



Nutritional and functional characteristics of barley-derived distillers dried grain and milled fractions as sausage extenders  
by Marjorie Christine Levine

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Home Economics  
Montana State University  
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**Abstract:**

Barley is being used extensively by biofuel plants in Montana. Identification of the nutritional qualities and functional properties of distillers dried grain (DDG) derived from barley would be helpful in determining its applicability in food products and value as a potential protein and fiber source.

DDG was milled into fine, coarse, and shorts fractions. These, along with whole DDG and a soy isolate control, were evaluated for proximate analysis (moisture, ash, crude fiber, fat, and protein), neutral and acid detergent fiber analysis, amino acid determination, and the functional properties protein solubility, water-binding, and emulsification. DDG and DDG milled fractions in Polish sausage substituted at 3.5 percent levels were evaluated by organoleptic testing. Emulsion stability, color, and texture were also evaluated.

The fine milled fraction contained almost double the protein of other milled fractions. Amino acid analysis revealed whole and fine DDG fractions to be deficient in lysine. Protein solubility and emulsifying activity were low in all milled fractions. The waterbinding capacity of DDG was comparable to other meat extenders.

Sausage extended with DDG fine milled fraction was similar to the control sausage in composition, cooking loss, and texture, but varied considerably in color.

DDG's application in meat products is limited by lack of adequate functional properties. DDG could be added at low replacement levels in a filler capacity, but would do little to improve texture and flavor of meat products. Distiller dried grain does represent a potential protein and fiber source but would be beneficial only in a product where protein solubility and other functional characteristics are not important in consumer acceptability.

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MONTANA STATE UNIVERSITY  
Bozeman, Montana

December 1983

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency, and is ready for submission to the College of Graduate Studies.

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## ACKNOWLEDGMENTS

Sincere thanks is extended to my graduate adviser, Dr. Rosemary Newman. I would also like to express appreciation to the other members of my committee, Dr. Ramona Marotz-Baden, Dr. Jacquelynn O'Palka and Dr. Charles McGuire, and to Dr. Peter Gras. Most of all, I acknowledge the unending support, patience and love of my husband, Chris.

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## ABSTRACT

Barley is being used extensively by biofuel plants in Montana. Identification of the nutritional qualities and functional properties of distillers dried grain (DDG) derived from barley would be helpful in determining its applicability in food products and value as a potential protein and fiber source.

DDG was milled into fine, coarse, and shorts fractions. These, along with whole DDG and a soy isolate control, were evaluated for proximate analysis (moisture, ash, crude fiber, fat, and protein), neutral and acid detergent fiber analysis, amino acid determination, and the functional properties protein solubility, water-binding, and emulsification. DDG and DDG milled fractions in Polish sausage substituted at 3.5 percent levels were evaluated by organoleptic testing. Emulsion stability, color, and texture were also evaluated.

The fine milled fraction contained almost double the protein of other milled fractions. Amino acid analysis revealed whole and fine DDG fractions to be deficient in lysine. Protein solubility and emulsifying activity were low in all milled fractions. The water-binding capacity of DDG was comparable to other meat extenders. Sausage extended with DDG fine milled fraction was similar to the control sausage in composition, cooking loss, and texture, but varied considerably in color.

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## CHAPTER 1

## INTRODUCTION

The need for an economical biofuel process has prompted renewed interest in distillers dried grain, DDG. DDG, the protein by-product remaining after the alcohol fermentation process, has potential both as a source of protein for human nutrition and as a marketable product to help defray some of the costs of the fermentation process. The "spent" grain has traditionally been utilized as a cattle feed supplement. The advent of the alcohol fuel process has brought about an increased need to find an acceptable food use for distillers dried grain.

Cooking, fermentation and distillation comprise the three main processes in alcohol fuel production (Figure 1). The cooking process converts carbohydrates to fermentable sugars by use of enzymes. In the fermentation process, these simple sugars are changed to alcohol by specific yeast varieties and yield a 6-12% ethanol-water mixture. This ethanol mixture is nonflammable because of the high water content. Therefore the ethanol must be concentrated. A series of evaporation and condensation steps is used to separate the ethanol from the less volatile water. The difference in boiling points of water and alcohol allows for this separation. The end product is a 96% proof alcohol solution (Hunt, 1981).

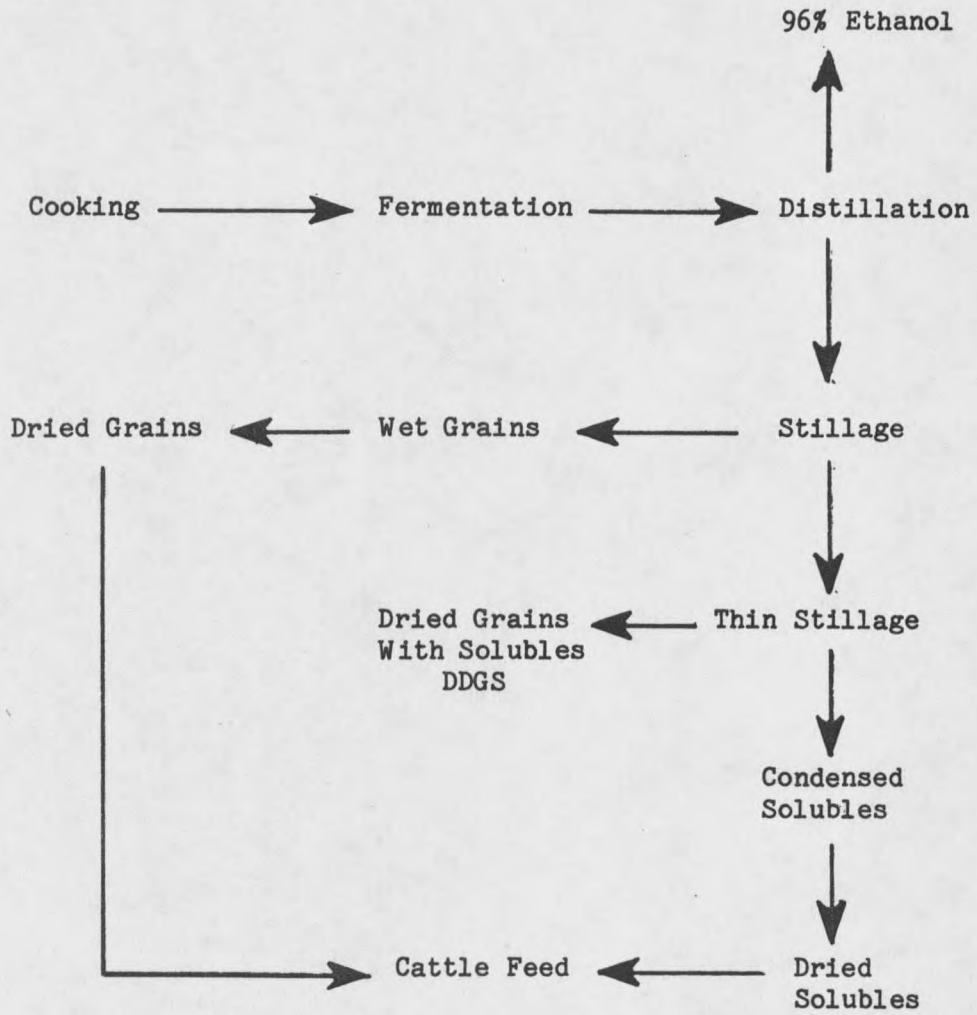


FIGURE 1: ETHANOL PRODUCTION (Derived from Hunt, 1981)

The residue in the bottom of the still is a substance called stillage, made up of 90% water and 10% suspended solids. The stillage is filtered or centrifuged to separate the wet grain from the thin stillage. The wet grain can then be dried for easier transportation and storage. The solubles can be condensed from the thin stillage and added to the dried grain to form distillers dried grain with solubles (Hunt, 1981).

#### Need to Study to DDG

As ethanol production increases, an enormous amount of DDG will be produced. One of the Department of Energy's goals was to produce 500 million gallons of ethanol by 1981, 2.3 billion by 1983, and 11.5 billion by 2000 (Solar Energy Research Institute, 1981). Production of this amount of ethanol leaves behind an extensive amount of DDG. A Pillsbury Company (1980) estimate is that 1 bushel of corn will produce 2.5 gallons of ethanol and 17 pounds of distillers dried grain or 6.8 pounds of DDG are produced per gallon of ethanol. Thus, in the production of 1 million gallons of ethanol requiring 400,000 bushels of corn, 3,400 tons of DDG would be produced. If the goal of 2.3 billion gallons of ethanol by 1983 is achieved utilizing corn as the starting grain, 7,360,000 tons of DDG would be available.

With the expansion of the alcohol fuel industry, a greater variety and quantity of feedstocks or starting grains are being utilized in alcohol fermentation. Some research has been done on distillers dried grain/brewers dried grain by the brewing industry. However, as the amount and variety of grains used in the liquid fuel industry increases,

the need increases to identify the potential of DDG derived from feedstocks other than those traditionally used in the brewing process. Barley which is presently being utilized as an alcohol fuel source in Montana, is one such grain that requires investigation.

A variety of feedstocks are now being utilized in alcohol production. Corn constitutes the largest potential feedstock supply (Solar Energy Research Institute, 1981), but other grains are being investigated to determine the alcohol and by-product yield and their economic value. The attributes of hardiness and adaptability to wide variations in soil and climate, genetic manipulability and its wide variation in nutritional composition (Truscott, 1980) increase barley's potential for becoming an important fermentation feedstock especially in climates too cool to grow corn.

Alcohol yield varies with the feedstock utilized in its production. Corn and wheat have the greatest yields of 2.5 and 2.4 gallons of ethanol per bushel respectively, while barley yields 2.1 gallons/bushel. When figured on a gallon per acre basis, corn is by far the most productive, yielding 228 gallons/acre. Barley yields 92 gallons/acre and wheat 74 gallons/acre (Solar Energy Research Institute, 1981).

Grain cost is one of the most important factors in the choice of feedstock. Significant price fluctuations occur seasonally and on a yearly basis due to supply and demand and government policies. The average price paid to farmers between 1963-1977 was \$2.69/bushel for corn, \$2.35/bushel for barley, and \$3.46/bushel for wheat. The lower feedstock cost of barley would make ethanol production from barley less

expensive than wheat (\$1.24/gallon versus \$1.36/gallon), but more costly than corn at \$1.14/gallon (Solar Energy Research Institute, 1981).

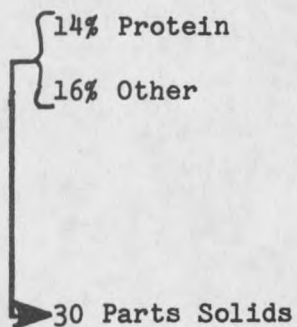
By-product or DDG value should also be considered in choice of feedstock as DDG can represent an additional source of income. The amount of by-product produced is determined mainly by the composition of the feedstock. Figure 2 shows the approximate compositions of wheat and barley and shows how the approximate yield of DDG may be calculated. The greater amount of by-product produced from barley feedstock makes it even more important that a marketable product be found for DDG.

The conversion of agricultural crops to liquid fuel is an expensive process, influenced by the yield of alcohol and by-product and the cost of feedstock and processing. Unless the by-product can be effectively utilized in marketable foods, the process is inefficient and uneconomical. Department of Energy figures estimate a net production cost of ethanol of \$1.29 per gallon which includes \$1.01 for raw materials, \$.45 for operating costs, \$.15 for fixed costs and taxes, and minus the \$.35 obtained from sale of stillage for cattle feed. Allowing \$.33 for dealer margin and \$.08 for transportation, gasohol would have to be sold at \$1.70 per gallon to break even (Solar Energy Research Institute, 1981). However, if more money could be obtained from sale of the by-product, ethanol production would be much more competitive. At the moment, DDG is sold at \$.07 per pound and utilized mainly for cattle feed. If it could be sold at the current

WHEAT

60% Starch

10% Moisture



$\frac{30}{.9}$  yields 33% DDGs

$\frac{14\% \text{ Protein}}{33.3\% \text{ DDGs}} = 42\% \text{ Protein}$

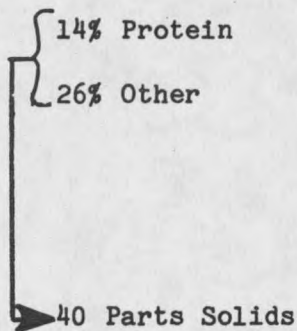
32 Pounds of Wheat Yields

4.8 Gallons Ethanol

BARLEY

50% Starch

10% Moisture



$\frac{40}{.9}$  yields 44.44% DDGs

$\frac{14\% \text{ Protein}}{44.44\% \text{ DDGs}} = 31.4\% \text{ Protein}$

32 Pounds of Barley Yields

4 Gallons Ethanol

Figure 2: Approximate DDG and Alcohol Yield from Wheat and Barley (Gras, 1982)

price for other protein concentrates for human consumption, i.e. sodium caseinate at \$1/pound, promine D (soy protein) at \$.60/pound, or Torutein (yeast) at \$.42/pound (Scheller and Mohr, 1976), the profit margin could be increased considerably.

#### Problem Statement

Distillers dried grain (DDG), the protein by-product from alcohol fermentation, has nutritional qualities which could make it a valuable product for human consumption. With the expansion of the alcohol fuel industry, a greater variety and quantity of DDG is being produced. DDG represents a potential protein and fiber source for human food. Besides nutritional quality, functional properties are important in identification of appropriate food systems for DDG incorporation. Evaluation of the physical characteristics would help in determining the extent of denaturation and in predicting applicability in food products. The objective of such an evaluation would be to find a product where the nutritional qualities could be utilized and where the functional properties would be adequate to produce an acceptable product.

DDG contain approximately 27-37% protein and 13-16% fiber, depending on the feedstock used in the alcohol fermentation process. At present, DDG is used mainly as a low-cost animal feed supplement. Several studies have been done to examine possible human food uses of DDG. Most of these studies have utilized brewers spent grain derived from corn and wheat. The advent of the biofuel industry, however, has encouraged the use of other cereal grains as a feedstock. Barley is

one grain that is being used more extensively. As each grain varies in structure and composition, nutritional quality and physical characteristics will vary accordingly. Identification of the nutritional quality and functional properties of each type is necessary to evaluate their potential in a food system.

DDG has been successfully incorporated into baked goods at levels up to 15% flour replacement. At higher substitutions, products exhibited poor functional characteristics as demonstrated by low volume and texture changes. Color and flavor also needed improvement. Products utilizing DDG at higher levels would require further processing and/or additives.

DDG might be utilized in other food systems such as comminuted meat products. Milling DDG into more uniform particle-sized fractions was suggested by Finley and Hanamoto (1980) to help optimize utilization in specific products. The high protein fraction after milling was predicted to have better application in extruded or fabricated foods. An acceptable meat extender from brewers spent grain press water has been produced when extruded with other cereal grains (Finley et al., 1976). Sausages with 1% brewers spent grain substitution were rated almost equal to control sausages in a study by Koivurinta et al. (1980). Incorporation of DDG at high levels in meat products still represents a problem.

The challenge of finding a human food product for DDG utilization requires a two-fold approach. First, nutritional qualities and functional properties need to be analyzed for the specific type of DDG being produced, as type of feedstock and processing method affect both

the nutritional and functional characteristics of DDG. Then, a product needs to be identified where the nutritional potential of DDG can be realized within the bounds of functional adequacy. The objective of such a study would be to find an organoleptically appealing product which could be produced with a minimum amount of additional processing.

### Objectives

The objectives of this study were as follows:

1. To assess the nutritional quality of DDG and DDG milled fractions from alcohol production from barley by selected analyses.
  - a. To measure the proximate composition of DDG and DDG milled fractions utilizing AOAC techniques. Moisture, ash, crude fiber, acid and neutral detergent fiber, fat, and protein will be analyzed.
  - b. To determine total amino acid composition.
2. To identify certain functional properties of barley DDG and DDG milled fractions as related to their use as a meat extender.
  - a. To determine extent of denaturation and amount of soluble protein in barley DDG and DDG milled fractions by measuring soluble nitrogen.
  - b. To determine emulsifying capacity and emulsifying stability of barley DDG and milled fractions.
  - c. To assess water-holding capacity of barley DDG and DDG milled fractions.

- d. To determine how different milled fractions of barley DDG will perform as a meat extender in sausage by organoleptic evaluation of test sausages.

#### Definition of Terms

1. Binder--meat extender that binds water and acts as an emulsifier (Price and Schweigert, 1971).
2. Binding--ability of protein to bind and absorb other food components and hold them together in solid, semi-solid, or fluid state (Kinsella, 1976).
3. Brewers spent grain (BSG)--the solid residue left after filtering the brewing mash consisting of husk, bran and embryo residues of malt kernel and corn bran if corn grits used as adjunct. Brewers spent grain may include yeast and trub, the coagulum which separates after wort boiling (Prentice and D'Appolonia, 1977).
4. Brewers dried grain (BDG)--brewers spent grain which have been dried by conveyer or drum drier (Prentice and D'Appolonia, 1977).
5. Comminution--the cutting of meat into smaller particles (Price and Schweigert, 1971).
6. Distiller's dried grain (DDG)--grain from which alcohol has been removed after fermentation (Ockerman, 1978). See also brewers dried grain.
7. Distillers protein concentrate (dpc)--protein products obtained by extracting protein from distiller's dried grain (Kendrick, 1976).

8. Emulsion capacity (EC)--the amount of oil that can be emulsified or held in solution by protein before phase inversion or collapse of emulsion (Kinsella, 1975).
9. Emulsion stability (ES)--the ability of a protein to form an emulsion that remains unchanged for a particular duration under specific conditions (Kinsella, 1976).
10. Extender--any ingredient, other than salt or water, that is added to meat in sufficient quantity to contribute materially to bulk and composition (Price and Schweigert, 1971).
11. Feedstock--cereal grain or other material used as a substrate for alcohol fuel production (Solar Energy Research Insititute, 1981).
12. Filler--starchy product adding bulk and sometimes water binding ability to meat products but not emulsification properties (Price and Schweigert, 1971).
13. Functional property--any characteristic of a substance, besides nutritional ones, that affects its utilization and determines its overall performance in foods during manufacturing, processing, storage, and consumption (Kinsella, 1976).
14. Organoleptic--A test of a food product evaluated by a sense perception (feeling, sight, smell, or taste) (Ockerman, 1978).
15. Protein Solubility--proportion of nitrogen capable of passing into solution (Kinsella, 1976).

### Limitations

Lack of testing equipment and cost of commercial tests have limited this study to the selected analyses. Amino acid analysis was limited to two samples of DDG. Protein solubility and emulsification tests were performed by methods appropriate to available laboratory equipment. Sausage type and quantity were adapted to fit available facilities.

### Delimitations

This study was delimited to the type of distillers dried grain being produced in the state of Montana. Barley DDG which has been drum dried will be utilized as this is the type currently produced by surrounding alcohol plants.

Incorporation of distiller's dried grains into meat products is delimited to 3.5% by the United States Department of Agriculture's regulations on Meats and Meat Products.

### Assumptions

1. Ethanol production from ethanol fermentation of barley will increase with greater energy demands and improved technology so that a great quantity of DDG will be available for food.
2. The demand for new protein forms will grow as living standards increase and as resources become more limited.
3. Vegetable forms of protein will gain more acceptance in fortified, extended and extruded food products.

4. The utilization of waste materials from alcohol production plants will have more advantages than starting with raw, unprocessed materials.

#### Hypothesis

It was hypothesized that distillers dried grain from barley would possess adequate nutritional qualities to make them a valuable addition to human food systems.

It was hypothesized that distillers dried grain and milled fractions would display functional properties that would make them suitable for incorporation into meat products.

It was hypothesized that distillers dried grain and milled fractions from barley would perform adequately as an emulsifier in a basic sausage formulation.

## CHAPTER 2

## LITERATURE REVIEW

The specific purpose of this section was to present an overview of literature concerning nutritional and functional characteristics of distiller's dried grain and its application to food systems. The literature selected for review will provide background information in these areas: (1) nutritional characteristics, (2) functional characteristics, (3) protein solubility, (4) binding, (5) emulsification, (6) DDG in baked goods, and (7) DDG in meat products.

Nutritional Characteristics

Distiller's dried grain contain essentially three components: a non-soluble protein, a fiber component, and cereal oils. The exact composition of DDG depends on the malt and adjuncts utilized by the brewer in the mash. The proximate analysis of the spent grain is 27-37% protein, 13-16% crude fiber, 4-5% ash, 6-7% fat, 31-45% carbohydrate depending the starting grain (Stiles and Hebert, 1977).

DDG's main contribution to foods is to increase protein content. Two methods have been utilized to concentrate the protein content-- either a mechanical method of centrifugal separation and/or screening or a chemical method using alkaline extraction. About two thirds of the protein present in "spent grain" may be isolated by the purely

mechanical methods of stirring, pressing, settling, and centrifuging (Loncin and Schornick, 1977).

Brewer's protein ranges in size from 1/4 micron to 100 microns with greater than 50% of the protein smaller than 8 microns (Stiles and Hebert, 1977). Being much smaller particles than the cellulose fiber, the protein may be separated by a screening centrifuge. Townsley (1979) found fractionation of spent grain could be achieved without expensive preliminary drying.

Chemical isolation of protein may be done by an alkaline extraction procedure utilizing caustic soda (Kendrick, 1975) or ammonia (Scheller and Mohr, 1976). After centrifuging the alkaline solution, protein is precipitated by either acetic, sulfuric, or hydrochloric acid. Wet solids yielded 1.5 to 3 times more DDG protein concentrate than the dried grain. The greatest amount of protein was extracted from corn at 80-90° C and with sonication, while wheat protein extraction was greatest at 23° C without sonication (Kendrick, 1975). The percentage of protein recovered was 54.0% for corn and 36.4% for wheat.

Protein quality may be affected by the processing DDG has undergone. Protein efficiency ratios (PER), feed efficiency ratios and gross digestibilities of wheat and corn and their protein concentrates from DDG were measured by Kendrick (1975). The PER of the distillers dried grain protein concentrates were lower than the values for the whole grain, but were still adequate. Digestibilities and feed efficiency ratios were slightly higher than their starting grain. Low protein quality was found by Ranhotra et al. (1982).

Amino acid analysis showed DDG had a amino acid profile similar to their starting grain (Satterlee et al., 1976). Even with their higher amino acid levels, DDG protein concentrates were deficient in lysine and corn distillers protein concentrate in tryptophan.

Several studies have shown that the protein content of foods may be enhanced by the addition of distiller's dried grain. Products utilizing 40% DDG have 74% more nitrogen than 100% wheat flour products (Prentice et al., 1978). By substituting just 15% of DDG in baked products, which is a more acceptable level, nitrogen content of flour has been increased by 27% (Prentice et al., 1978). Prentice and D'Appolonia (1977), substituted 15% DDG in flour and increased protein by 15%.

Another marketable characteristic of food products containing DDG is the benefit derived from a higher fiber content. Breads and cereals with added fiber are becoming increasingly popular since dietary fiber might have a role in prevention of certain diseases such as diverticulosis, colon cancer, hemorrhoids, arteriosclerosis, varicose veins, and appendicitis (Burkitt and Trowell, 1975). A product with 40% DDG exhibits a ten-fold increase in crude fiber (Prentice et al., 1978). Prentice and D'Appolonia (1977) concluded that a minimum of 10% replacement of wheat flour would be necessary to achieve fiber increases relative to 30% whole wheat bread. At this substitution level, crude fiber and acid detergent fiber were approximately doubled. Prentice (1978) increased the fiber in muffins by replacing up to 15% of flour with brewers spent grain.

Satterlee et al. (1976) found mineral levels in DDG were lower than in soy isolates. When compared to soy isolates, corn and wheat distiller protein concentrates were lower in calcium, magnesium, zinc, and manganese. Iron contents were similar. Wheat had more copper than corn or soy and lead levels were low in all three. Amounts of potassium, magnesium, phosphorous, zinc, copper, iron, chromium, thiamin, riboflavin, and niacin were found to be comparable to the bran fraction of grains (Ranhotra et al., 1982).

#### Functional Properties

A substance must have adequate functional properties to be of value in food systems in addition to nutritional quality. Functional properties of proteins are the intrinsic physiochemical characteristics which affect the behavior of protein in food systems during processing, storage and preparation. Factors which influence these properties are the composition and conformation of the proteins, interactions with other components ( $H_2O$ , ions, protein, carbohydrate, and flavors), amount and type of processing, and the environment (temperature, pH, and ionic strength) (Kinsella, 1979). A determination of functional properties is essential to evaluate potential application and limitations of proteins and to predict their acceptability. Nutritional value is of little consequence if the protein is not acceptable for eating.

Different functional characteristics are important for specific food systems. Water absorption and binding, cohesion/adhesion,

emulsification and fat adsorption are important in sausage and meat systems, while water adsorption and binding, cohesion/adhesion, color and flavor are important in baked products (Kinsella, 1979).

Processing may have affected the physical properties of DDG. The functional properties remaining will determine what food system DDG may be best suited for.

Different laboratory procedures may be used to identify these physio-chemical characteristics. The only real test, however, to determine how an ingredient will function in a system is to incorporate that ingredient into a formulation and produce the finished product (Johnson, 1970). Improved techniques and standardization of testing methods in measuring functionality in proteins will increase the extrapolative ability of functional property measurements to food applications. Good correlations between functional properties and their performance in model meat systems have been found by Hermansson and Akesson (1975b).

#### Protein Solubility

One of the most critical functional properties for application of a protein into any food system is protein solubility. The solubility of a protein depends on the attraction between molecules of the solute compared to the intensity of solvent-solute interactions. When the forces of attraction between solute molecules is greater than interaction forces between solvent and solute, dissolution results. The chemical structure of a protein which includes its kind, number and sequence of amino acids determines solubility. The distribution

of ionic and nonpolar groups of amino acids affect the affinity of protein molecules for water. Solubility is markedly affected by pH. Protein solubility is at a minimum at the isoelectric point and increases with increasing acidity or alkalinity. Other factors affecting solubility are processing, heating, temperature, and ion concentration (Kinsella, 1976).

Different methods may be employed to determine solubility, including protein dispersability and nitrogen solubility (AOAC, 1975). The basic test involves dispersing protein in water, adjusting pH values with hydrochloric acid or sodium hydroxide to obtain desired pH, centrifuging, and determining nitrogen content of the supernatant. Most researchers include a solubility profile at pH ranges from 2 to 12.

Solubility is of paramount importance in food production. Since solubility relates directly to emulsifying ability, foaming capacity, and gelation quality, solubility provides a good index to protein functionality. DDG have undergone heat and other processing treatments. Protein solubility will be helpful in determining their state of denaturation (Kinsella, 1976). Solubility may also be a good indicator of emulsification capacity and stability which would be beneficial in comminuted meat systems (Inkelaar & Fortuin, 1969). Hermansson (1974), however, cautions that solubilities in complex food systems may differ from laboratory profiles due to denaturation, complexing with quaternary structures, the presence of proteins with different net charges, or complex binding between proteins and other components.

The pH solubility profiles of brewers grain reported by Koivurinta et al. (1980) showed poor solubility between pH 3 and 8 increasing slightly over pH 8. Neither increased temperature nor NaCl (2%) had significant effects on the solubility. Finley et al. (1976) found the protein concentrate recovered from spent grain press water to be essentially insoluble at neutral or acidic pH's and therefore suggested uses in food applications where solubility was not critical, i.e., extruded vegetable protein.

### Binding

Binding is an important property in the formation of many foods, especially sausage meats, cheeses and custards (Kinsella, 1976). Water binding or absorption is a measure of the water retained by a protein following filtration and application of mild pressure or centrifugation (Janicke and Walczak, 1954). The water binding capacity is important in adjusting the water ratio for optimum viscosity in food formulations where different proteins have been substituted. For example, soy protein in meat products absorb a different amount of moisture than would the myosin protein.

Many studies have been done to determine the factors affecting binding capacity. The pH and ionic concentration which affect the surface area and properties, presence of carbohydrates and lipids, degree of fat emulsification, particle size of protein, extent of mechanical agitation, modification by enzymes or chemicals and temperature treatments, are all factors influencing binding characteristics (Kinsella, 1976). Berlin et al. (1973) showed that

water absorption increased with the number of components in the system, including nonprotein material. Pure protein was found to bind .5 grams water/gram protein (dry milk). Huffmann et al. (1975) found the water absorption of sunflower meals increased as solubility decreased. Denaturation enhanced the water-holding capacity. Water absorption increased as protein concentration increased and decreased slightly with longer mixing time (Fleming et al., 1975).

Higher water absorption was observed between pH 5 and 6 in comminuted meats. The addition of soy protein or sodium caseinate lessened the effect of pH and increased water retention (Hermansson and Akesson, 1975b). Moisture loss from meat was affected by type of meat added, protein type and amount, prior heat treatment and salt content (Hermansson, 1975a). Salts up to 4% enhanced water binding (Hermansson, 1975a), but had varied effects in systems that had added protein or had been heated.

Koivurinta et al. (1980) found water binding to be low in drum dried and conveyor dried brewers grain (1.1 and 1.5/gram dry matter, respectively). Sodium chloride (2%) did not raise the binding capacity. Higher pH (pH 7) slightly increased the value of water absorption. The method used measured water binding of non-soluble components which may account for the low figures.

#### Emulsification

Emulsification is a surfactant property related to the ability of a protein to lower the interfacial tensions between the hydrophobic and hydrophilic components. Emulsifying properties are measured as

emulsifying capacity (EC), emulsifying stability (ES), or emulsifying activity (EA). Factors which affect emulsification are equipment design, shape of container, blender speed, rate of oil addition, temperature, pH, protein source, solubility of protein, concentration of protein, kind of oil, salt (type and concentration), sugars, and water content. Increased concentration of NaCl caused increased emulsifying capacity of water-soluble proteins and salt-soluble proteins (Saffle, 1968). Carpenter and Saffle (1964) showed that emulsion capacity in meat emulsions was determined by amount of solubility. Protein concentration was inversely related to EC at low and high protein levels, and stability was dependent on the ratio of interfacial film thickness to droplet size (Ivey, et al, 1970). EC and ES were reduced by denaturation of protein caused by processing factors such as cyclic freezing and thawing, high temperature, and low pH (Kinsella, 1976). Ivey et al (1970) found that higher blender speed reduced EC.

Three basic methods measure emulsifying ability of proteins. Pearson et al. (1965) added oil to a blending aqueous protein mixture and determined by visual inspection the point at which the emulsion broke. The emulsion capacity was expressed as g of oil emulsified/mg of protein nitrogen. Oil and water were added to a protein and then centrifuged in a method by Yasumatsu et al. (1972). Emulsifying activity was calculated by measuring the height of the emulsified layer over the height of the entire layer in the centrifuge tube. Heat stability could be tested by heating the emulsion, centrifuging and calculating the ratio as described above. The most objective

emulsification test utilized electrical conductivity to measure the inversion point. Oil was slowly added to a protein suspension until the emulsion broke. A sudden drop in viscosity as demonstrated by a sharp drop in amperage precisely determined the inversion point. Crenwelge et al. (1974) used this method to compare emulsification capacity of four proteins after having optimized the effects of blender speed, oil addition rate, and pH for each sample.

Emulsifying stability and capacity of DDG have been determined in several studies. Koivurinta et al. (1980) reported that brewers grain did not form emulsions under their experimental conditions. A study by Kendrick (1975) showed that the emulsion capacity decreased as the amount of soluble protein decreased. Type of washing also affected emulsification properties (Kendrick 1975). Alcohol-washed corn distillers protein concentrate had greater emulsion capacity than textured soy protein and other distillers protein concentrate, but less than soy isolate. Emulsifying stability of water-washed corn distillers protein concentrate was poorer than the stability of non-fat dry milk and soy isolate since more water and solids were released during sausage cooking (Kendrick, 1975).

#### DDG in Baked Goods

Even though DDG exhibit poor functional properties, low levels of DDG have been successfully incorporated into baked products. Utilization of DDG in baked goods could increase the nutritional components of fiber and protein. Reduced volume, unfavorable color changes, and "feedy" flavor were the main problems for DDG's use in

baked products (Finley et al., 1976; Prentice & D'Appolonia, 1977; Townsley, 1979, Finley & Hanamoto, 1980).

Consumers accepted bread made with brewers dried grain (corn) at 5 and 10% levels if the brewers dried grain had not been dried additionally at 100° C and 150°C. The upper limit for sugar cookies and specialty cookies was 25% incorporation, but organoleptic qualities were lowered significantly if these levels were surpassed (Prentice et al., 1978). Finley et al. (1976) found brewers dried grain protein concentrate from press water was unsatisfactory even at the 5% level in baked goods.

One of the most noticeable effects of DDG on yeast-raised products was a decrease in volume. Volume of bread decreased as DDG addition was increased (Prentice and D'Appolonia, 1977). Kendrick (1975) correlated decreased bread volume with increased protein percentages when the protein came from DDG. Wheat distillers protein concentrate affected loaf volume less than did corn distillers protein concentrate or soy isolates (Kendrick, 1975). Satterlee (1976) also found that wheat distiller protein concentrate had the least detrimental effect on loaf volume when compared with corn and soy protein concentrate. Bread volume was reduced 17% when DDG from corn-malt mash were substituted at 15%, 11% at 10% substitution, and equal in volume at 5% substitution (Prentice and D'Appolonia, 1977). Bread made with 6 and 12% substitution levels of protein concentrate from press water was poor in volume, even lower than 6 and 12% soy fortified bread (Finley et al., 1976). Bran-enriched bread was found superior in loaf volume to bread enriched with ground crude all-malt

or malt-corn grit spent grain (Pomeranz et al., 1976). When DDG was split into different mill fractions, all fractions at 6% levels caused a reduction in volume, shorts decreasing volume the greatest and bran portions, the least. At a 12% substitution, loaf volume was depressed even more with the fine bran bread having the lowest volume (Finley and Hanamoto, 1980). It was concluded that a replacement of flour with DDG at greater than 12% DDG would not yield a satisfactory baked product.

Color is another important product characteristic that was unfavorably changed with DDG addition. All breads made with various milling fractions even at 6% flour replacement, were considerably darker than controls (Finley and Hanamoto, 1980). Color was reduced when spent grain were heat treated at 45° C before being added to bread (Prentice & D'Appolonia, 1977). Townsley (1979) recognized the color limitation and used DDG in products that were not white. DDG could be successfully used in combination with dark flour such as rye, or with molasses and spices. DDG addition was found suitable for dark colored cookies by Tsen et al. (1982).

Flavor intensity increased in products with 10% substitution or more DDG, especially if DDG had been heat treated at 150° C (Prentice and D'Appolonia, 1977). Cookies and breads from presswater protein concentrate were described as tasting "feedy" (Finley et al., 1976). Flavor of chocolate chip cookies with 15% DDG flour was not found any different from controls, however spice and bar cookies were rated lower (Tsen et al., 1982).

Other baking characteristics of DDG have been described in studies done with bread and cookies. Water absorption increased with DDG addition to breads (Finley et al., 1976; Prentice and D'Appolonia, 1977). Poor crumb quality was noticed by Finley et al. (1976), but no change in crumb texture or mouthfeel at 10% levels were recorded by Prentice and D'Appolonia (1977). Dough stability increased with higher DDG level, unless heat treated, and dough extensibility decreased with higher levels. Cookies made from flour with 5% DDG added were smaller, darker, and had poor top grain quality, and off-flavor compared to the control (Finley et al., 1976). Cookie width and thickness were decreased and spread ratio improved with 15-25% DDG substitution (Tsen et al., 1982). Brewers grain levels could be increased to 20% if 2% soy lecithin was added as a surfactant. Cookies baked with this modification had acceptable physical characteristics, but organoleptic quality deteriorated after 15% substitution. Sugar and specialty cookies (chocolate chip, oatmeal and Jan Hagels) were acceptable at the 15% substitution levels (Prentice et al., 1978).

Because of the poor functional characteristics of DDG, additives and separation techniques to enhance desirable traits of DDG have been studied. Lecithin was found beneficial in improving physical properties of volume of cookies (Prentice, et al., 1978). Decreased loaf volume and poor crumb grain were improved by addition of sodium stearoyl lactylate and/or increasing shortening in bread utilizing 15% substitution with the bran fraction of brewers spent grain (Dreese & Hosney, 1982). Finley and Hanamoto (1980) suggested that milling DDG

into more uniform particle sized fractions without varying nitrogen content might help optimize utilization for specific products. Brewers grain was milled at different moisture levels and separated into high protein flour, fine and coarse bran, and shorts fractions. The amount of flour fraction was increased six-fold and coarse bran was decreased when milling was done at low moisture levels (7.4%). The bran fractions were more suited to baked products than the high protein flour fraction which appeared to have more potential for fabricated foods and extruded products.

#### DDG In Meat Products

Comminuted meat products offer an alternate use for DDG. If emulsification and binding properties of DDG are adequate, DDG could make a valuable nutritional contribution to meat systems in the form of fillers, binders, or emulsifiers.

The terms extender, binder, emulsifier, and stabilizer are often used interchangeably, but each may take on a more specific meaning. Extender is a non-specific term that refers to "any non-meat product that is added with the exception of water and salt to sausage in sufficient quantity to contribute materially to sausage bulk and composition" (Price and Schweigert, 1971). Binders must possess the ability to hold fat and water, while fillers need to contribute only bulk. In actual practice, however, cereal fillers bind several times their weight in water and the main difference is the ability to emulsify a fat as well as bind water. Nonmeat binders are "insurance" ingredients added to reduce the need for skeletal meats high in myosin

which is the protein primarily responsible for emulsification (Price and Schweigert, 1971). Emulsifier refers to any protein capable of keeping fat dispersed in a medium. A stabilizer's main function is to bind water and strengthen unstable emulsions by producing interfacial films between two emulsion phases (Price and Schweigert, 1971).

Meat extenders have a valuable contribution to make. Not only do they reduce formula costs, but also may improve emulsion stability, improve cooking yield, improve slicing characteristics and improve flavor (Price and Schweigert, 1971). Lower cost is perhaps the major incentive.

Amounts of these non-meat materials are regulated by federal meat inspection regulations. Up to 3.5% of cereal and/or animal extender products are permitted in sausage products. Products containing more than 3.5% of extenders must be identified as "imitation" (USDA, 1977). Plant proteins may be used at higher levels (10-21%) in other processed meat products such as meat loaves or chili con carne (Gallimore, 1972). Projected volumes of processed meat items and the possible replacement by plant protein foods in 1980 (Duda, 1974) show that these products could have a great impact on the meat market, replacing as much as 1,668.14 metric tons of meat.

Many types of extenders are on the market. Nonfat dry milk, calcium-reduced non-fat dry milk, dried whey and sodium caseinate are the animal products commonly used as binders. Various plant proteins could be utilized as functional ingredients in meat products, i.e. corn, wheat, oats, rye, rice, soy, and potato (Bird, 1974). Soy protein is the only binder of vegetable origin which is extensively

used in processed meats (Price and Schweigert, 1971). With the increase in food fabrication technology, more vegetable meat substitutes are being investigated. Wills and Kabirullah (1981) recently found that sausages prepared with sunflower seed flour produced a more stable emulsion than gluten and soy protein isolate and were equally acceptable to a taste panel. The efficacy of various vegetable and animal protein additives have been evaluated by Smith et al. (1973) and Terrell et al. (1979). Vegetable proteins were generally found less effective as emulsion stabilizers than animal proteins (Smith et al., 1973).

Several studies have examined the use of DDG in meat systems. Finley and Hanamoto (1980) found that brewers grain derived from corn could be dry milled to produce a high protein product which could have application in extruded products or fabricated foods. A protein concentrate obtained from brewers spent grain press water was found to make an acceptable meat extender when extruded with other cereal proteins (Finley et al., 1976). Junilla et al. (1981) found quality of sausage with 1% brewers grain, brewers yeast, or stillage rated almost equal to the control. When replacement levels were above 5%, the products were clearly inferior. Brewers grain displayed poor functional properties when incorporated into sausage or meatballs. Consistency of all test sausages was harder than the control sausages when measured by texturometer. Hardness was decreased by addition of water or water and fat. Addition of water and fat decreased all

scores (appearance, grain texture, odor, and flavor) except color. In preliminary tests, Mahoney (1982) found that high protein flour from milled DDG from barley would be accepted by consumers when incorporated into sausage at 5% levels.

#### Emulsification Properties in Sausage

Emulsification properties are important in determining the potential of a vegetable protein as a meat extender. An emulsion is a two-phase system of two liquids dispersed in a colloidal state. Although not a true emulsion, sausage is often regarded as one, with water forming the continuous phase and fat the discontinuous phase. Solubilized proteins act as emulsifiers by surrounding the fat globules (Price and Schweigert, 1971). A stable emulsion is one in which these fat globules do not separate.

To form an emulsion, protein must be dissolved by subjecting lean meats to a dilute brine solution and by the cutting action of knives. Seasoning and cure agents are added to aid dispersion and color development. Non-meat binders are added during the beginning of chopping to be ready for emulsification. The fatty meats are then added and comminuted until the emulsion is stabilized and desired texture is obtained. Non-meat starchy extenders may be added after fatty meats as they serve only as moisture binders, not fat binders. During cooking, fat not melted by heat of chopping is melted and is held in bounds by the protein membrane (Price and Schweigert, 1971).

Several factors affect emulsification of sausage. Emulsifying capacity of the salt-soluble protein (myosin and actin in meats) is

increased as pH and/or salt is increased (Price and Schweigert, 1971). Water soluble proteins have limited emulsification capacity which may be increased in the presence of salt. Emulsification is also affected by pH. The neutral pH of meat before slaughter falls to a pH of 5.3 - 5.7 after rigor mortis. As the isoelectric point, or point of least solubility, for actomyosin is pH 5, solubility must be increased to insure emulsification. Several procedures may be used to increase the soluble protein as much as 50%: 1) comminute hot-boned meat, 2) bone meat hot, freeze rapidly and comminute in frozen state, and 3) bone meat hot, add salt cure, and ice to maintain low temperature and hold for several hours (Price and Schweigert, 1971).

Chopping time and meat temperature also affect emulsification. Comminution must be sufficient for maximum solubilization of protein, yet not too long to decrease emulsification. Prior to adding fat, a temperature between 3° and 11° C is best for maximum emulsion stability. Final emulsification temperature should range from 10 ° to 16 °C.

If emulsification is adequate, no unemulsified fats, unbound moisture or gelatin on the surface or interior of stable sausage would be found. Air pockets sometimes cause pockets of free fat or gelatin in the interior but may be remedied by subjecting sausage to uniform compression and constant tension by use of tight casings or molds. Fat caps, or free, unemulsified fat is usually evidenced at the ends of sausages or as a coating on the body the sausage. To avoid fat caps, the formulation must be balanced and contain sufficient solubilized protein to emulsify all fat. Reducing relative humidity

in the smokehouse will also help eliminate surface grease (Price and Schweigert, 1971). Gelatin on the exterior or in pockets in the interior of sausage, is caused by heating collagen past its transition point in the presence of moisture. This "jelly" may be minimized by restricting high collagen meats or by comminution. Less jelly is formed during dry heat cooking than moist heat cooking (Price and Schweigert, 1971).

#### Summary

Distillers dried grain may have potential as a human food source of protein and fiber. Nutritional analyses have shown high protein and fiber levels and some minerals present in the brewers spent grain. Successful incorporation of these nutritional components into food products depends on the functional characteristics of the protein. Functional properties of DDG have generally been poor causing poor texture and lower volume in baked goods. Color and flavor changes have also discouraged substitution of DDG in baked products. Use of DDG in other food systems such as comminuted meat products will be determined largely by solubility, emulsification, and water-binding properties. Thus, identification of both nutritional and functional properties are important in finding an organoleptically appealing food use for distillers dried grain. Such a product would have implications not only in supplementing the world's food supply, but in subsidizing the biofuel industry as well.

## CHAPTER 3

## METHODS

Distillers dried grain (DDG) was obtained and subjected to a series of analyses to determine future application and acceptability in meat systems. Nutritional analyses and determination of certain functional properties for barley-derived DDG and milled fractions were accomplished through procedures described below.

Materials

Distillers dried grain was obtained from the Alcotec biofuel plant, Ringling, Montana which utilizes 100% barley in its fermentation process. The distillers grain was centrifuged to separate spent grain from the stillage and then dried by a direct heated rotating drum.

Milling was performed at the Montana State University Cereal Quality Laboratory on a Buhler mill at 10% moisture level. A high protein flour or fine fraction, a coarse bran fraction and a shorts fraction of finely ground bran and adherent endosperm were obtained from the milling process. Representative milling data for DDG showed an average yield of 33% flour, 32% bran and 35% shorts (McGuire, 1983). These fractions along with whole unmilled DDG were evaluated.

### Nutritional Analysis

Proximate analysis of the whole DDG and fine, coarse, and shorts milled fractions were performed in duplicate. AOAC, 1975 methods for moisture (14.058), ash (14.006), crude fiber (14.060), fat (7.045), and protein (2.049) were used. A conversion factor of 6.25 was used to calculate protein percentage from nitrogen. Neutral and acid detergent fiber content were analyzed by the Goering/Van Soest method adapted from the U.S.D.A. Handbook (1970).

Amino acid analysis was performed on DDG Whole and Fine samples by AAA Laboratory, 6206 89th Avenue, Mercer Island, Washington. Dionex Analyzers, Models D-500 and D-502 and Beckman-Spinco Analyzer, Model 120B were used to determine amino acid composition. All analyzers used the ion-exchange chromatographic methods developed by Spackman et al. (1958).

### Functional Properties

Functional properties identified for whole DDG and milled fractions included protein solubility, emulsifying capacity and water-holding capacity. Protein solubility of DDG and milled fractions were determined by a procedure outlined by Inklaar and Fortuin (1969). Protein (2.5 g) was added to 100 ml distilled water. The pH was adjusted by addition of NaOH or HCl for a solubility range from pH 2 to 10. The pH was measured on a Model 12, Corning pH Meter. The mixture was stirred for 1 hour at a controlled temperature of 50° C and then centrifuged. Protein in the supernatant was measured by the AACC (1962) Biuret Method 46-15 for wheat and other grains. This was

expressed as the ratio of water soluble protein to total protein times 100.

The method of Yasumatsu et al. (1972) was used for determining emulsifying activity. Protein (1.4 g) was suspended in water (20 ml), and soy oil (20 ml) was added. This mixture was blended at high speed in an Osterizer blender for one minute. The emulsion was divided into three 15 ml centrifuge tubes and centrifuged at 3,000 rpm for 5 minutes. The emulsifying activity was calculated as

$$\frac{\text{Height of emulsified layer (mm)}}{\text{Height of whole layer in centrifuge tube}} \times 100$$

Water-binding capacity was measured by the method described by Smith et al. (1973). Each protein additive (1 g) was dispersed into 30 ml of 3.5% NaCl solution (to approximate the salt concentration of commercial frankfurters). After agitation, heating to 85° and cooling, this mixture was centrifuged at 5,000 rpm for 15 minutes at 25° C. The protein additive was weighed and then dried for 20 hours at 105° C. Water holding capacity (WHC) was expressed as the ratio of the wet weight of the additive to the dry weight of the additive.

#### Performance in Meat Products

The performance of DDG and DDG milled fractions in meat systems was evaluated by organoleptic evaluation of sausage substituted at 3.5 percent levels. A mildly seasoned Polish sausage formulation (Table 1) was utilized so the effect of DDG addition on flavor could be evaluated. The boneless pork butt contained approximately 30% fat.

The protein extender was replaced by distillers dried grain and fine, coarse and shorts milled fractions. Soy protein isolate

obtained from Richard S. Kutas Company, New York was utilized as the control. The extender is added at the 3.5% level allowed by Federal regulations. The meat was ground through a 1/4" grinder plate and all the fat meat through a 1/8" grinder plate. Ingredients were mixed by hand until evenly distributed (three minutes). The mixture was stuffed into 40-42 MM hog casings. Smoking was performed by a commercial meat processor.

Table 1. Polish Sausage Formulation

<u>Ingredient</u>	<u>Quantity (Grams)</u>
Boneless pork butts	2 kg
Ice water	1 pt
Protein extender	68 g
Salt	50 g
Sugar	5.6 g
Cure (Prague Powder #2)	1 g
Black Pepper (Coarse ground)	5.5 g
Garlic (Fresh)	1 large bud
Marjoram	1 g

Modified from Kutas, 1976

Emulsion stability in boiling water was measured by the method described by Lin et al. (1974). Half sausages were boiled for twenty-five minutes in 200 ml distilled water. Cooking water was evaporated

and remaining fat was measured. Percent cooking loss was tabulated for each sausage.

Color analysis was performed on the Agtron M-500 A color analyzer (Magnuson Engineering, Inc., San Jose, CA)<sup>1</sup>. Readings were taken at the red spectral measurement utilizing a 640 nanometer line of neon.

#### Texture Evaluation

Texture was analyzed on cooked sausage without casings using the Warner-Bratzler Shear Tester (G.R. Electric Manufacturing Company, 1317 Collins Lane, Manhattan, KS 66502)<sup>1</sup>. Samples were prepared with the .5 inch core borer. Penetration of a 20 g cone and 47.5 g test rod in 5 seconds was taken with a Universal Penetrometer (Precision Scientific Company, 3737 W. Cortland, Chicago, IL 60647)<sup>1</sup>.

#### Nutritional Evaluation

Total protein of the uncooked sausage was analyzed using the AOAC (1975) Kjeldahl method. Moisture, ash and ether extract were also measured by AOAC (1975) methods.

#### Organoleptic Evaluation

Organoleptic evaluation was performed by 8 untrained panels consisting of 8 panelists each. Ages ranged from 18 to 63. Eighteen of the panelists were male. Testing was performed in partitioned

<sup>1</sup>Mention of a firm name or product does not constitute endorsement by Montana State University over others of a similar nature.

booths lighted with 25 watt red bulbs. Panels were held between 1 and 4 p.m. Sausage samples were cut in 2 cm slices, precooked to 150° F and reheated in a microwave oven for testing. Samples were coded with randomly selected three digit numbers and were rotated so each sample had equal exposure in the first position. Samples were scored using a hedonic scale (see Appendix A) of liked extremely (9 points) to disliked extremely (1 point). One-way analysis of variance and LSD at .05 was performed to find significance between the scores.

## CHAPTER 4

## RESULTS AND DISCUSSION

Nutritional Analysis

Results of the proximate analyses are shown in Table 2. The milled DDG fractions had a protein range from 20% for the shorts fraction to 41% for the fine fraction. Whole DDG contained 26% protein which is double the protein content in barley grain. Protein content was much lower in all of the DDG fractions than in the soy isolate control which contained 67% protein. The fine milled fraction displayed the lowest ash and fiber values of the DDG samples (3.3% and 9.3% respectively) and the highest lipid value (4.2%). The ash content was much higher than the .5% maximum specification for bread flour or 1.5% specification for barley flour (Matz, 1959). The lipid content was slightly higher than the 3% specification for barley

Table 2

Proximate Analysis of Whole DDG and Milled Fractions<sup>1</sup>

Protein Additive	% Protein a.s.	% Dry Matter	% Ash a.s.	% Ether Extract	% Crude Fiber a.s.	% ADF	% NDF
Whole DDGs	25.70	95.3	3.7	3.45	16.65	28.3	59.5
Fine DDG Fraction	40.75	93.9	3.3	4.20	9.30	26.9	34.1
Coarse DDG Fraction	26.00	96.5	3.8	3.90	17.15	29.1	57.1
Shorts DDG Fraction	20.00	94.5	3.8	4.10	19.9	29.2	65.5
Soy Isolate Control	67.00	95.8		.4	.7	4.4	6.0

<sup>1</sup>Each value is the mean of duplicate samples.

flour. The soy isolate contained much less fiber and lipid than did the DDG samples. Acid detergent values were similar for all DDG samples. The fine DDG fraction was lower in neutral detergent fiber than the other DDG milled fractions which contained more bran. As bran contains a high proportion of the hemicellulose of a grain, neutral detergent fiber figures were higher for bran containing fractions.

Amino acid analyses for whole DDG and Fine DDG are shown in Table 3. Lysine is the limiting amino acid as it is in most cereal grains. The other essential amino acids are present in amounts similar to the FAO reference protein.

#### Functional Properties

Functional properties are displayed in Table 4. Total protein and solubility differed significantly between all samples. Even though the fine fraction had almost double the protein of other DDG fractions, the protein was not very water soluble. The soy isolate control contained more than three times the soluble protein in the DDG samples. As solubility reflects denaturation of protein (Kinsella, 1976), the low solubility values may be the result of the processing the protein has undergone. From the low solubility levels found in the DDG fractions, it could be assumed that functional properties, such as emulsification, gelation, and foaming capacity, are somewhat restricted (Kinsella, 1976).

Table 3

## Amino Acid Analysis\* of Whole Barley DDG and Fine Milled Fraction

Amino Acid	FAO Reference** Protein mg/g N	Whole DDG mg/g N	% FAO Protein	Fine DDG mg/g N	% FAO Protein
<b>Essential Amino Acids:</b>					
Isoleucine	250	267	107	265	106
Leucine	440	491	112	478	109
Lysine	340	189	56	159	47
Methionine*** and tyrosine	220	86 177	120	108 192	136
Phenylalanine	380	363	142	380	151
Threonine	250	233	93	213	85
Valine	310	305	98	294	95
<b>Nonessential Amino Acids:</b>					
Alanine		242		213	
Arginine		328		230	
Aspartic Acid		349		313	
Glutamic acid		179		192	
Glycine		189		159	
Histidine		125		118	
Proline		872		948	
Serine		<u>253</u>		<u>253</u>	
Total		3440		5820	

\* 24-hour 6N-HCL hydrolysis @ 110° C; serine increased by 10% and threonine increased by 5% to compensate for destruction by acid. 1 crystal of phenol added before acid hydrolysis.

\*\* Provisional amino acid pattern for scoring protein quality based on essential amino acid needs of preschool child (WHO, 1973)

\*\*\* Found as methionine sulfoxide.

Table 4

## Functional Properties of DDG and Milled Fractions

Protein Additive	Total Protein <sup>1</sup>	Protein Solubility <sup>2</sup>	Water-holding Capacity <sup>3</sup>	Emulsifying Capacity <sup>4</sup>
	(N=2)	% (N=4)	(N=3)	(N=4)
DDG Whole	26 <sup>a</sup>	1.2 <sup>c</sup>	4.9 <sup>ab</sup>	0
DDG Fine	41 <sup>b</sup>	1.8 <sup>b</sup>	4.7 <sup>a</sup>	0
DDG Coarse	26 <sup>c</sup>	1.5 <sup>d</sup>	4.5 <sup>a</sup>	0
DDG Shorts	20 <sup>d</sup>	.8 <sup>a</sup>	5.5 <sup>bc</sup>	0
Soy Isolate Control	67.5 <sup>e</sup>	5.9 <sup>c</sup>	--	0

<sup>1</sup>Kjeldahl Analysis

<sup>2</sup>Amount of soluble protein found at natural pH of 3.9 by AACC (1962) Biuret Method

<sup>3</sup>Method by Smith et al. (1973), expressed as ratio of wet wt/dry wt.

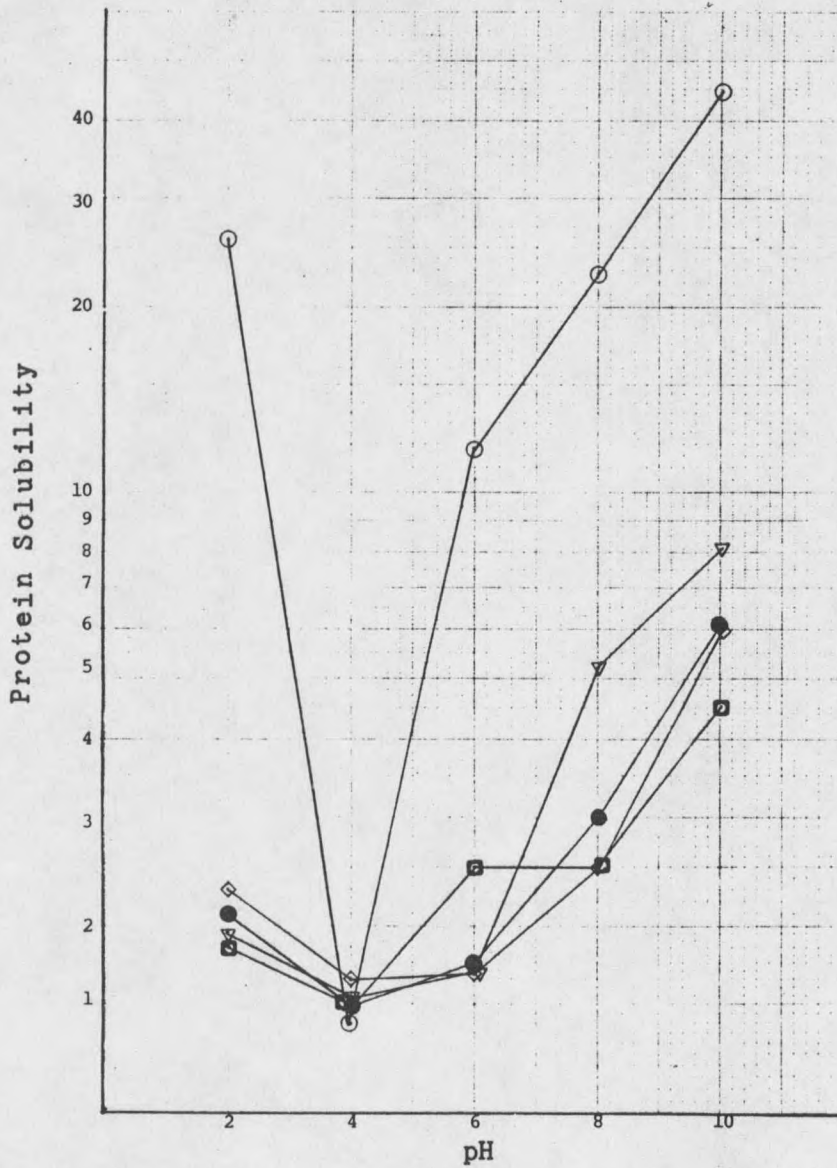
<sup>4</sup>Method by Yasumatsu et al. (1972). No emulsifying capacity was measureable at the natural pH of the DDG fractions by this method.

abcde Means in the same column with unlike superscripts are significantly different ( $p < 0.05$ ).

Solubility profiles (Figure 3) showed an increase in soluble protein as pH increased. Solubility was lowest at pH 4 which is close to the natural pH of the DDG fractions. An increase in solubility was also noted at pH 2. These findings were similar to those found by Kinsella (1976). As most meat products have a pH between 5-6, the increase in soluble protein at higher pH levels would have little applicability in meat products.

Figure 3

Protein Solubility<sup>1</sup> of DDG and Soy Control as a Function of Ph



KEY:  
● DDG Whole  
▽ DDG Fine  
■ DDG Coarse  
◇ DDG Shorts  
○ Soy Isolate Control

<sup>1</sup>Method by Inklaar and Fortuin (1969).

Water-binding capacity was highest in the soy isolate and DDG shorts milled fraction. The higher water-binding capacity of the soy isolate control can be explained by its higher protein content as water-binding increases with protein content (Fleming et al., 1975). Denaturation also enhances water-binding capacity (Huffman et al., 1975) which explains the higher value for the DDG shorts fraction. All samples displayed similar water binding capacity to other protein additives tested by Smith et al. (1973).

No emulsification capacity was exhibited by any of the samples including the soy protein isolate. The soy isolate did form a milky emulsion that did not remain stable after centrifugation. Large fat globules remained unmixed in all of the DDG samples. Koivurinta et al. (1980) found the same results using brewers dried grains. Non-fat dried milk was tested and had an emulsion capacity of 35 (height of emulsion/height of whole layer X 100). Whole DDG at a pH of 10 was also tested and demonstrated a emulsion capacity of 15. This finding agrees with work by Carpenter and Saffle (1964) that demonstrated that emulsification increased with protein solubility. These tests showed that distillers dried grain would not be a valuable emulsifier in meat systems. DDG exhibited emulsifying capacity only at a high pH not applicable to meat products.

Sausages were analyzed for protein, moisture, ash, and fat (Table 5). The fine fraction and soy isolate control had comparable protein and moisture levels. The higher protein level of these sausages reflect the higher protein content of the extender itself. The fine fraction had the lowest value for ether extract and highest

value for ash. No significant differences in cooking losses were found between the DDG sausages and the soy sausages. This is not surprising as none of the samples exhibited emulsifying capacity. A good emulsifier would prevent moisture and fat loss during cooking by bounding fat molecules with a protein coat. Color was much darker in the DDG sausages than in the soy sausages. The DDG fine fraction sausages had the darkest readings on the Agtron color analyzer. The fine fraction also had the highest ash content which often is an indicator of flour grade (Matz, 1959). Ash content of sausage did not correlate with ash content of the milled fractions.

Table 5

Characteristics of Polish Sausage Made with DDG and Milled Fractions

Protein Additive	Protein <sup>1</sup> %	Moisture %	Ash %	Ether Extract	Cooking Loss <sup>2</sup> %	Color <sup>3</sup>
	(N=2)	(N=2)	(N=2)	(N=2)	(N=3)	(N=3)
DDG Whole	13.8 <sup>a</sup>	51.6 <sup>c</sup>	2.8 <sup>b</sup>	27.5 <sup>c</sup>	8 <sup>a</sup>	37 <sup>c</sup>
DDG Fine	16.9 <sup>c</sup>	55.1 <sup>ab</sup>	3.0 <sup>c</sup>	20.5 <sup>a</sup>	10 <sup>a</sup>	30 <sup>a</sup>
DDG Coarse	13.3 <sup>a</sup>	49.3 <sup>d</sup>	2.7 <sup>a</sup>	30.4 <sup>d</sup>	13 <sup>a</sup>	38 <sup>c</sup>
DDG Shorts	15.1 <sup>b</sup>	55.6 <sup>a</sup>	2.8 <sup>b</sup>	22.5 <sup>b</sup>	10 <sup>a</sup>	34 <sup>b</sup>
Soy Isolate	16.3 <sup>c</sup>	54.0 <sup>b</sup>	3.0 <sup>c</sup>	23.9 <sup>b</sup>	7 <sup>a</sup>	51 <sup>d</sup>

<sup>1</sup>Proximate analysis by AOAC methods (1975).

<sup>2</sup>Method by Lin et al. (1975).

<sup>3</sup>Agtron M500, red setting

<sup>abcd</sup>Means in the same column with unlike superscripts are significantly different at  $p < .05$ .

Texture was measured by the Universal Precision Penetrometer and Warner-Bratzler Shear Tester (Table 6). No significant differences were noted in the Warner-Bratzler Shear testing, even in the commercial sample of Polish sausage that was included in the testing. However, the penetrometer showed less penetration in the soy isolate sample indicating a harder sausage. The DDG fine and coarse samples had the greatest penetration. Koivurinta et al. (1980) had found the BDG sausage to be harder than their control sausage. Addition of water and fat softened the consistency of sausage in their study. The data was insufficient in the present study to correlate fat and water content to sausage hardness.

TABLE 6

## Texture Analysis of Sausage Extended with DDG

Sausage Sample	Penetrometer (N=10)	Warner-Bratzler Shear Tester (N=12)
DDG whole	41 <sup>b</sup>	1.66 <sup>a</sup>
DDG fine	44 <sup>cd</sup>	2.07 <sup>a</sup>
DDG coarse	46 <sup>d</sup>	1.47 <sup>a</sup>
DDG shorts	42 <sup>cb</sup>	2.17 <sup>a</sup>
Soy isolate	39 <sup>a</sup>	1.33 <sup>a</sup>
Commercial		1.74 <sup>a</sup>

abcd Means in the same column with unlike superscripts are significantly different at  $p < .05$ .

In organoleptic testing (Table 7), the panelists showed a slight preference for the soy extended sausage. The sausage containing milled DDG fractions had a mean score rating of 5 (neither liked nor disliked). The sausage containing different milled DDG fractions received similar ratings (Table 8). The soy isolate control with a mean of 7 (liked moderately) was significantly different from the DDG samples. Several panelists expressed a preference for spicier sausage which may account for the lower scores. DDG addition slightly lowered acceptability of sausage. As the panel was conducted under red lights, sausage was rated only on texture and taste and not color or appearance. Milling did not seem to affect the rating even though milled fractions varied in protein content, fiber and other functional properties.

Table 7

Mean Scores of Taste Panel Ratings<sup>1</sup> of Polish Sausage  
Extended with DDG Milled Fractions

Panel	N	DDG Whole	DDG Fine	DDG Coarse	DDG Shorts	Soy Isolate
1	8	6	5	5	6	7
2	8	6	5	5	5	6
3	8	5	5	6	5	7
4	9	6	4	5	5	6
5	8	4	4	5	4	6
6	7	4	6	5	4	7
7	8	5	6	7	6	6
8	8	5	5	6	6	6
Mean	8	5	5	5	5	7

<sup>1</sup>Rated on a hedonic scale from 1 (dislike extremely) to 9 (like extremely).

<sup>ab</sup>Means with unlike superscripts are significantly different (p < 0.05).

Table 8

Analysis of Variance Table for Taste Panel Ratings of DDG Polish Sausage

Source of Variation	Degrees of Freedom	Sum of Squares	Mean of Square	F-Value
Between	4	11.82	2.955	5.857 *
Within	35	17.66	.05046	
Total	39	29.48		

\*F value is significant at  $p < 0.05$ .Conclusions

The hypothesis that distillers dried grain would possess adequate nutritional qualities to make them a valuable addition to human food systems was accepted. Distillers dried grain did possess two nutritionally beneficial components, fiber and protein. The fine milled DDG fraction would contribute the most protein having almost double the protein of other milled fractions. Amino acid analysis revealed whole and fine DDG fractions to be deficient in lysine having only 50% of the FAO reference protein. Although a high lysine content is not a necessary characteristic for meat additives, it is important for an additive in cereal products which are lysine deficient. Further testing of protein quality of DDG needs to be done. The other milled fractions (shorts and coarse fractions) would be beneficial in adding fiber to foods.

The hypothesis that distillers dried grain and milled fractions would display functional properties that would make them suitable for

incorporation into meat products was rejected. Water solubility of protein was low, increasing only at very basic or acid pH levels. This poor solubility affected emulsifying capacity. No emulsifying activity was exhibited by any of the DDG samples. DDG fractions might be useful as meat fillers as water binding capacity was similar to scores of other protein extenders.

The hypothesis that distillers dried grain and milled fractions from barley would perform adequately as an emulsifier in a basic sausage formulation was rejected. Overall, acceptability was slightly lower for the DDG sausage than the soy isolate control sausage. Sausages with DDG and milled fractions received a neutral rating by panelists while the soy sausage was liked moderately. The DDG sausages were slightly less firm than the soy control sausage. The fine fraction had the more favorable characteristics of the milled fractions. Sausage with the fine fraction and soy isolate were similar in composition, cooking loss, and texture, but varied considerably in color.

Distiller dried grain does represent a potential source of fiber and protein for human consumption. DDG's application in meat products, however, is limited by lack of adequate functional properties. Finding a way to improve protein solubility and other functional properties of distiller dried grain would greatly enhance the probability of its utilization in human food products.

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Appendix

Hedonic Scale Utilized for Taste Panels on Sausage

Booth Number \_\_\_\_\_

Food Evaluation Test

Product \_\_\_\_\_

Check the appropriate block:

SAMPLE NUMBER

- Like Extremely
- Like Very Much
- Like Moderately
- Like Slightly
- Neither Like nor Dislike
- Dislike Slightly
- Dislike Moderately
- Dislike Very Much
- Dislike Extremely

SAMPLE NUMBER					

Comments:

Name \_\_\_\_\_

Date \_\_\_\_\_

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