis.

Table 10
Yields^a and Protein Contents^b of Sifted Fractions
From DDGS-F (a) by Processes I and IV

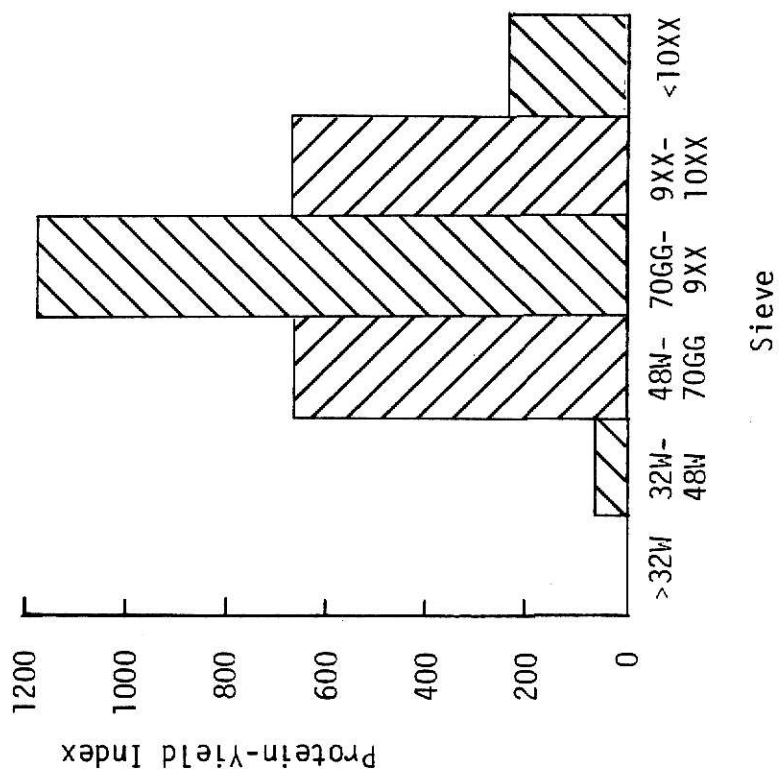
Process	Sieve Size	Moisture Levels (%)					
		"as is"		15.0		20.0	
		Yield (%)	Protein (%)	Yield (%)	Protein (%)	Yield (%)	Protein (%)
I	Over 32W	1.1	18.9	6.8	17.0	10.7	18.6
	32W-48W	11.0	20.0	29.2	24.5	33.9	28.7
	48W-70GG	28.9	27.5	29.7	33.2	28.3	35.3
	70GG-9XX	48.0	35.8	31.2	36.1	23.7	36.2
	9XX-10XX	8.9	35.1	2.7	35.9	3.4	36.3
	Thru 10XX	2.1	37.0	0.4	36.0	0	----
IV	Over 32W	1.0	20.8	3.8	22.7	6.8	23.9
	32W-48W	8.3	25.9	20.2	30.1	22.9	34.5
	48W-70GG	18.9	31.5	22.3	37.3	21.9	37.1
	70GG-9XX	18.4	38.2	15.0	40.1	14.9	39.3
	9XX-10XX	13.1	39.5	7.7	40.3	7.1	39.6
	Thru 10XX	15.5	38.9	6.0	40.3	1.5	40.8

^aYields indicate percent against starting material.

^bData are on a dry basis.

Figure 14
Protein-Yield Index of DDGS-B(a) at "As-is"
Moisture. Processes I and IV

IV



I

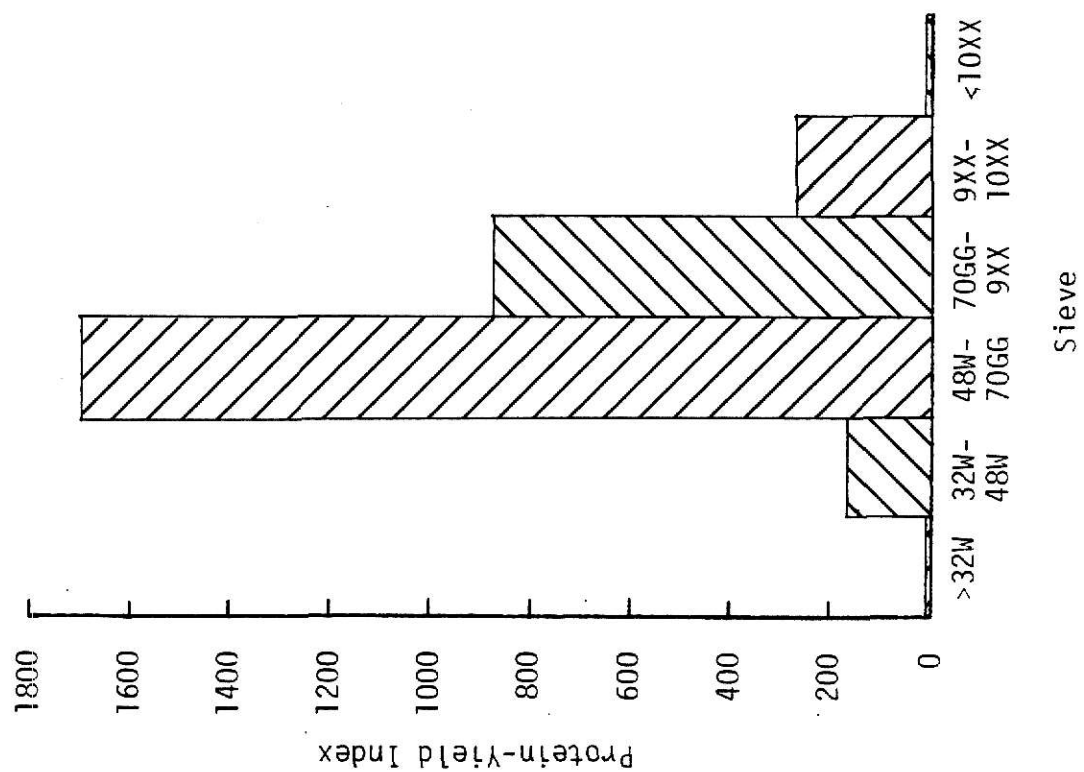
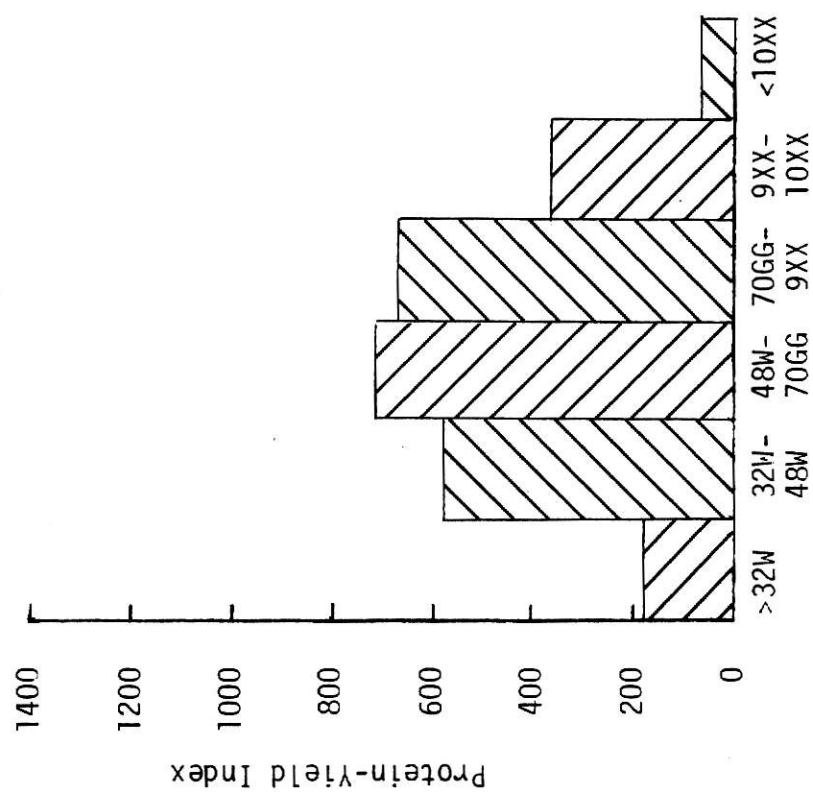


Figure 15
Protein-Yield Index of DDGS-B(a) at 15% Moisture
Processes I and IV

IV



I

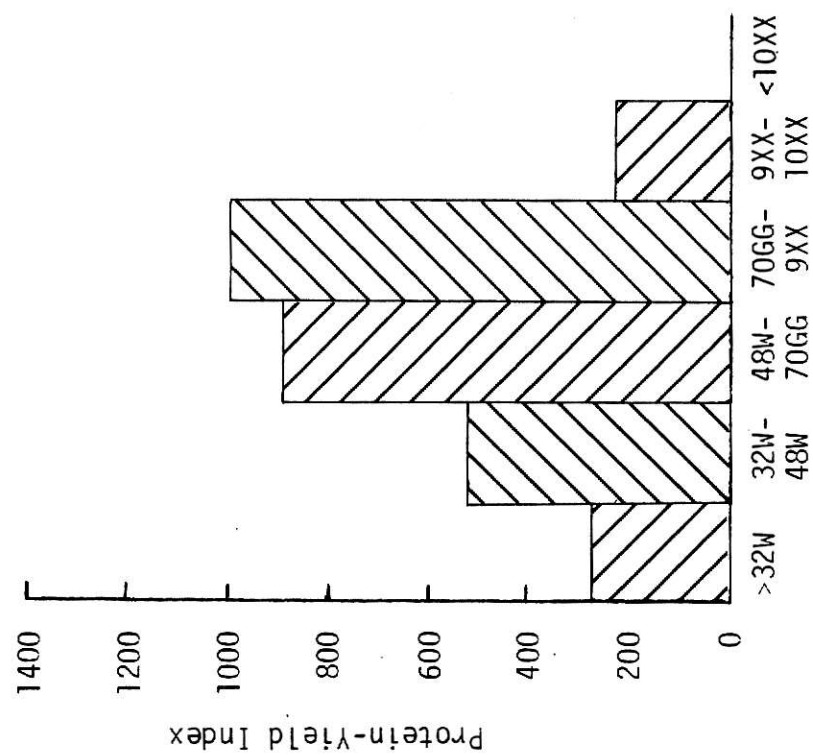


Figure 16
Protein-Yield Index of DDGS-B(a) at 20% Moisture
Processes I and IV

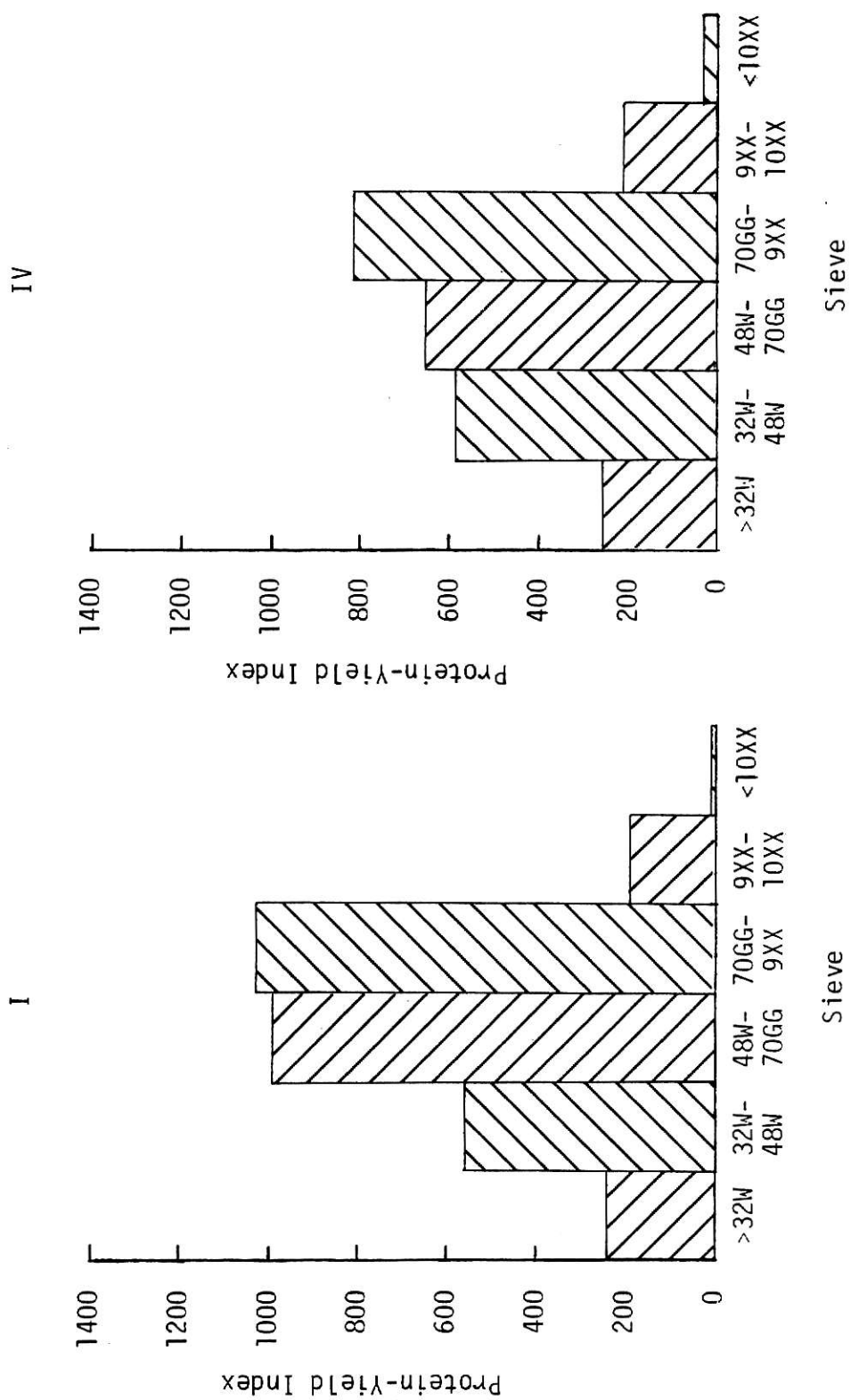
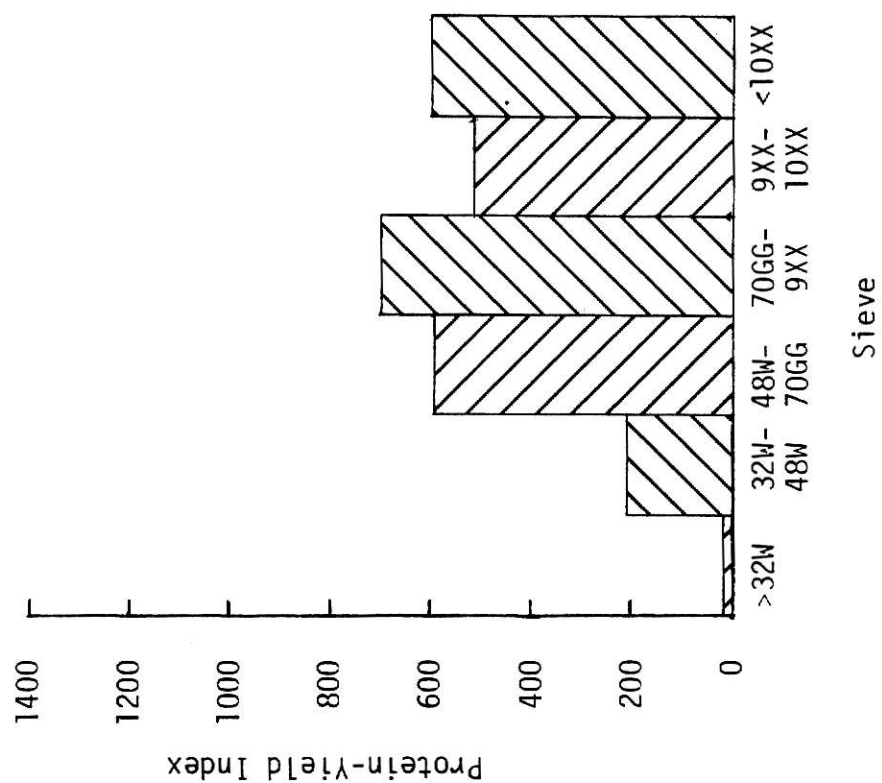


Figure 17
Protein-Yield Index of DDGS-F(a) at "As-is"
Moisture. Processes I and IV

IV



I

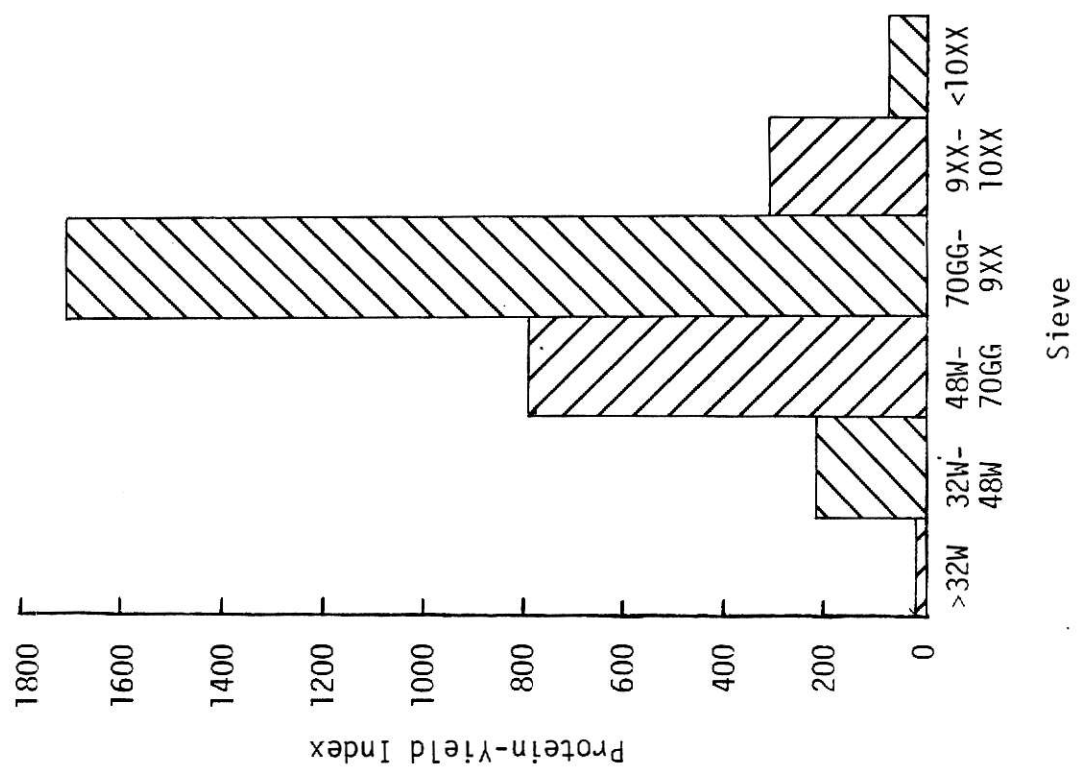


Figure 18
Protein-Yield Index of DDGS-F(a) at 15% Moisture
Processes I and IV

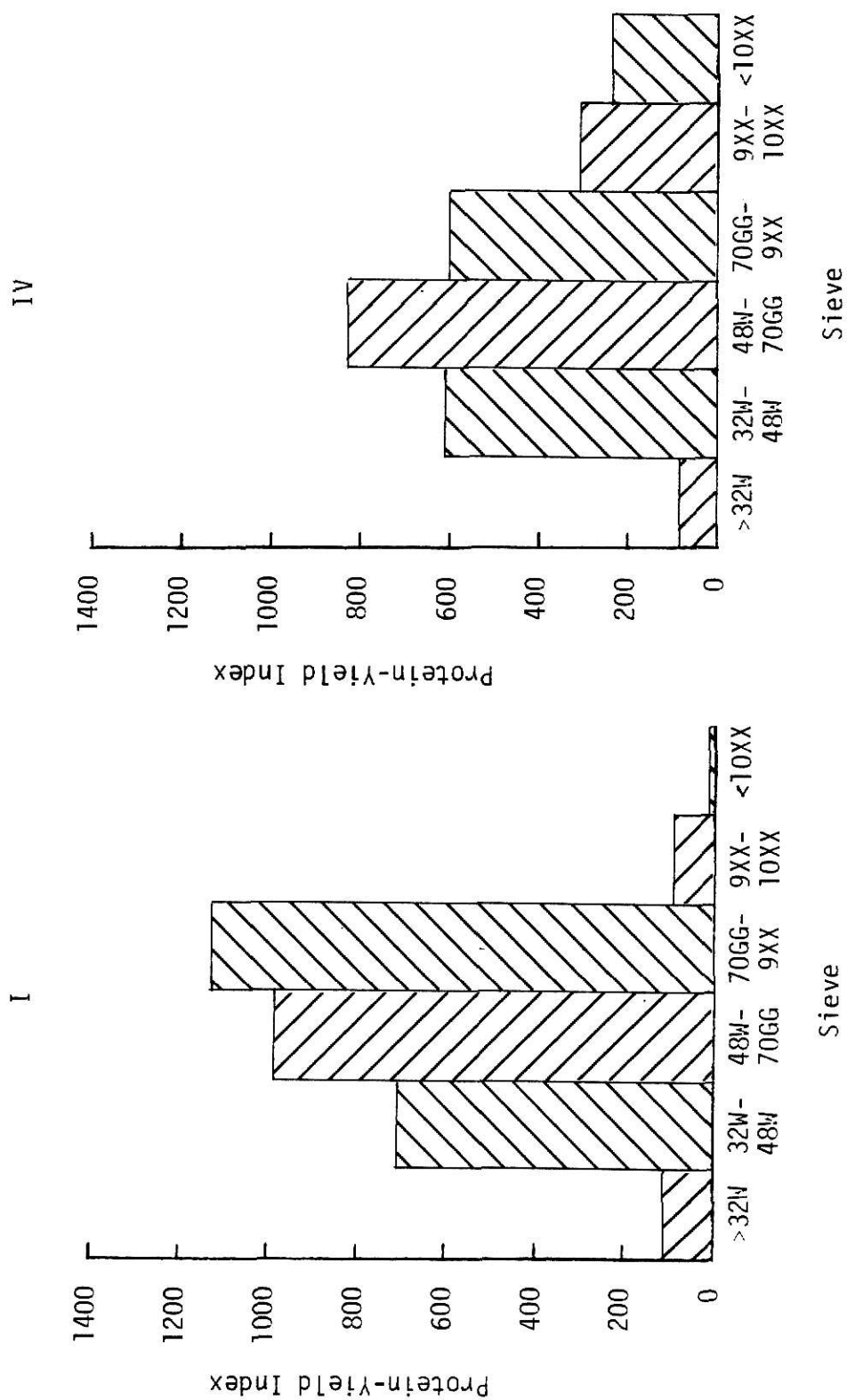


Figure 19
Protein-Yield Index of DDGS-F(a) at 20% Moisture
Processes I and IV

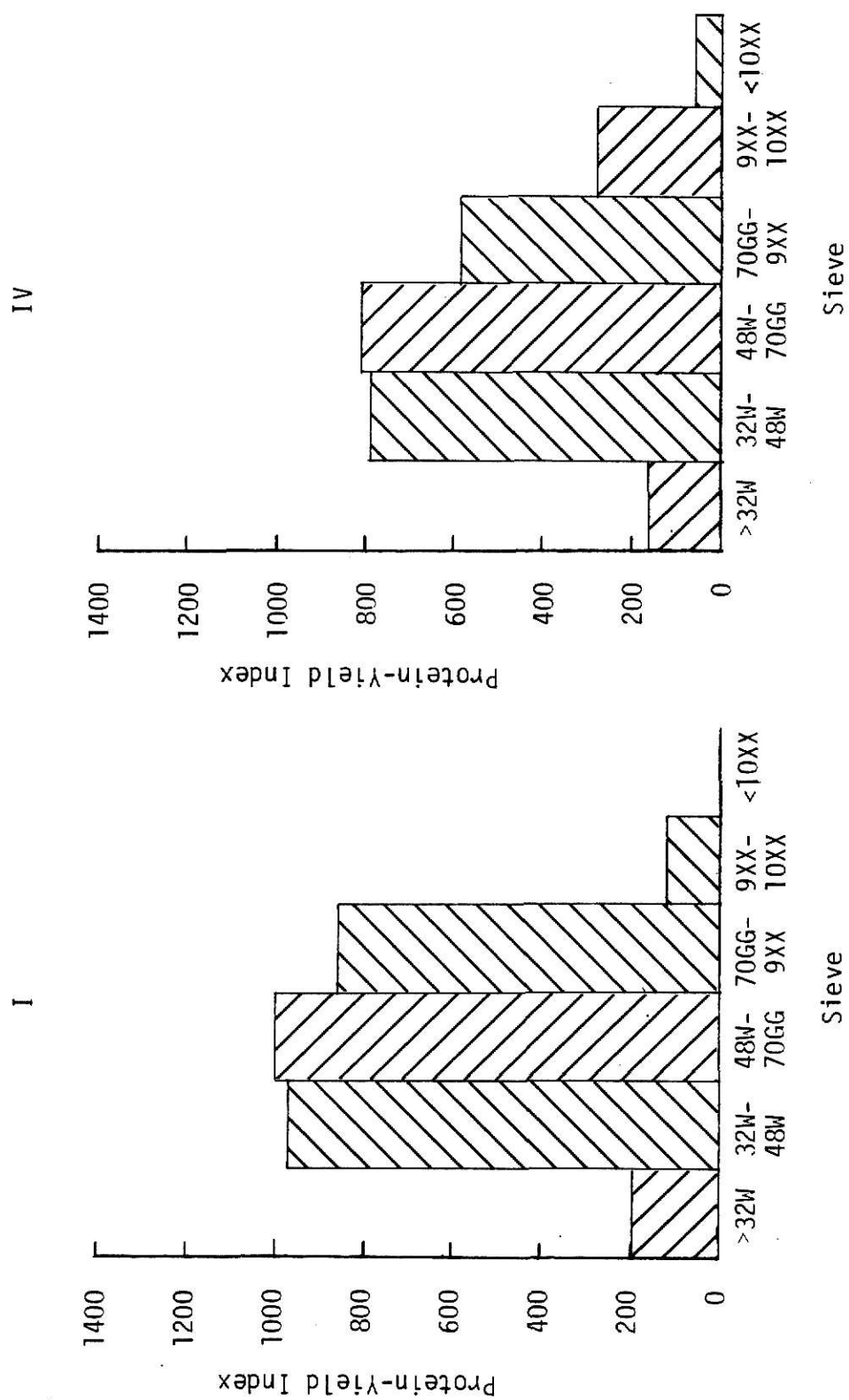


Table 11

Total Yield and Calculated Protein Contents of High Protein (HP)
Fractions of Each Sample Type, Process,
and Moisture Level

Sample	Method	"as is"		15%		20%	
		Yield (%)	Protein (%)	Yield (%)	Protein (%)	Yield (%)	Protein (%)
DDGS-B	I	49.1	37.1	37.9	40.2	25.3	42.0
	II	44.2	44.1	36.9	45.3	20.0	47.1
	III	45.5	38.1	38.4	41.0	25.5	42.8
	IV	50.2	44.9	22.0	45.2	19.0	47.0
DDGS-F	I	45.9	34.2	37.4	36.7	27.1	36.2
	II	54.9	38.4	39.4	39.6	23.0	40.5
	III	30.8	34.8	30.4	36.6	19.9	36.3
	IV	59.6	38.3	31.6	40.3	27.1	40.0
DDGS-B(a)	I	37.3	36.4	32.2	38.4	29.5	41.9
	IV	55.4	37.7	23.1	47.7	21.4	49.8
DDGS-F(a)	I	59.0	35.7	34.3	36.1	27.1	36.2
	IV	47.0	38.8	28.7	40.2	23.5	39.5

From the results obtained in this experiment, several conclusions can be drawn as to the mechanism and magnitude of the effects produced by the defatting, air-separation, and water addition processes.

Defatting

The defatting process reduced the fat content of DDGS-B and DDGS-F from 11.8 to 2.5%, and from 7.4 to 1.0%, respectively. Fat removal has the effect of decreasing particle size distribution by reducing agglomeration caused by the "stickiness" of the fat. This produces an increase in the yield percent of the small HP fractions. Increased yield may also result from an increased efficiency of the sieving process, since full-fat DDGS has a tendency to clog up the sieves.

Defatting may also increase protein content of the HP fractions for several reasons. First, protein percent increased by simple concentration of materials after fat removal. Also, the corn protein may become loosened or detached from other components of the grain after defatting, allowing more of the protein to become part of the smaller HP fractions. Finally, defatting seems to increase efficiency of the air separation process, since the increase when both defatting and air separation were used was greater than the sum of the increases when either one was used alone (Processes II and III); but at "as is" moisture only.

Air Separation

This operation reduced yields in most all of the fractions produced in Process III, because a portion of the starting material is discarded in the process. Material discarded was higher in non-protein components (especially fiber), so protein level would be increased due to concentration. This appeared

to occur in DDGS-B, perhaps because the larger fibrous particles could be separated to a certain extent by air. But a protein increase was not seen in DDGS-F, again possible due to the initial smaller particle size which made this process much less efficient.

Water Addition

Water addition to 15% and 20% moisture resulted in increased yields of larger particle fractions, and decreased yields of the smaller particle, HP fractions. But the protein percentage of almost all fractions of DDGS-B and DDGS-F increased with increased moisture content. The water evidently caused an agglomeration of particles, reversing somewhat the effect of the defatting process. The particles adhered to each other, increasing "apparent" particle size. Also, as with the full fat DDGS flour, the higher moisture samples clogged the sieves and adhered to the bolting cloth, decreasing sieving efficiency. The size separation cuts were then made at smaller "actual" particle sizes; i.e., the material in each fraction was actually of smaller mean particle size than the same fractions at lower moisture content. Thus, consistent with other results, the smaller particle sizes had greater protein contents. In the case of the HP fractions, the resulting smaller size particles were, of course, reduced in quantity, thus the lower yield values.

This work has been that a high protein product (38 - 45%) from DDGS can be produced at a satisfactory yield (50 - 60%) by this fractionation procedure. Ultimately, the economics of this dry separation procedure would determine if the process, or which separation techniques, could be used to produce a protein concentrated distillers by-product. Equipment necessary to make such a product on a large scale would require a substantial capital investment, but processing and labor costs of the operation would be

comparatively low. Of course, all would depend on the eventual selling price of the higher protein grain product.

Higher proteins and yields than obtained simply by milling and sifting can be achieved using the defatting, or defatting and air-separation procedures, but each additional operation would increase the cost of production. Final values would also be dependent on initial particle size and protein content. Water addition would increase protein content of the HP fractions at a relatively low cost, but would only be used if yield is not an important factor.

TOTAL AMINO ACID

Total amino acid analysis for the DDGS samples appears in Tables 12 and 13. DDGS-F had the higher initial lysine content of 3.21 g/100 g protein, with DDGS-B at 2.04 g/100 g lysine. The DDGS-G value of 1.46 g/100 g protein was unexpectedly low. It appears that DDGS-B and DDGS-G had undergone more lysine damage during the drying process than DDGS-F samples. This agrees with the assumption that the darker the DDGS grain, the more heat damage. For both DDGS-F and DDGS-B, total lysine decreases with further heating. This decrease is shown graphically in Figure 20. When DDGS-F was heated, little change occurred until after the first 30 minutes. Then there was a very rapid decrease up to 180 minutes. DDGS-B when heated further, appeared to have no lag time, with decreases in lysine occurring within the first 30 minutes. Subsequent heating decreased lysine content but not at as fast a rate as with DDGS-F. It appears from the curves that perhaps DDGS-B is actually in a later stage of heat damage. It may already have gone through most of the rapid loss phase during the drying process of recovery.

Besides lysine, several other changes occurred with increased heating. Methionine appears to decrease slightly; this has been known to occur in

Table 12
Grams of Amino Acids per 100g Protein in DDGS Samples
Corrected to 100% Protein Recovery Basis

Amino Acid	DDGS-F -0	DDGS-F -30	DDGS-F -60	DDGS-F -120	DDGS-F -180	DDGS-F -360	DDGS-B -0	DDGS-B -30	DDGS-B -60	DDGS-B -120	DDGS-B -180	DDGS-B -360
Aspartic Acid	7.74	7.66	7.74	8.28	8.44	8.72	7.74	8.03	7.98	8.38	8.49	8.68
Threonine	3.98	3.89	3.81	4.05	3.97	3.74	3.77	4.18	3.80	4.13	4.13	3.87
Serine	5.37	5.26	5.41	5.56	5.85	5.85	5.71	5.85	5.80	5.91	5.81	6.05
Glutamic Acid	18.03	17.90	18.09	18.76	19.47	19.15	18.18	18.68	18.30	19.33	18.53	19.36
Proline	10.11	9.99	9.74	10.31	9.12	8.20	8.75	8.94	8.47	9.05	9.75	8.45
Glycine	4.32	4.27	4.30	4.35	4.45	4.59	4.28	4.49	4.34	4.33	4.42	4.67
Alanine	7.75	7.67	7.70	7.86	8.00	7.31	8.24	8.62	8.45	7.45	8.22	8.02
Half Cystine	0.49	0.47	0.47	0.54	0.65	1.65	0.61	0.58	0.56	0.60	0.59	1.49
Valine	3.33	3.43	3.45	3.78	3.78	3.91	3.80	4.17	3.75	3.70	3.85	3.75
Methionine	2.03	2.15	2.09	1.97	1.75	1.85	2.21	2.55	2.56	2.08	2.09	1.89
Isoleucine	2.37	2.46	2.44	2.52	2.49	2.55	2.54	2.89	2.64	2.56	2.82	2.50
Leucine	9.99	9.87	9.99	10.26	10.62	10.27	11.16	7.92	11.48	11.69	11.68	11.73
Tyrosine	4.47	4.86	4.90	4.84	4.73	5.10	5.26	5.65	5.41	5.29	5.36	5.37
Phenylalanine	4.74	5.22	5.21	5.09	5.01	5.52	5.47	6.04	5.54	5.72	5.54	5.45
Histidine	3.81	4.19	4.50	3.73	3.87	4.10	4.44	5.03	4.43	4.21	4.32	4.14
Lysine	3.21	3.17	2.69	1.95	1.59	1.93	2.04	1.66	1.75	1.22	0.88	0.96
Ammonia	2.74	2.40	2.62	2.10	2.08	2.19	1.84	1.48	1.47	1.30	1.11	1.75
Arginine	5.52	5.13	4.87	4.06	4.12	3.40	3.95	3.26	3.25	3.06	2.43	1.87
% Recovered Protein	92.74	95.67	95.18	94.95	91.78	82.27	86.99	86.15	89.26	89.53	86.55	77.73
% Nitrogen	3.60	3.64	3.65	3.47	3.36	3.33	3.18	3.08	3.17	3.11	2.96	3.02

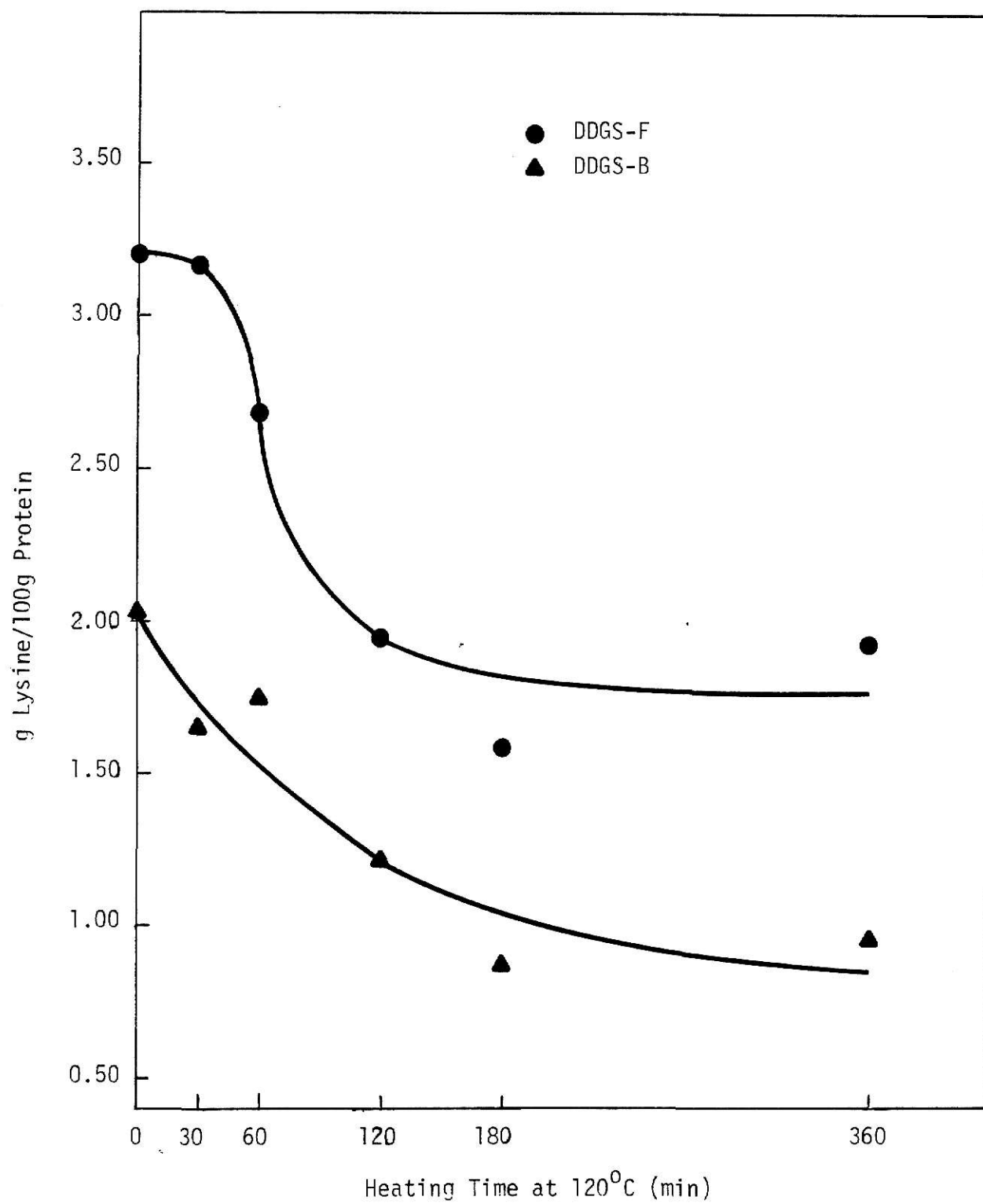
Table 13

Grams of Amino Acids per 100g Protein in DDGS-G, DDGS-E and Corn Meal Samples
Corrected to 100% Protein Recovery Basis

Amino Acid	DDGS-G	DDGS-E	CM-0	CM-60	CM-180	CM-360	CM-720
Aspartic Acid	8.79	8.12	6.50	7.17	6.93	7.03	7.86
Threonine	3.77	3.74	2.88	3.18	3.11	3.15	3.40
Serine	5.89	5.83	5.76	6.04	6.07	6.08	0.65
Glutamic Acid	20.04	18.58	18.78	19.81	20.09	20.23	21.26
Proline	8.48	9.43	10.57	11.52	11.03	10.90	10.86
Glycine	4.34	4.39	3.19	3.26	3.35	3.35	3.63
Alanine	8.84	7.08	8.57	8.90	9.17	9.21	8.79
Half Cystine	1.36	1.83	1.16	1.24	1.12	1.16	1.72
Valine	3.84	3.75	3.11	2.97	3.05	3.11	3.37
Methionine	1.85	2.35	2.21	1.86	2.05	1.97	2.06
Isoleucine	2.69	2.62	2.23	2.12	2.19	2.19	2.40
Leucine	11.49	9.92	13.05	13.47	13.67	13.74	14.30
Tyrosine	5.00	5.00	5.09	4.95	4.98	5.02	5.35
Phenylalanine	5.13	5.66	5.91	5.36	5.30	5.18	5.72
Histidine	3.53	4.28	3.92	3.20	3.93	3.91	4.34
Lysine	1.46	3.06	2.02	1.03	0.98	0.91	0.99
Ammonia	1.51	2.41	3.95	2.72	1.90	1.76	2.20
Arginine	1.98	1.94	1.12	1.20	1.08	1.11	1.09
% Recovered Protein	79.80	85.02	88.52	88.72	85.92	86.83	82.18
% Nitrogen	3.41	3.31	1.18	1.10	1.03	1.03	0.99

Figure 20

Heating Time at 120°C vs Total Lysine: DDGS



heated foods (Rao and McLaughlin 1967). With further heating time, glutamic acid content increases somewhat. No explanation can be given for this phenomenon. Interestingly though, DDGS-G, which had a very low initial lysine level, indicating heat damage, also had a relatively high glutamic acid value of 20.04. Conversely, DDGS-E and G, with higher initial lysine values, had lower glutamic acid values of 18.58 and 18.03, respectively.

There are also some minor differences in the amino acid contents between DDGS-B and DDGS-F samples. They are probably due to varietal difference of the original grains used, or to differences in mash ingredients, although corn was the major component of all samples.

In Table 12, percent recovered protein decreases slightly with heating, and is considerably lower in the case of DDGS-B compared to DDGS-F. This may be an effect of the destruction of protein by heat induced reactions.

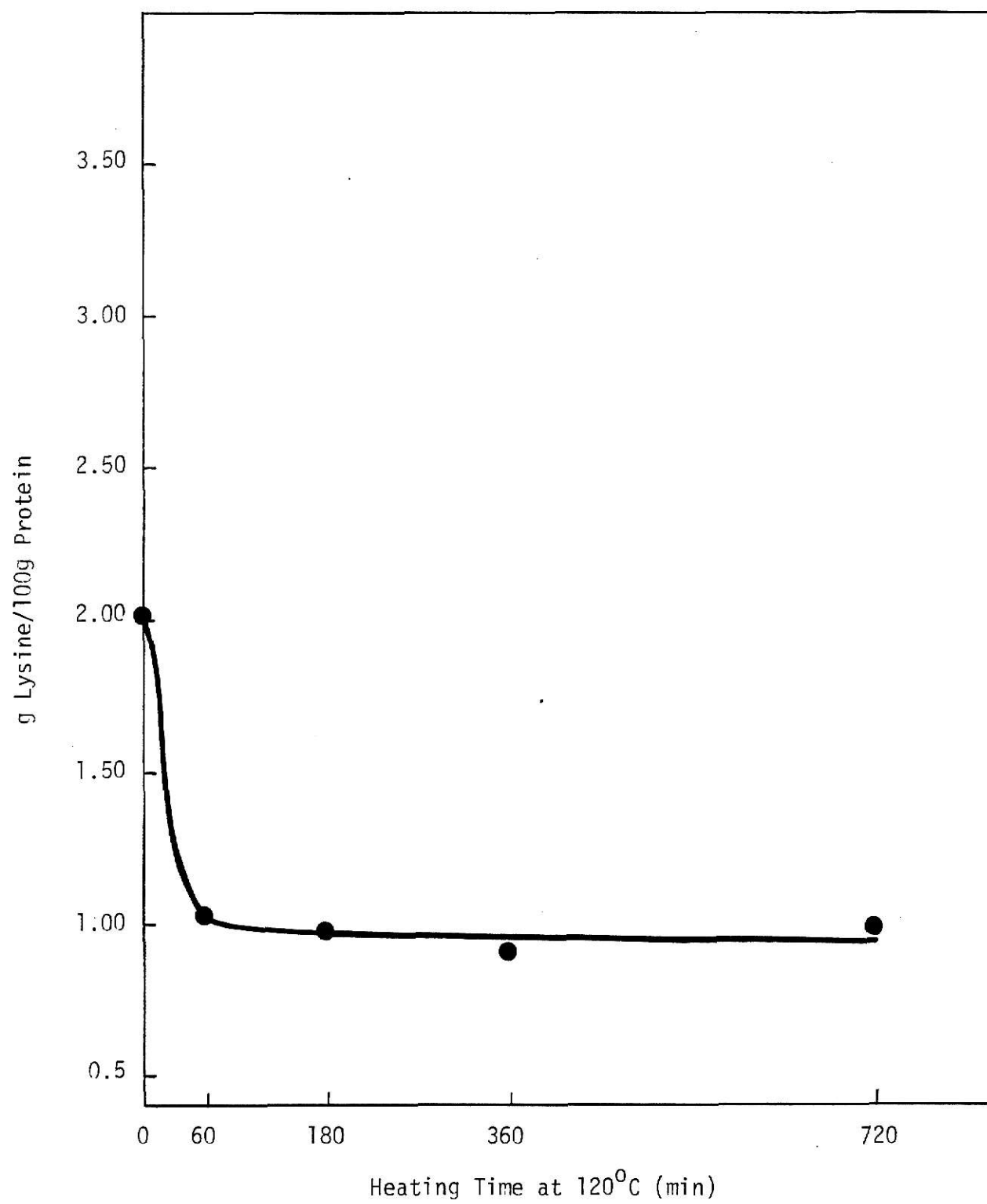
The corn meal amino acid results appear in Table 13. The most significant change that occurs with heating is, of course, the decrease in lysine from 2.02 to 0.99 g /100 g protein (Figure 21). Also, as with DDGS samples, glutamic acid appears to increase with heating; but in addition, aspartic acid, threonine, and leucine also increase slightly.

If the amino acid analysis of the DDGS samples are compared with the corn meal, several dissimilarities exist. Most noticeably, the lysine level is higher in two of the four DDGS samples even after being heated during the drying process. In addition, proline and leucine are noticeably higher in the corn meal sample, and glycine is lower. The amino acid results of the DDGS samples agree well with those obtained by Carpenter (1970) and Ranhotra et al. (1981), and corn meal amino acid contents coincide with those reported by Wall and Paulis (1978). Therefore, there must be an explanation for these noted differences.

In this study, the corn meal sample used was degermed for increased

Figure 21

Heating Time at 120⁰C vs Total Lysine: Corn Meal



stability. Thus, the corn analyzed was mainly endosperm and the DDGS was derived from whole corn. Wall and Paulis (1978) report differences in the amino acid content of endosperm versus corn germ. They state that endosperm of normal corn is low in many essential amino acids, especially lysine and tryptophan, due to its high level of zein. In contrast, germ has high levels of these amino acids. Since the DDGS samples contained germ, their analyses would naturally be somewhat higher in these amino acids compared to the corn meal, which did not include the germ.

Wall and Paulis also showed endosperm proteins to be higher in proline and leucine than germ proteins; thus, the protein of the corn meal had slightly higher value of proline and leucine than did the DDGS samples.

In addition, some variation was undoubtedly due to varietal differences between the corn types. The small amount of yeast protein that was in the DDG may have altered the amino acid content.

DYE-BINDING OF REACTIVE LYSINE

DDGS

Samples were analyzed for reactive (available) lysine using the Udy-Carpenter dye-binding method. Values obtained are given in Table 14, and shown in Figure 22. All final results are an average of two to four determinations.

Initially, DDGS-F was greater in reactive lysine than DDGS-B, indicating less initial heat damage. Values for heated DDGS samples follow closely the trend set by the total lysine values. DDGS-F had a very sharp decline until 60 - 120 minutes, where the rate of loss began to slow. DDGS-B also decreased quickly at first, but began to level out after 30 minutes. Again, this gives evidence that DDGS-B was heated more extensively than DDGS-F during the drying

Table 14
Results of Udy-Carpenter Dye-Binding Procedure
on DDGS and Corn Meal (CM) Samples

Sample	# of Runs	Avg. g Reactive Lysine/100 g Samples	Moisture (%)	Protein (%)	Ave. g Reactive Lysine/100 g Protein
DDGSF-0	4	1.078	6.90	28.1	4.12
DDGSF-30	2	.958	2.95	28.1	3.51
DDGSF-60	2	.754	2.43	28.1	2.75
DDGSF-120	2	.695	2.57	28.1	2.54
DDGSF-180	2	.578	4.18	28.1	2.15
DDGSF-360	2	.388	7.06	28.1	1.48
DDGSB-0	3	.683	8.97	28.3	2.65
DDGSB-30	4	.534	4.26	28.3	1.97
DDGSB-60	4	.483	4.54	28.3	1.79
DDGSB-120	4	.450	3.74	28.3	1.65
DDGSB-160	4	.388	3.33	28.3	1.42
DDGSB-360	2	.314	8.08	28.3	1.21
DDGS-G	2	.666	7.3	34.1	2.11
DDGS-E	2	.716	6.5	29.0	2.64
CM-0	4	.200	11.42	8.8	2.57
CM-60	2	.219	5.45	8.8	2.63
CM-180	2	.205	5.26	8.8	2.46
CM-360	2	.168	7.44	8.8	2.06
CM-720	2	.176	6.98	8.8	2.15

^adata are on a dry basis

Figure 22
Heating Time at 120°C vs Reactive Lysine: DDGS

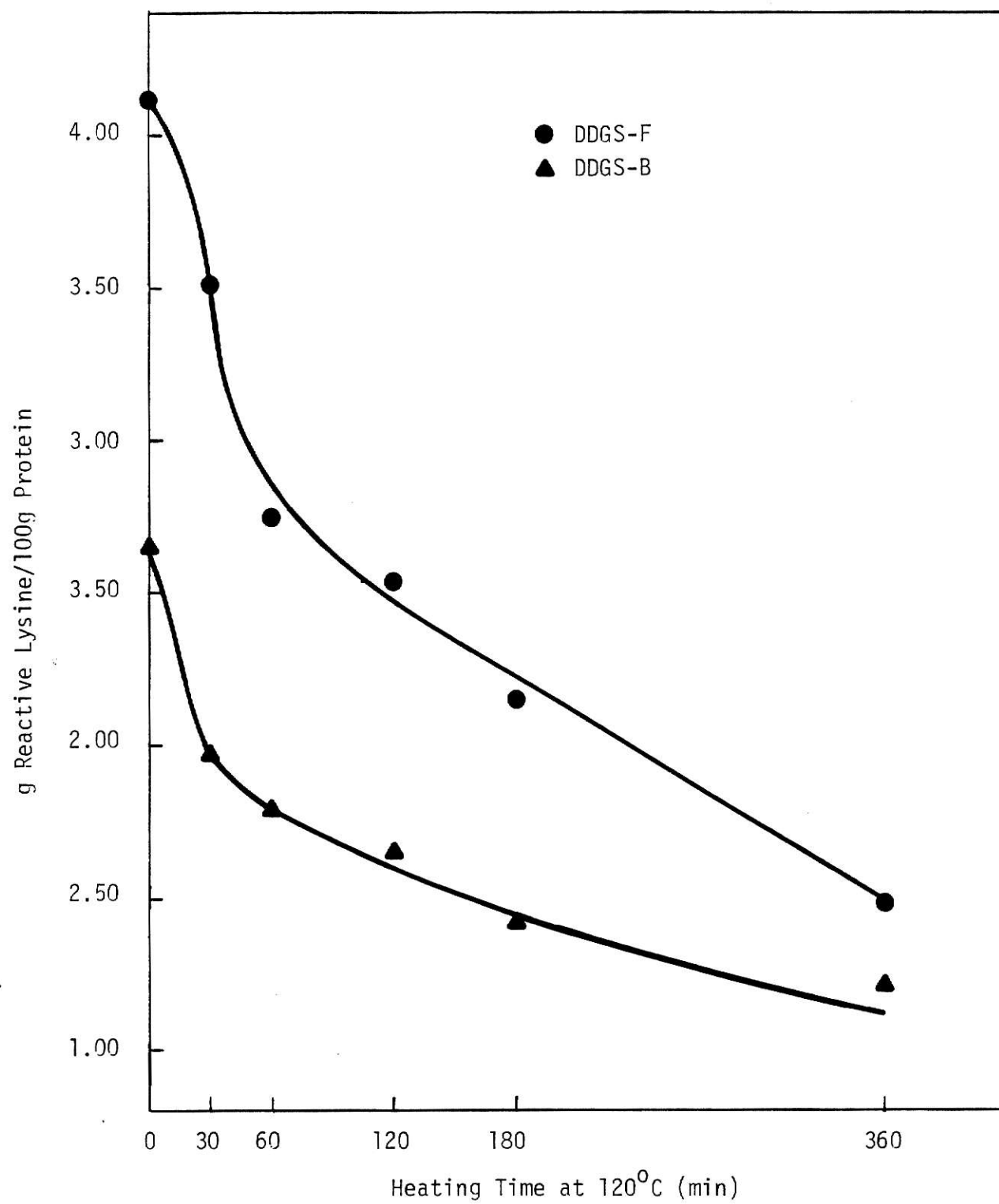


Table 15
Values of Total Lysine versus Reactive Lysine

Sample	g Total Lysine/100 g Protein	g Reactive Lysine/100 g Protein
F-0	3.21	4.12
F-30	3.17	3.51
F-60	2.69	2.75
F-120	1.95	2.54
F-180	1.59	2.15
F-360	1.93	1.48
B-0	2.04	2.65
B-30	1.66	1.97
B-60	1.75	1.79
B-120	1.22	1.65
B-180	0.88	1.42
B-360	0.96	1.21
DDGS-G	1.46	2.11
DDGS-E	3.06	2.64
CM-0	2.02	2.57
CM-60	1.03	2.63
CM-180	0.98	2.46
CM-360	0.91	2.06
CM-720	0.99	2.15

process of its recovery.

Unexpectedly though, the values of reactive lysine are higher than values obtained for total lysine (Table 15). This is the reverse of what was anticipated because by definition total lysine should be a greater amount.

Corn Meal

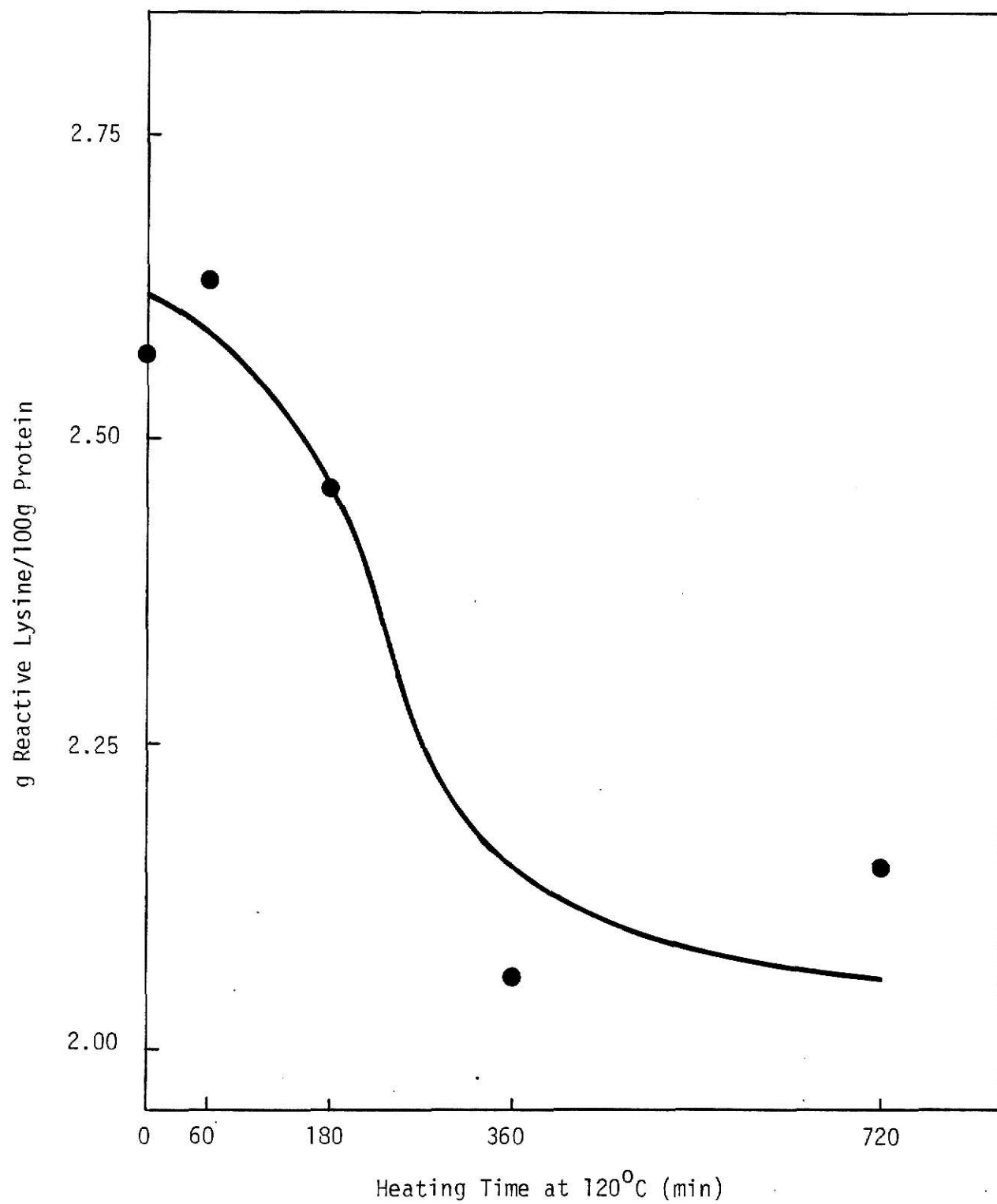
Again, reactive lysine results were greater than those of total lysine for the corn meal at every heating time (Table 14 and 15). But unlike the DDGS results, the decrease in corn meal reactive lysine was somewhat different than the total lysine (Figure 23). Reactive lysine did not decrease immediately, but remained at approximately the same level after 60 minutes of heating. Subsequent heating decreased the lysine value, but at a slower rate and to a much lesser extent than total lysine was reduced. Total lysine dropped to less than half its original value; reactive lysine only dropped about 20%.

No positive explanation is available to easily explain why the reactive lysine values were greater than total lysine. By definition, reactive (or available) lysine should be less, since some complexed or crosslinked lysine would still be determined as total lysine. Possible, the Acid Orange 12 dye is binding with some other compound or compounds besides lysine. But to be determined as reactive lysine, the propionic anhydride would also have to block these groups, resulting in a decrease in bound dye per gram of sample in the 'B' reading, and thus interpreted as blocked lysine groups, i.e., reactive lysine.

Several investigators have found that starch may bind a certain amount of dye (Hurrel and Carpenter 1975, 1979, Udy 1956, 1971). But there is disagreement as to how much is bound. Udy (1956) reports that 1 gram of starch binds 4.6 mg of Acid Orange 10 dye. If this value is assumed for Acid Orange 12 dye,

Figure 23

Heating Time at 120°C vs Reactive Lysine: Corn Meal



the starch in the corn meal used (approximately 80%) may be responsible for approximately 20% of the dye binding in the unheated corn sample. It then must be assumed that the propionic anhydride blocks these binding sites to prevent dye binding in the 'B' reading, resulting in an overestimated value for reactive lysine. Hurrel and Carpenter (1979) though, showed that addition of starch to protein has no net effect on reactive lysine value, since the increase in the amount of dye bound before and after propionation was the same. Thus, there was no net effect on lysine value since the results of the two determinations were the same.

Udy (1956) also reported that bran binds significant amounts of dye. This could affect the results in this experiment, especially in the case of the DDGS samples, which were high in bran and fiber.

Nevertheless, the data from the dye-binding experiment revealed some insight into what types of heat damage may have occurred in the DDGS and corn meal samples. If the average μ moles of dye bound per gram of sample for both the 'A' and 'B' readings of the DDGS and corn meal samples are examined, an interesting difference is noted (Table 16). Conversely, for the corn meal series, the 'B' readings remain relatively stable, and the 'A' readings decreased somewhat, except for the unheated samples.

The following explanation is based on evidence of heat induced reactions presented by Hurrel and Carpenter (1979). When the DDGS was heated, reducing sugars present (from starch hydrolysis) combined with the proteins in the Maillard reaction. Early Maillard products are formed, such as the deoxyketosyl or Amadori compound (Mauron 1981), which would still be positively charged at the epsilon amino group of the lysine unit. The substitution at this group prevents approximately 50% of the reaction with propionic anhydride (Hurrel and Carpenter 1981). The complex remained positively charged, and bound the Acid Orange 12 dye in the 'B' determination. This then resulted in an increase

Table 16
Average Mmoles of Dye Bound/g Sample

Sample	'A' Reading	'B' Reading
F-0	.220	.146
F-30	.224	.158
F-60	.218	.166
F-120	.223	.176
F-180	.215	.176
F-360	.189	.162
B-0	.202	.155
B-30	.197	.160
B-60	.195	.162
B-120	.200	.170
B-180	.198	.172
B-360	.180	.158
CM-0	.053	.039
CM-60	.058	.043
CM-180	.055	.041
CM-360	.052	.040
CM-720	.052	.040

in the 'B' determinations over the zero heating time controls.

Since the 'B' readings in the corn meal results did not increase, extensive Maillard reactions may not be taking place. For some reason though, the 'A' readings appeared to decrease with heating. The heat process may have caused crosslink formation between lysine and other amino acids, such as asparagine and glutamine (Hurrel and Carpenter 1979). In this case, the lysine unit would no longer be positively charged. Thus, the dye was not bound, resulting in decreases in the 'A' readings with heating. There would be no change in the 'B' readings, so long as arginine and histidine units remain unaffected. This occurrence seems likely, since corn meal is high in starch, but low in free reducing sugars for the protein to react with. Further evidence of this was the fact that when the corn meal was heated, there was minimal color change in the samples, indicating little Maillard browning taking place.

The decrease in both the 'A' and 'B' readings of the DDGS at 360 minutes may be due to some experimental error, or to effects of late Maillard reaction products. After 360 minutes, advanced Maillard reactions may have become more predominant. The deoxyketosyl lysine compound may have decomposed further, and the lysine, then was no longer partially determined as reactive. Further loss of lysine may then have occurred due to reaction of lysine with aldehydes formed in the advanced Maillard reactions (Hurrel and Carpenter 1979). This effect would then have decreased 'B' toward its initial value, and also decreased 'A' additionally.

Extraction of Proteins

Extraction of protein from DDGS-B and DDGS-F by the alkaline extraction procedure of Wu and Sexson (1976a) gave the results shown in Table 17. Yield percentages were low, due to the denaturation of proteins when heated. Plots of heating time versus percent of extracted protein of DDGS-F and DDGS-B samples are shown in Figure 24. The highest yield was obtained for DDGS-F control, 16.2%, and the lowest yield, 2.6%, was from DDGS-F after 360 minutes at 120°C. DDGS-F protein yield decreased more, and at a faster rate, than did that of DDGS-B. DDGS-B solubility did not decrease to any great extent after 60 minutes of heating.

It can be seen that DDGS-F extraction falls below the DDGS-B value after 360 minutes of heating. As with the lysine destruction results, DDGS-F initially started out with less heat damage, but the protein appears to be more heat labile. Wu and Sexson (1976a) state that the variety of corn may affect extraction of protein. This is a possible explanation for the dissimilar solubility characteristics of the two DDGS samples.

Extraction results of DDGS-G and DDGS-E are also give in Table 17. Percentage of protein extracted was similar to the DDGS-B-0 sample.

Extraction of corn meal resulted in much higher protein yield percentages than the DDGS samples. Values ranged from a high of 81.5% extraction at zero heating time, to 52.7% at 720 minutes (Table 18). A graph of heating time versus extraction percent is shown in Figure 25. The greatest decrease occurs between 60 and 180 minutes.

When DDGS-B and F, and corn meal extraction percentages are graphed on the same axis (Figure 26), it is very evident that the distillery and/or drying process of recovery of DDGS greatly decreases the solubility of corn proteins in an alkaline solvent. McGuire and Earle (1958) found that total nitrogen extractable with 0.01 N sodium hydroxide decreased with increased temperature of the drying.

Table 17

Extraction Percentage of Protein Concentrate (PC) from DDGS Samples

Sample	g	%H ₂ O	% Protein (db)	g Protein in DDGS	g PC	%H ₂ O	% Protein in PC(wb)	% Protein in PC (db)	g Protein in PC	% Extraction
DDGS-F-0	80.0	9.40	28.1	20.4	39.86	67.9	8.3	25.8	3.31	16.2
DDGS-F-60	80.0	7.14	28.1	20.9	28.47	80.2	6.9	34.8	1.96	9.4
DDGS-F-180	80.0	5.06	28.1	21.4	17.49	78.0	7.2	32.7	1.26	5.9
DDGS-F-360	80.0	2.95	28.1	21.8	8.10	78.0	6.9	31.4	0.56	2.6
DDGS-B-0	80.0	9.74	28.3	20.4	20.51	78.1	8.5	38.8	1.74	8.5
DDGS-B-60	80.0	8.48	28.3	20.7	14.82	75.1	8.6	34.5	1.27	6.1
DDGS-B-180	80.0	5.60	28.3	21.4	10.94	71.4	11.1	38.8	1.21	5.6
DDGS-B-360	80.0	4.78	28.3	21.6	12.58	74.1	8.6	33.2	1.08	5.0
DDGS-G	80.0	7.3	34.1	25.3	36.00	78.4	6.8	31.5	2.45	9.7
DDGS-E	80.0	6.5	29.0	21.7	21.51	80.5	7.6	39.0	1.63	7.5

Figure 24

Heating Time at 120°C vs Percent Protein Extracted: DDGS

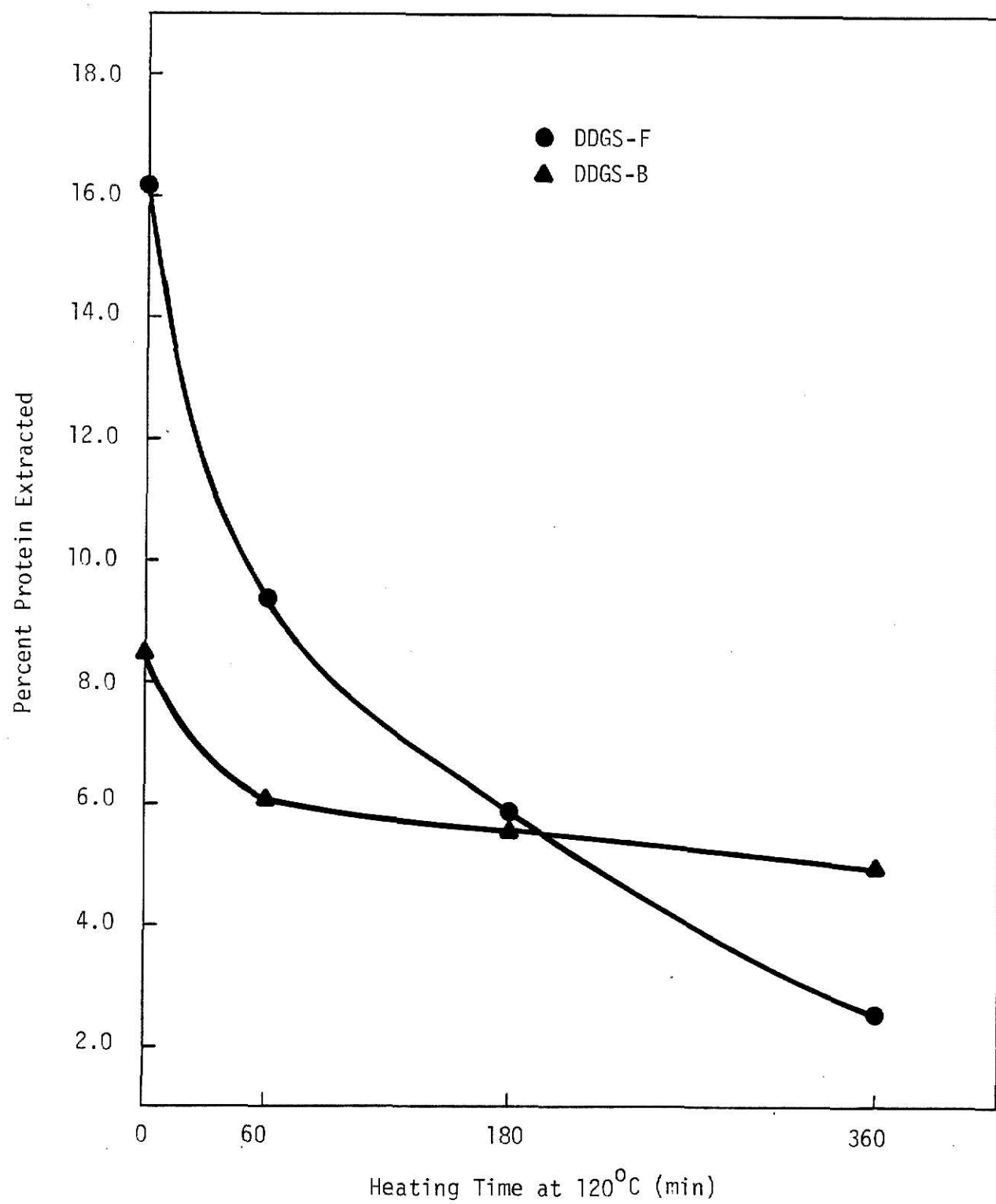


Table 18

Extraction Percentage of Protein Concentrate (PC) from Corn Meal (CM) Samples

Sample	g	%H ₂ O	% Protein (db)	g Protein in CM	g PC	%H ₂ O	% Protein in PC (wb)	% Protein in PC (db)	g Protein in PC	% Extraction
CM-0	80.0	10.70	8.8	6.28	38.52	82.2	13.3	74.7	5.12	81.5
CM-60	80.0	5.45	8.8	6.65	29.27	78.4	17.8	82.4	5.21	78.3
CM-180	80.0	5.26	8.8	6.67	24.07	76.7	18.0	77.2	4.33	64.9
CM-360	80.0	7.44	8.8	6.51	22.43	77.6	17.2	76.8	3.86	59.3
CM-720	80.0	7.0	8.8	6.54	20.79	78.2	16.6	76.1	3.45	52.7

1 2 3 4

Figure 26

Heating Time vs Percent Protein Extracted: Corn Meal

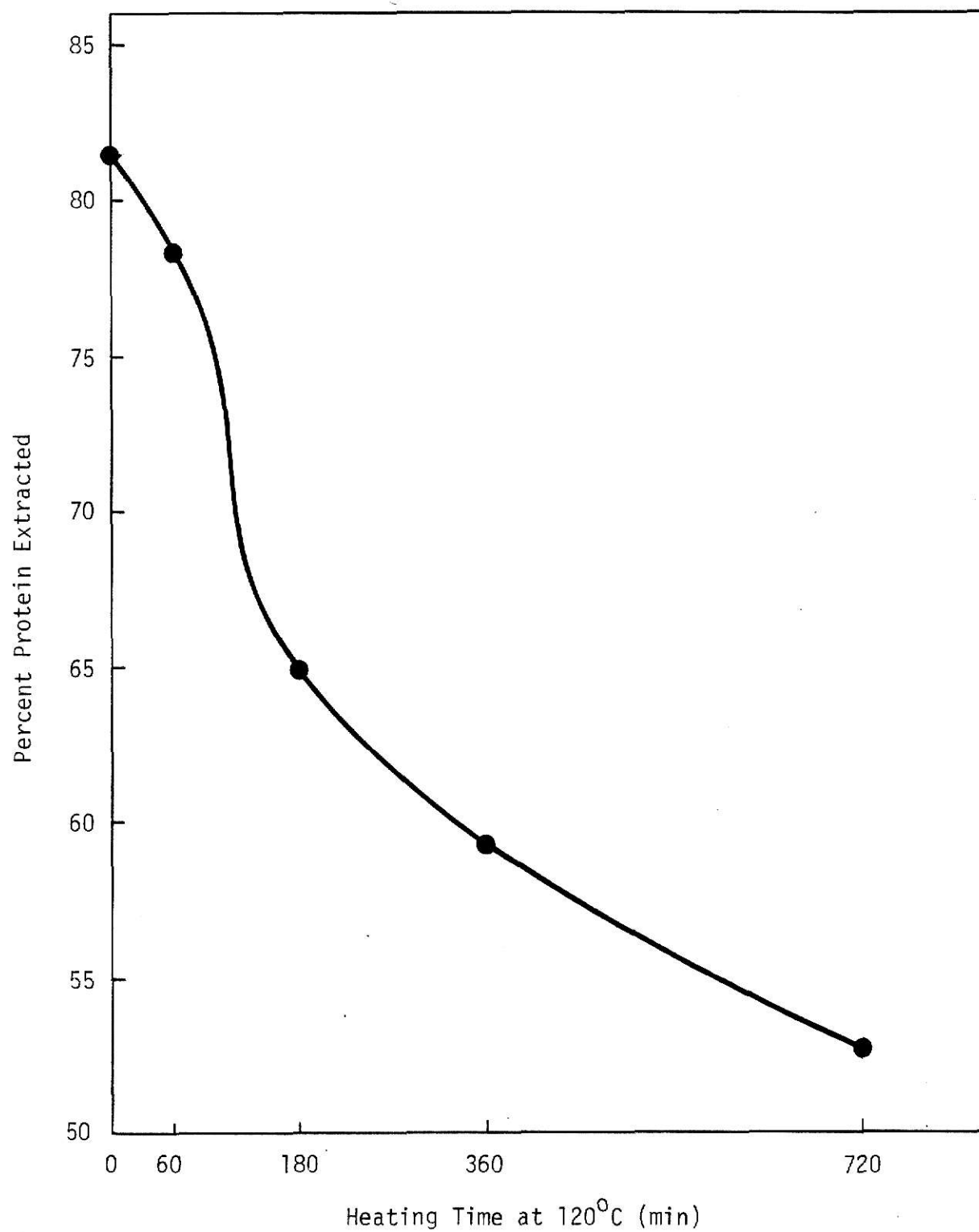
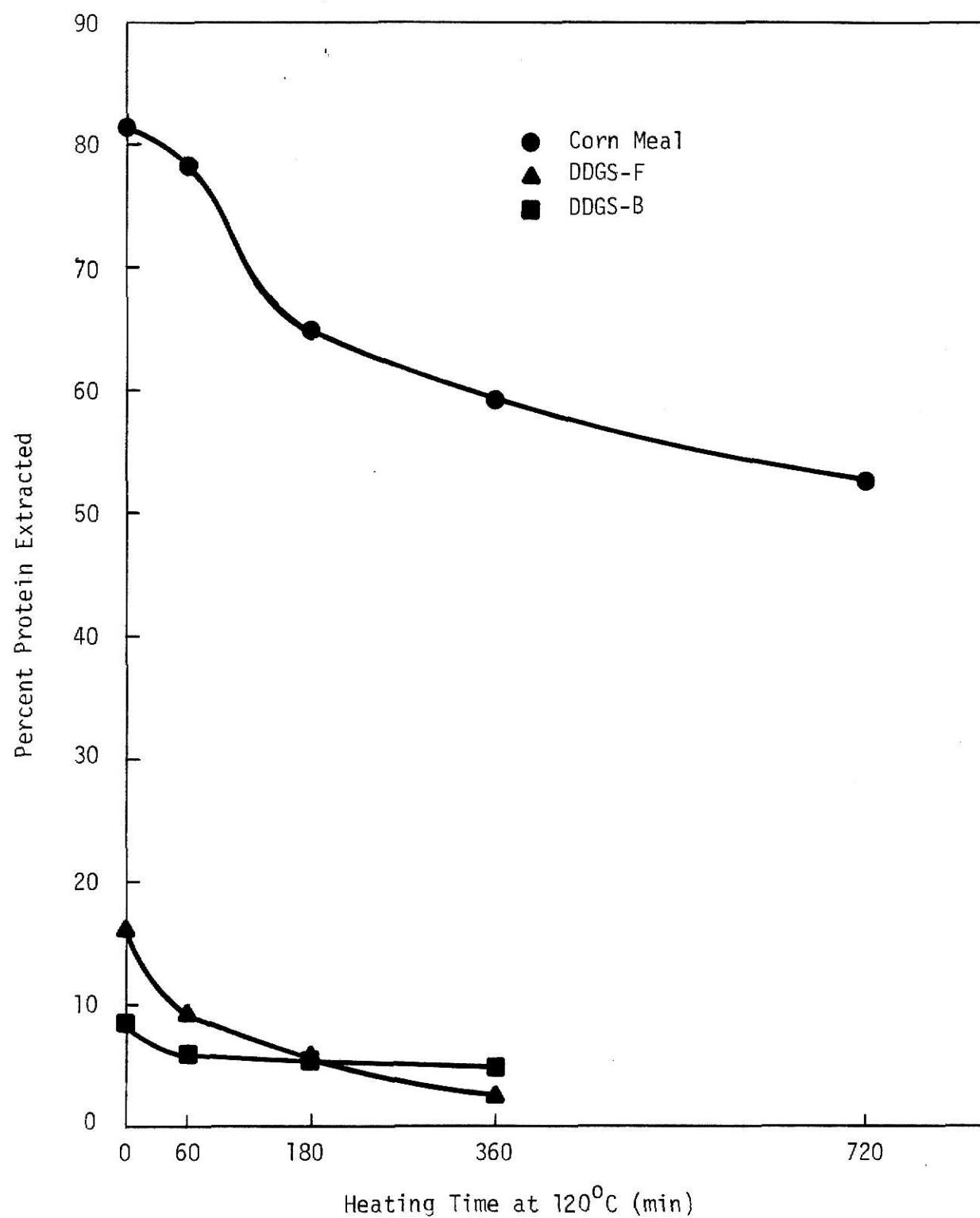


Figure 26
Heating Time at 120°C vs Percent Protein Extracted
DDGS and Corn Meal



air. They also showed that protein extracted by water and 5% sodium chloride decreased with higher temperatures.

In the alkaline extraction process, the protein removed from the sample would be mostly corn glutelin, although some zein and other proteins would also be removed. Alkali splits disulfide bonds, rendering the protein soluble and thus extractable.

When the corn (corn meal or DDGS) is heated, many complex reactions take place, including the Maillard reaction, as well as numerous types of crosslinking (Dworschak 1980, Hurrel and Carpenter 1975). The result is a highly complex and altered protein system much lower in solubility. Wall et al. (1975) studied the effects of drying on the solubility of corn proteins. They used several protein dissociating agents (0.5% sodium dodecyl sulfate, CM guanidine hydrochloride, and 0.6% B-mercaptoethanol) in attempts to determine what types of interactions occur in the corn proteins. It appeared that heat denaturation of proteins involved changes from stable conformations to more random structures that permit hydrogen bonding, hydrophobic interactions, as well as disulfide bond formation from sulfhydryl groups, and conversion of intramolecular disulfide bonds to intermolecular disulfide bonds. The formation of these new intermolecular disulfide bonds contributes greatly to the decreased solubility of heated proteins.

Gel Filtration

Peak recordings of the gel filtration separations of DDGS-F protein concentrates are shown in Figure 27. Through the series, from zero heating time to 360 minutes, there appears to be a slight shift from large peptides to smaller peptides. Most prominent is the decrease in height of area II, and change of shape of area III. Peak I, appearing at the front, has a molecular weight calcu-

lated to be 450,000; peak II, 250,000; area II, 130,000 - 50,000; and peak IV, 30,500.

This is also the case with the protein concentrates from DDGS-B when separated on the Sephadex G-200 gel column (Figure 28). Calculated peak molecular weights were very similar to the DDGS-F proteins. There is a change in the shape of the curve, again a decrease in area II, and a shift in area III toward smaller peptides. Compared to DDGS-F, the peak in area II is less prevalent, and the peak in area IV is more prevalent in DDGS-B.

The above printouts reveal that the protein concentrates from the more severely heated samples had a greater proportion of lower molecular weight proteins or peptides. This procedure was originally designed to reveal any structure changes that may have taken place due to heating; thus, one might hastily conclude that heating caused some type of breakdown in the protein structure. But the great decrease in protein solubility was not foreseen, and it must be accounted for. A more plausible explanation would be that with heating of the corn proteins, the overall solubility decreases, but the solubility decrease of the larger molecular weight peptides is much greater than that of the smaller peptides. Thus, the protein concentrates of the more severely heated samples contain a higher proportion of smaller molecular weight peptides than do the unheated DDGS samples. When equal amounts of both unheated and heated proteins concentrates are separated, the heated sample has a higher concentration of lower molecular weight polypeptides.

The sample preparation method used with the corn meal proteins gives more evidence for this assumption. This alternate method was used because of the difficulty in suspending the protein concentrate in the Tris buffer. At zero heating (Figure 29), the low molecular weight peptides (area IV) made up a small proportion of the sample. With increased heating, the total amount of protein

solubilized decreased. There was little difference after 60 minutes heating, but after 720 minutes, the height of the large protein peak (area 1) had decreased significantly, but peak area 4 was nearly the same size as with zero heating. Evidently, some of the smaller proteins remained soluble after heating. In addition, there was a decrease in height of area 2, and relative increase in area 3. Calculated molecular weights are for peak I, 450,000; peak II, 320,000; area III, 110,000 - 76,000; and peak IV, 30,500.

Difference in solubility, though, may not be the only reason for the increase in proportion of lower molecular peptides. Wu et al. (1981) shows that proteolysis of proteins occurs during the production of alcohol from corn. They used an ultrafiltration procedure on off-screen supernatant, and found more low molecular weight nitrogen compounds present than is normally found from unprocessed corn.

Figure 27
Gel Filtration Separations of DDGS-F Protein Extractions

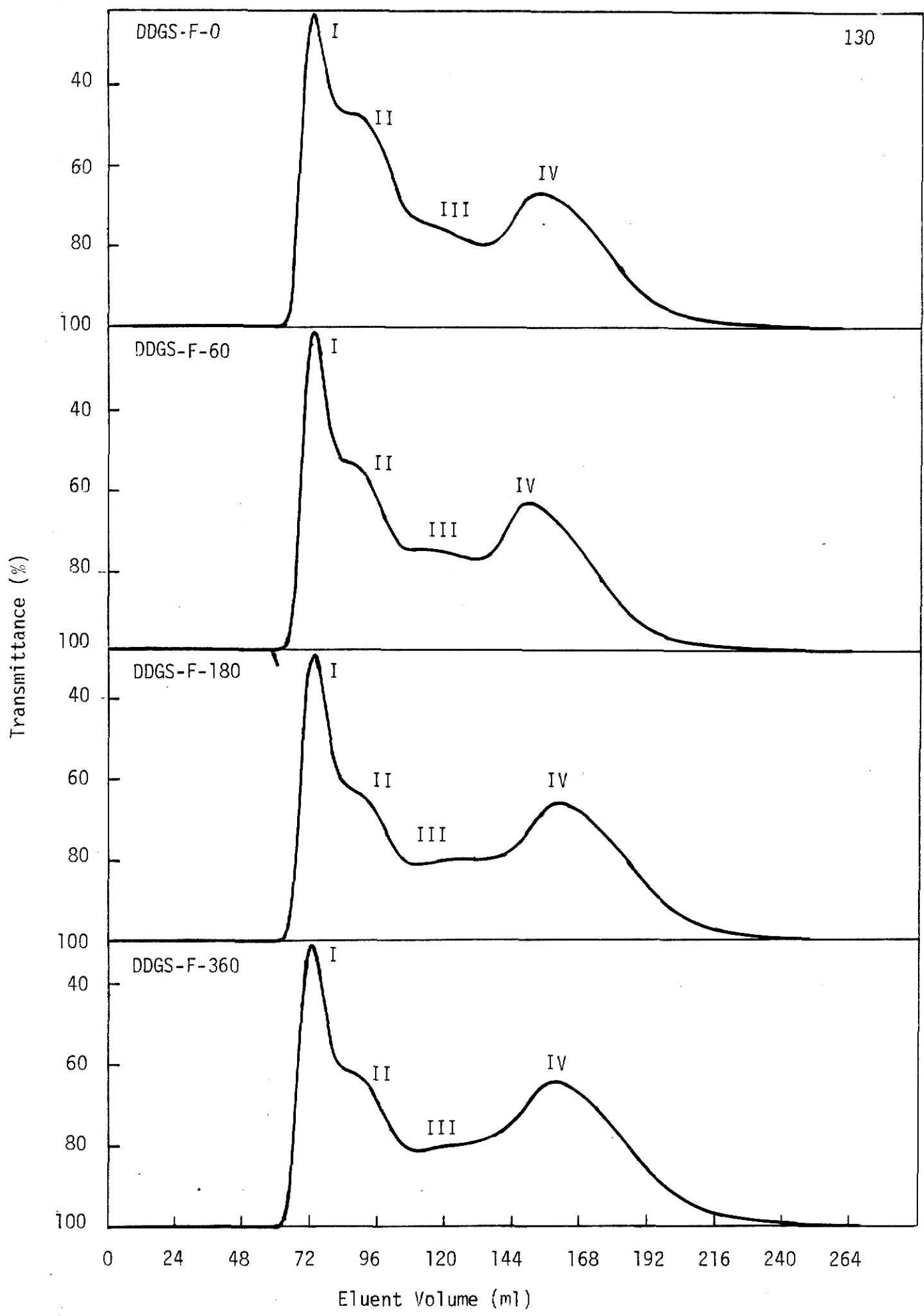


Figure 28

Gel Filtration Separations of DDGS-B Protein Extractions

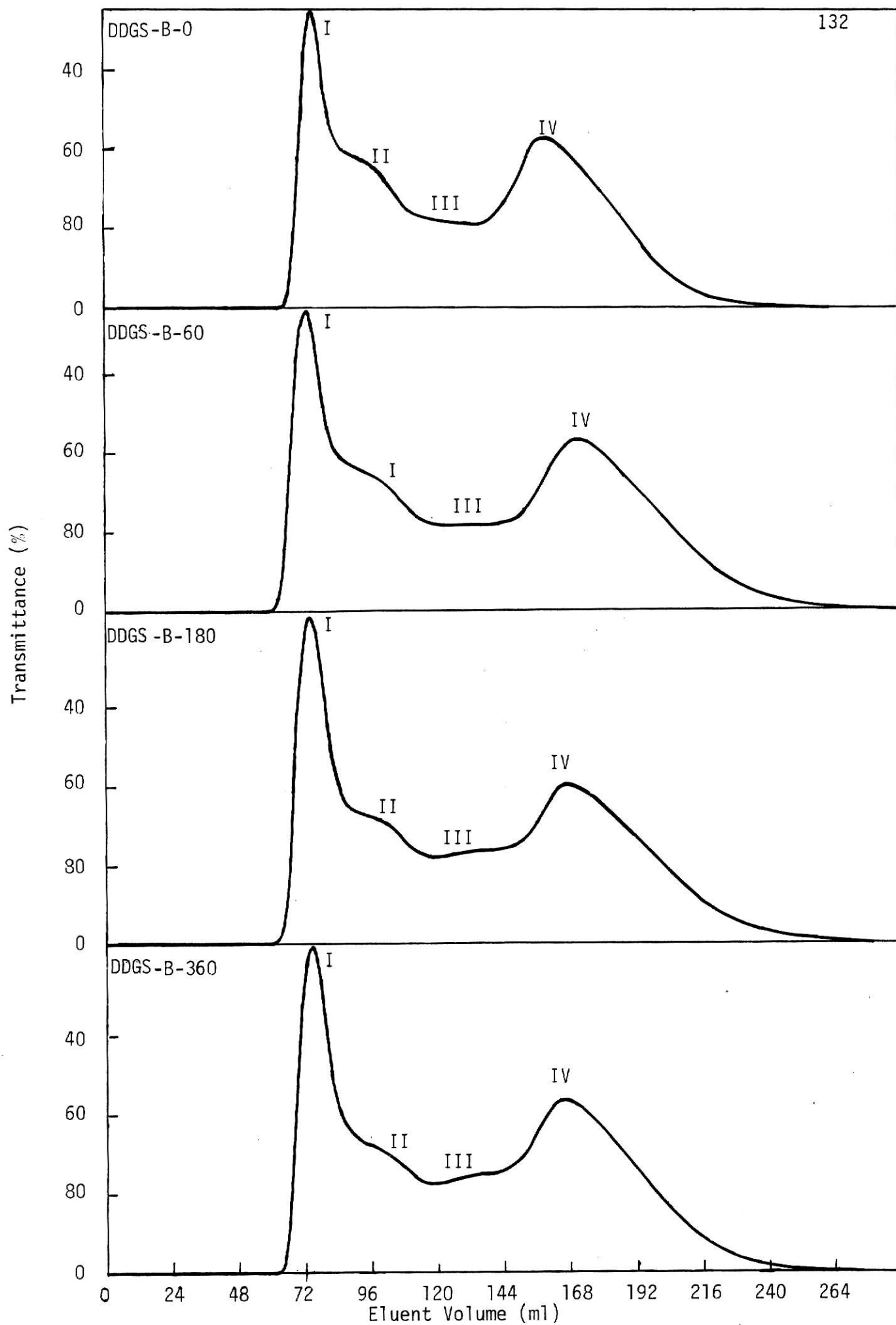
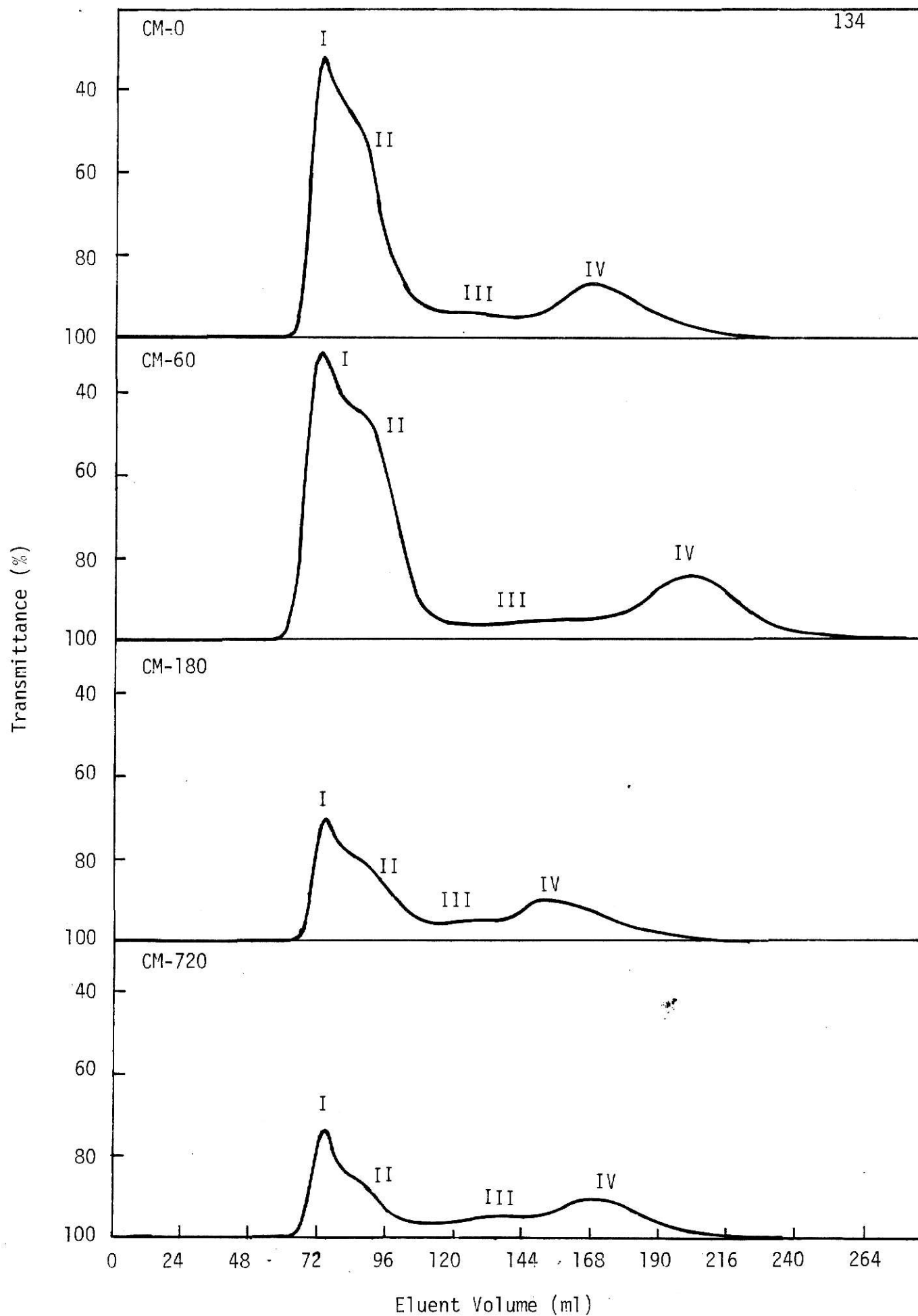


Figure 29

Gel Filtration Separations of Corn Meal Protein Extractions



CONCLUSIONS

A procedure for separating high protein fractions from DDGS was developed in an attempt to increase the value of the distillery by-product as food ingredient. By dry separation methods, it was found possible to recover fractions at relatively high protein contents and yields. One sample that was analyzed produced a fraction containing 45% protein at 50% yield, compared to 28% protein in the starting material.

The final results appear to depend on several factors. Besides initial protein content, one of the most important factors is initial particle size of the DDGS. Larger particle size material produced more satisfactory results. In this case, as with DDGS-B, the fibrous particles were separated more effectively by the sifting (48W) and air separation procedures. In addition, higher moisture content produces higher protein material but at a lower yield; and higher fat content of the DDGS (if not defatted) may decrease efficiency of final sifting procedure by clogging the sieves.

Heat processing greatly affected the properties of the corn proteins. Heated DDGS samples had much lower total and available lysine values. This was most likely a major cause of the reduced PER and apparent digestibilities of heated DDGS found by Tsen (1982) in rat feeding studies. Thus, protein from severely heated DDGS is of much lower quality. The decrease in total lysine indicates that advanced stages of the Maillard reactions have taken place.

Decreases in alkaline solubility of DDGS and corn meal proteins also resulted after heating. It is obvious the distillery and drying processes greatly reduced solubility if the solubilities of corn meal and DDGS proteins are compared. From the gel filtration results, it appears that the larger proteins and peptides were affected to a greater extent than the smaller ones.

Many types of heat induced reactions may be taking place, resulting in molecular aggregation and reduced solubility. Covalent linkages are formed, and hydrogen bonding or hydrophobic group interactions occur non-covalently (Wall et al. 1975). Corn proteins in particular may be susceptible to oxidation of sulfhydryl groups and/or disulfide interchange. These linkages that are formed may also be enzyme resistant, thus would also be responsible for decreased digestibility.

Before DDGS high protein fractions or DDGS protein concentrates can be used in foods as a protein or fiber source, more work would need to be done concerning the functional properties and compatibilities of the DDGS products in different food systems. Methods of counteracting any deleterious effects, such as color or flavor changes, volume decreases etc., must be developed.

In addition, the feasibility of high protein fraction or protein concentrate recovery must be studied. Capital costs of equipment, labor and production costs, and possible selling price of the product would have to be considered in determining economical possibilities of the products.

If distillery by-products are to be used in human foods, it would be desirable to maintain the protein quality at as high a level as possible. The existing drying methods could possibly be modified to improve the resulting protein nutritional value. Lower temperatures or shorter drying times could prove beneficial to decrease the severity of the process. Another possibility would be to have a more complete removal of water before the DDGS entered the dryer, as a way of reducing drying time.

It is clear from this study and others that heat processing severely decreases the nutritional quality of the protein, mainly due to the destruction of the limiting amino acid, lysine. To maximize the usefulness of the DDGS in food applications, the amount of protein damage must be as low as possible.

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DISTILLERS DRIED GRAINS WITH SOLUBLES: SEPARATION OF
HIGH PROTEIN FRACTIONS AND EVALUATION OF PROTEIN CHARACTERISTICS

by

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Distillers dried grains with solubles (DDGS) is a by-product of commercial alcoholic distillation. At the present time, distillers grains are used only in animal feeds, with no commercial use of DDGS in human foods. But since DDGS is high in protein and other nutrients, it does exist as a possible food ingredient.

This study of DDGS was divided into two parts. The first experiment was the separation of high protein fractions from DDGS by dry fractionation methods; the second was the study of the protein characteristics of DDGS, and the effect of heat on the proteins. DDGS samples used had protein contents of 28.1% to 29.6%.

Several variations of a procedure to separate high protein fractions from DDGS were used and compared. The procedure included defatting of the DDGS, sifting and air separation to remove low density material.

Yield and protein contents of four procedures, four samples, and three moisture levels were compared. It was found possible to produce a fraction containing 45% protein at 50% yield, compared to 28% protein in the starting material. Final results appear to depend on initial protein content, moisture content, fat content, but most importantly, initial particle size.

In the second part of the study of two DDGS samples were heated at 102°C from 30 - 360 minutes. The extent of decreases in total and available lysine after heating were determined. Total amino acid analyses was run on a Dionex D-300 single column analyzer. Available lysine was found using the Udy-Carpenter dye binding procedure utilizing Acid Orange 12 Dye. Both lysine values of the DDGS samples decreased substantially when heated further. The light colored sample and dark colored sample dropped from 3.21 to 1.93, and 2.04 to 0.96 g lysine/100 g protein, respectively, and available lysine values dropped from 4.12 to 1.48, and 3.65 to 1.21 g available lysine/100 g protein, respectively. Unexpectedly, available lysine values were higher

than total lysine in almost every case. This may be due to the dye complexing with other compounds, such as starch or fiber.

Protein in the DDGS samples were then subjected to an alkaline extraction process. The light colored DDGS fell from 16.2% protein extracted to 2.6% after 360 minutes of heating. The dark sample decreased from 8.5 to 5.0% protein extracted. By comparison, 84% of the protein in corn meal was extracted. Thus, the distillery and drying processes greatly affected the solubility and perhaps availability of the corn proteins.

The extracted proteins were run through a gel-filtration column (Sephadex G-200) using 0.1M Tris buffer. Results showed that the protein extracted from more extensively heated DDGS had a greater proportion of smaller molecular weight peptides. It appeared that the heating process greatly decreased solubility of the larger peptides, and affected the smaller peptides to a lesser extent. In addition, some breakdown of the protein due to the heating may have taken place.