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# A STUDY ON THE QUALITY OF WHOLE STILLAGE WHEN DAMAGED GRAINS ARE USED AS FEEDSTOCKS FOR ALCOHOL PRODUCTION

by

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# Introduction

Legislative initiatives of 1980 along with a growing interest in the use of agricultural products to produce alcohol for fuel use will result in an increase in the supply of fermentation by-products available to the feed industry. It has been estimated that a supply of 1.8 million tons in 1981 may rise to 6.5 million tons by the mid-1980's (Klopfenstein and Abrams, 1981). Sale of these by-products is an economic necessity to alcohol producers since the return can offset conventional feedstock costs by as much as 50 percent (Black et al., 1981). As much as 40 percent of the energy used in commercial distilling operations may be used for drying these by-products (Klopfenstein and Abrams, 1981). Such costs are economically prohibitive in "on farm" type operations suggesting that wet distillers by-products or whole stillage be utilized as a feed ingredient.

Good quality conventional feedstocks have traditionally been used by the beverage alcohol industry and information on the quality of the dry by-products as livestock feed is readily available. Although the beverage alcohol industry requires a good food grade feedstock and has used mainly corn and grain sorghum to make ethanol, such is not the case with the fuel alcohol industry. Modern technology permits the use of many nonconventional feedstocks, potentially making fuel alcohol production more economically feasible. It is also possible to use many types of "damaged" grains; those with physical damage from handling, those sustaining insect damage during storage, sprout damaged grains, or grains having fungal damage and possible contamination from mycotoxins. Osweiler (1981) conservatively estimated the monetary loss in 1977 due to mycotoxin contamination in grain at $500 million.

It appears that fuel alcohol producers could realize substantial savings if some of these damaged grains were utilized as feedstocks. Although the alcohol can be produced, information is needed about the quality of the resulting wet by-products.

The purpose of this investigation was to examine some of the nutritional parameters of the resulting whole stillage when several nonconventional and damaged grains were utilized as feedstocks in an "on farm" type ethanol production process.

# Literature Review

There are four basic steps required for the production of ethanol: feedstock preparation, starch conversion, fermentation, and distillation. Although not a basic step in the procedure, the collection and further processing of the fermentation by-products is usually an integral part of fuel alcohol production. Figure 1 is flow diagram of the ethanol production process.

Feedstock Preparation. Yeast converts simple sugars to alcohol and carbon dioxide. These sugars may come from a variety of sources and the preparation required will depend on the type of source. The first step, regardless of source, is to remove foreign materials from the feedstock. Although pressing, washing, clarification, and concentration of sugar crops such as sugarcane or sugar beets yields sugar solutions, this process is costly and likely will occur only at large alcohol plants (NRC, 1981).

Whole grains must be mechanically reduced to make the starch more accessible to the enzymes which are used in the conversion of the starch to the mono- and disaccharides required by the yeast. Grinding is the most common method of particle size reduction. While some people advocate the use of roller mills in order to reduce fines (Nellis, 1979), most grains are ground through a hammermill. Particle size is important as too fine a grind affects the feed recovery process while too coarse a grind increases the time and energy required for starch gelatinization (Maisch et al., 1979). Suggested particle sizes include "chick feed size" or through a 3/16 in. screen (Titus, 1980; Shelton and Rider, 1980), meal that will pass a 40-mesh screen (Solar Energy Research Institute, 1980), and 80% passing a 20-mesh screen (Yang et al., 1982).

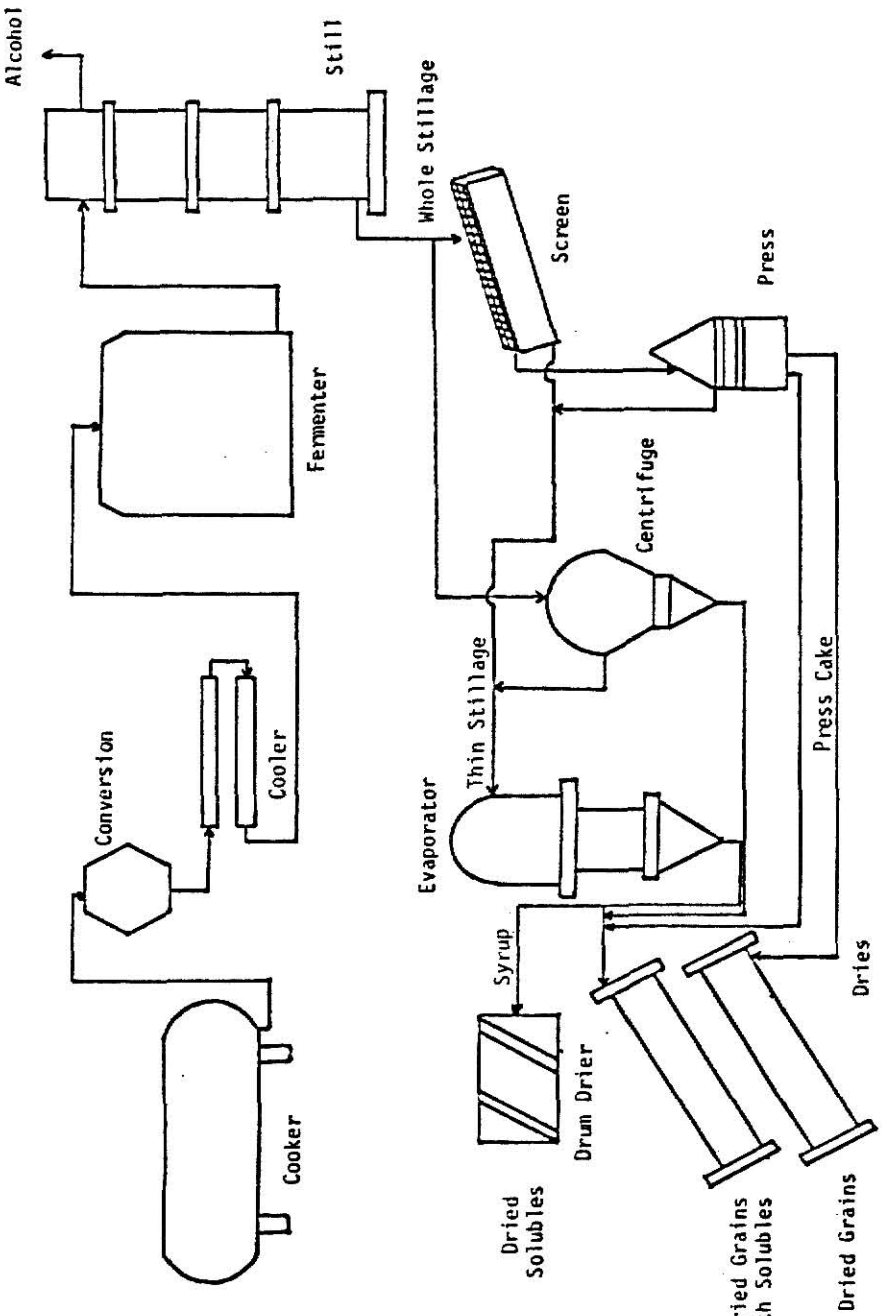


Figure 1. ETHANOL PRODUCTION PROCESS

Liquification and Saccharification. The feedstock is mixed with water to form a slurry or mash. When the temperature is raised the mash will thicken as the starch begins to gelatinize, usually between 60 and 72 C depending on the feedstock. Amylase from malt or microbial sources is added to reduce the mash viscosity. Alpha-amylase hydrolyzes the $\alpha$1-4 bonds in the long starch molecules resulting in chains of 6 to 30 glucose units called dextrins (Titus, 1980). Following hydrolysis the temperature of the mash is normally raised to 98 C for 30 min to destroy undesirable yeasts, bacteria, and enzymes.

Saccharification is the conversion of the dextrins to the simple sugars utilized by the yeast. Glucoamylase (amyloglucosidase) breaks both $\alpha$1-4 and $\alpha$1-6 links to yield single glucose units. Distiller's yeast can utilize glucose more readily than maltose allowing fermentation to be completed more rapidly (Maisch et al., 1979). Temperature and pH must be monitored carefully to maintain optimum conditions for the enzymes.

Fermentation. Yeasts convert sugars to ethanol, carbon dioxide, and heat in the stoichiometric ratio of 2 moles each of ethanol and carbon dioxide for each mole of glucose (NRC, 1981). The yeasts normally used in ethanol production are top fermenting facultative anaerobes belonging to the genus Saccharomyces. If the sugar concentration of the mash exceeds 22% by weight, the high osmotic pressure will cause the yeast cells to rupture and yeast growth will be greatly inhibited (Solar Energy Research Institute, 1980). Fermentation will continue until the substrate is depleted or the ethanol concentration is high enough to destroy the yeast, greater than 12 to 14% by weight (Maisch et al., 1979).

Distillation. The purpose of distillation is to separate the ethanol from the mash. The formation of an azeotropic mixture of water and alcohol at 1 atmosphere limits the concentration to 95.6% by weight of ethanol (National Alcohol Fuel Producers Assoc., 1979). Anhydrous alcohol can be obtained by azeotropic distillation using benzene (NRC, 1981).

Conventional distillation procedures use a system of two columns: a stripping column to separate ethanol from the mash and a rectifying column to concentrate the ethanol. Sieve trays in the columns improve liquid-vapor contact and encourage refluxing (Solar Energy Research Institute, 1980).

Feedstock Recovery. There are several points in the production process at which by-products may be removed depending upon the physical design of the system and/or the desired use of the by-products. Figure 2 is a simplified flow diagram of an ethanol production process indicating these points. Removal of the solids following saccharification can reduce the size of the fermentor as well as allow for the use of a packed bed distillation column. Removal of grains following fermentation also allows a packed bed column to be used. Ethanol yield, however, may be reduced up to 40% because of the loss of sugars or ethanol if the solids are removed prior to distillation (NRC, 1981).

Dry matter content of whole stillage normally ranges from 5 to 10%, however, the solids content can be increased to 13.9 % if 50 percent of the stillage is recycled and used as a heat source (NRC, 1981). Solids are usually removed by screening or centrifugation, increasing the solids content to 15 to 35%. Solids are then dried

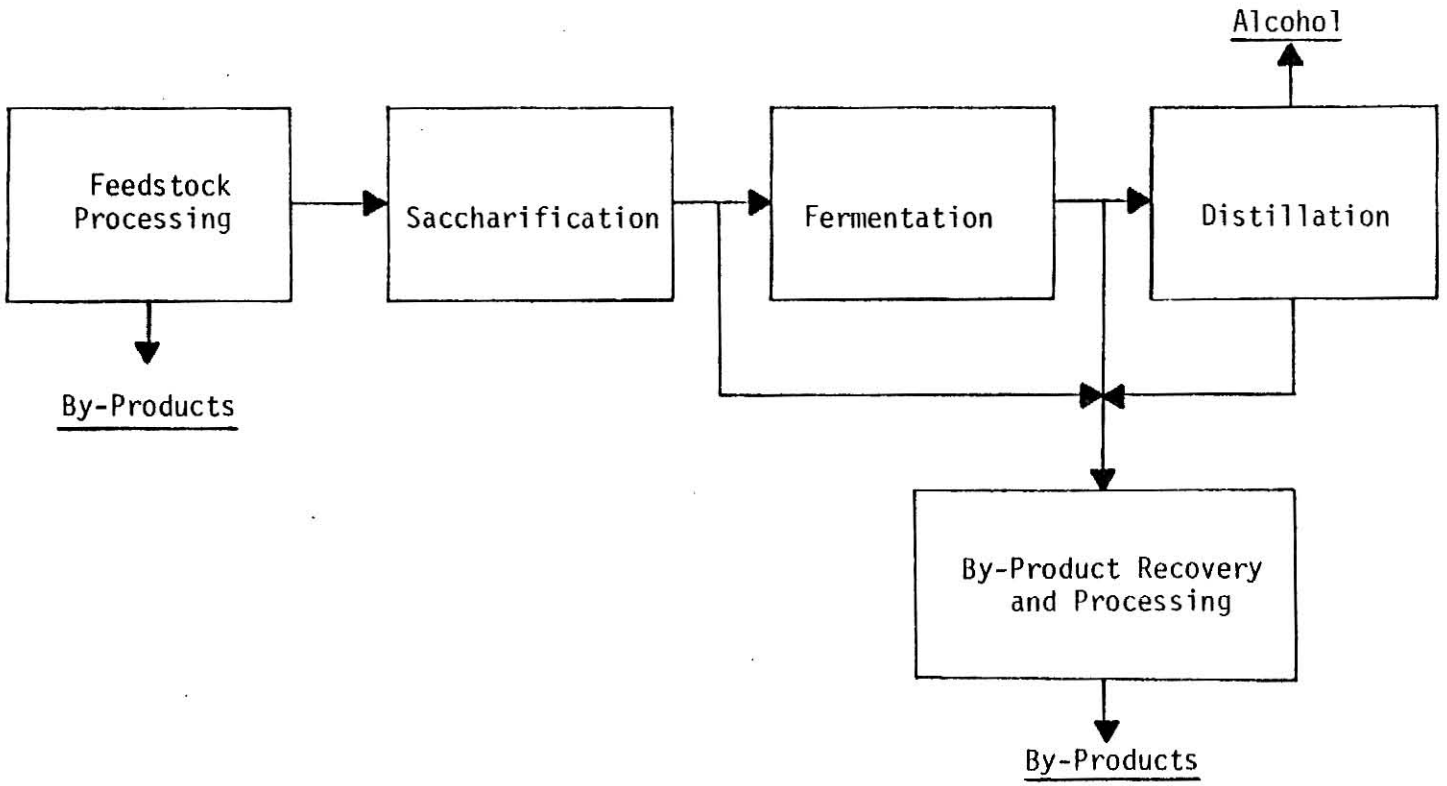


Figure 2. FLOW DIAGRAM OF THE ETHANOL PRODUCTION PROCESS AND POTENTIAL BY-PRODUCTS.

NRC, 1981

and sold as distillers dried grains (DDG). The liquid (thin stillage), consisting of yeast cells, sugars, alcohols, and other soluble nutrients, ranges from 2 to 5% dry matter. Commercially this is condensed in an evaporator and marketed as condensed distiller solubles (CDS) with a dry matter content of 20 to 40%. CDS can be further dried and sold as distillers dried solubles (DDS) or can be mixed back with the grains and dried to make distillers dried grains with solubles (DDGS). Nearly one-third of the original feedstock dry matter can be recovered in the by-products with 40% of this dry matter as solubles and 60% as dried grains (Berger, 1981).

The composition of ethanol production by-products is influenced by several factors related to raw materials, processing procedures, and types of equipment. De Becze (1949) identified several of the factors affecting nutrient composition of distillers by-products (table 1). Variation within a production facility can be minimized because processes and equipment remain relatively constant. The most important influence on the nutrient composition of the by-product is the type and quality of the feedstock (Carpenter, 1970). Bauernfeind et al. (1944) showed that the nutrient composition of whole stillage can be predicted fairly accurately if the nutrient content of the raw ingredient is known. Compositions of dried distillers grains from several grain sources are presented in Appendix G.

Table 1. FACTORS INFLUENCING THE COMPOSITION OF DISTILLERS BY-PRODUCTS

Raw Materials

1. Kind of cereal grain
2. Variety of grains
3. Quality of grains
    a. soil
    b. fertilizer
    c. meteorological conditions
    d. method of production
    e. crop year
    f. harvesting methods
    g. drying methods
    h. storage
4. Grain formula or mash bill
    a. fermentable starch content

Processing

1. Grinding procedure
2. Cooking
    a. amount of water
    b. amount of pre-malt
    c. temperature and time
    d. continuous or batch
    e. cooling time
3. Conversion
    a. type, amount and quality of malt
    b. fungal amylase (malt and fungal amylase)
    c. time and temperature
4. Diltuion of converted grain
    a. volume in gallon per bushel of grain bill
    b. quality and amounts of grain products
5. Fermentation
    a. yeast - quality and quantity
    b. temperature
    c. cooling
    d. agitation
    e. time
    f. acidity and control

Table 1. (Continued)

6. Distillation

   a. type-vacuum or atmospheric batch or continuous
   b. direct or indirect heating
   c. change in volume during distillation

7. Processing of spent or dealcoholized stillage

   a. type of screen-stationary, rotating or vibrating
   b. use of centrifuges
   c. type of presses
   d. evaporators - temperature and number
   e. dryers - type, time and temperature
   f. amount of evaporator syrup mixed with fibrous grain portion.

Source:

DeBecze (1949)

## Literature Cited

Bauernfeind, J.C., M.B. Smith, S.C. Garey, W. Bauemgarten, F.A. Gustoff, and L. Stone. 1944. Nutrient content of alcohol fermentation by-products from various grains. Cereal Chem. 21:421.

Berger, L.L. 1981. Nutritional value of distillers feeds for livestock. Thirty-sixth Kansas Formula Feed Conf., A-1.

Black, J.R., B.W. Wilkinson, and J.C. Waller. 1981. Economic feasibility of ethanol production in large-scale plants. Agricultural Economics Res. Rep., Michigan State University.

Carpenter, L.E. 1970. Nutrient composition of distillers feeds. Proc. Distill. Feed Res. Council Conf. 25:54.

DeBecze, Geo. I. Factor affecting the composition of distillers feeds. Proc. Fourth Conference on Feeds of the Grain Distilleries, 1949.

Klopfenstein, T. and S.M. Abrams. 1981. Distillers by-products use - a review. Nebraska Cattle Day Rep. EC 81-218, p. 2.

Maisch, W.F., M. Sobolov, and A.J. Petricola. 1979. Distilled beverages In H.J. Peppler and D. Perlmau (Ed.) Microbial Technology. Vol. II. Academic Press, New York.

National Alcohol Fuel Producers Association. 1979. A learning guide for alcohol fuel production. Lincoln, Nebraska (Published by Colby Community College).

Nellis, M. 1979. Makin' it on the farm. American Agriculture News, Iredell, Texas.

NRC. 1981. Feeding Value of Ethanol Production By-products. National Academy of Sciences - National Research Council. Washington, D.C.

Osweiler, G.D. 1981. Handling mycotoxin problems in livestock production. Anim. Nutr. and Health, March 1981, 19-21.

Shelton, D.P. and A.R. Rider. 1980. Ethanol production equipment and processes. Ethanol Production and Utilization for Fuel. Cooperative Extension Service, University of Nebraska-Lincoln. p. 23.

Solar Energy Research Institute. 1980. Fuel From Farms - A Guide to Small-Scale Ethanol Production. SERI/SP-451-519. The National Technical Information Service, Springfield, Virginia.

Titus, T.C. 1980. Making on-farm ethanol fuel. Fermentation Chemistry and Microbiology. Extension Service, Clemson University, South Carolina.

Yang, R.D., D.A. Grow, and W.E. Goldstein. 1982. Pilot plant studies of ethanol production from whole ground corn, corn flour, and starch. Fuel Alcohol U.S.A., p. 13.

# QUALITY OF WHOLE STILLAGE WHEN AFLATOXIN CONTAMINATED CORN IS USED FOR PRODUCTION OF ALCOHOL

## Summary

Aflatoxin contaminated corn was used to investigate the quality of whole stillage from alcohol produced from damaged grain. Treatments included a control (US No. 2 yellow dent) and corn containing 426 ppb aflatoxin. Aliquots of stillage were either freeze dried or filtered and oven dried at 56 C. Grains and stillages were analyzed for crude protein, ether extract, crude fiber, ash, gross energy, neutral detergent fiber, and amino acid composition. Ergosterol levels were used as an indication of fungal activity. A particle size analysis was also conducted on the feedstocks.

Analysis of the stillage and alcohol for aflatoxin, a toxic metabolite produced by A. flavus, showed no detectable amount in the alcohol, however 362 ppb were found in the freeze dried stillage. Although the toxin is concentrated in the post-fermentation solids, some degredation did occur.

A determination of nutrient levels in the stillage showed no differences due to treatment however the amount of protein in the oven dried sample was higher than in the freeze dried sample. This was due to a loss of dry matter in the filtering process prior to the oven drying. Analysis of amino acid composition showed a slight decrease in the amounts of cystine and arginine in the stillage while no change was seen in the remaining amino acids.

## Introduction

The majority of the nutritional information available on distillers by-products pertains to corn distillers by-products since corn is the grain most frequently used for alcohol production (Berger, 1981). Nearly 30 million bushels of corn are fermented to neutral spirits and alcoholic beverages yearly. A typical kernel is approximately 90% dry matter consisting of 72% starch, 10% protein, 4.4% lipid, 2.2% crude fiber, and 1.2% ash (Inglett, 1970).

Traditionally alcohol is made from good quality corn. Each year, however, a portion of the crop is damaged by insects and field or storage fungi. Many of the storage fungi adapted to life at conditions of low water availability belong to the species Aspergillus. Aflatoxins are a group of metabolites produced by two fungal species of the Aspergillus flavus group. These metabolities are extremely toxic to many species of animals. As little as .4 ppb has been shown to cause liver tumors in trout, 250 ppb will cause death in turkey poults, and 700 to 1000 ppb can result in liver damage and reduced growth and feed efficiency in beef cattle (Wilcox, 1972). The FDA has established an action level of 20 ppb in animal feed whereas in foods intended for human use, the permitted tolerance is zero. (Mirocha and Christensen, 1982). Several surveys have been conducted to ascertain the level of aflatoxin contamination in corn. Between 1964 and 1969 corn samples from the midwest showed a relatively low incidence of contamination (Shotwell et al., 1971). In the southeastern states, however, both a high incidence and concentration have been reported (Shotwell, 1977a; Edds, 1979).

Lillehoj et al. (1979), Dam et al. (1977), Chu et al. (1975) and others have shown that no toxin appeared in the distilled alcohol

following fermentation of a mycotoxin contaminated grain, however, toxins were concentrated in the post-fermentation solids. These results indicate that a viable alternative for the use of aflatoxin contaminated corn, in light of the zero tolerance permitted in human food, would be in alcohol production.

The objective of this study was to examine some of the nutritional qualities of the whole stillage when sound and aflatoxin contaminated corn were used as feedstocks for ethanol production.

## Materials and Methods

Corn used in this study was obtained from the Kansas State University Department of Grain Science and Industry pilot feed mill. Treatments included a control, yellow dent corn (U.S. No. 2) and corn contaminated with aflatoxin.

The contaminated corn was produced by spraying kernels with spores from strains of A. flavus known to produce predominantly $B_1$ toxin. The grain was placed in a growth chamber for 6 days at a temperature of 27 to 30 C and an equilibrium relative humidity of 85 to 90%. Grain moisture content ranged from 18.2 to 19.5%.

All grains were ground through a hammermill having a 3.2 mm screen and placed in airtight containers in cold storage. Analysis of feedstock particle size followed procedures outlined by Pfost and Headley (1976). Duplicate 2 kg samples were randomly processed through the pilot scale alcohol plant (Appendix A) using the procedure described in Appendix B. Representative aliquots of whole stillage were either freeze dried or filtered through cheesecloth and oven dried at 56 C for 48 hours.

AOAC (1975) methods were used to determine dry matter, crude protein, ash, crude fiber, and ether extract. A modification of the method of Goering and Van Soest (1970) was used for determination of neutral detergent fiber. A detergent stable alpha-amylase solution (Robertson and Van Soest, 1977) was used to eliminate the possibility of residual starch in the sample. Analyses were run on a Tecator Fibertech$^R$ System[1]. Gross energy values were obtained using a bomb calorimeter (Parr Instrument Co., 1978)[2]. Proteins were

[1]Tecator, 2814 Towerview Rd., Herndon, VA 22070

[2]Parr Instrument Co., Moline, Illinois.

hydrolyzed with p-toluensulfonic acid and amino acids determined on a Dionex[1] Kit amino acid analyzer (Liu and Chang, 1971). Sugars were extracted in water and soluble carbohydrates were analyzed for using the method of Parker (Undated). Ergosterol is the predominant sterol component of most fungi and has been used to estimate fungal growth in cereal grains. Ergosterol levels were determined using a method described by Seitz et al. (1979) (Appendix C). Quantitative analysis of aflatoxin were conducted on feedstocks, stillages, and alcohols using a modification of the method described by Seitz et al. (1982) (Appendix D).

[1]Dionex Corporation, 1228 Titan Way, Sunnyvale, CA 94086

## Results and Discussion

The control and aflatoxin contaminated corn, ground through a 1 hp hammermill with a 3.2 mm screen, show no differences between the treatments in the particle size analysis (table 2). Analysis of the nutrient composition of the feedstocks (table 3) shows few differences between the control and contaminated corn. The protein level of the aflatoxin contaminated corn was slightly lower than the control. A slight decrease in protein levels may be expected because A. flavus destroys the germ and decays and discolors whole kernels (Christensen and Sauer, 1982). Ergosterol level, an indicator of fungal invasion, was higher in the contaminated corn than in the control. Analysis of the grain for aflatoxin showed a level of 426 ppb in the contaminated corn while none was detected in the control. This level of aflatoxin is considerably higher than that normally encountered in corn which is naturally contaminated in the field, but for the purposes of this experiment, it was desirable to determine if such high levels would cause aflatoxin to move with the distilled alcohol.

No aflatoxin was detected in the alcohol (table 5) but 362 ppb was found in the freeze dried stillage (table 7). It appears that there was a slight degradation of aflatoxin during the alcohol production process. Although Chu et al. (1975) showed that aflatoxin was not substantially modified by the cooking process used in brewing, work by Nip et al. (1975) showed that some degredation did occur during the mashing and brewing stages with the initial concentration of toxin in the substrate determining the quantity of toxin found in the beer. Investigations by Dam et al. (1977) and Lillehoj et al. (1979) of the fate of aflatoxin in distilled products

showed that no toxin appeared in the alcohol but the toxin was concentrated in the post-fermentation solids.

Tables 5 and 6 list the parameters of the alcohol and stillage produced. The percent of theoretical value was calculated on a stoichiometric basis as described by Maisch et al. (1979). The hydrolysis of 1 kg of starch (dry basis) yields 1.11 kg of glucose which can then be converted to .551 kg alcohol. The specific gravity of ethanol (.794) can be used to convert to volume. The value obtained from this calculation is for pure ethanol and does not take into account the formation of the azeotropic mixture of ethanol and water which forms during distillation. The Solar Energy Research Institute (1980) indicate the expected alcohol yield from a bushel of corn would be 9.8 l. If this basis was used, the percent of expected yield in this experiment would be 50% for the control and 91% for the aflatoxin contaminated corn.

In the early stages of this research the distillation column was packed with 6 mm glass beads and the column was insulated which allowed the column to flood rather than reflux thus resulting in poor yields. The beads were replaced with glass marbles (approximately 15 mm) and the percent of theoretical obtained was increased by 25 to 30%.

There is a large difference in solids content of the whole stillage between the freeze dried and oven dried samples. The stillage to be oven dried was filtered through cheesecloth, thus removing a portion of the liquid. In many operations some type of filtration or centrifugation is used to remove liquid and lower the cost of drying. Since the freeze dried sample was not filtered, there is a lower percentage of solids than in the oven dried sample.

During fermentation of corn, the starch is converted to alcohol and $CO_2$ leaving protein, fat, fiber, and ash. As starch makes up nearly 70% of the kernel, the disappearance of this fraction should result in nearly a three fold increase of the remaining nutrients in the by-products essentially changing an energy source to a protein source (Klopfenstein and Abrams, 1981). The chemical composition of freeze dried (table 7) and oven dried (table 9) corn stillages showed an increase of protein, fat, fiber, and ash of 2.5 to 3 times that found in the feedstocks. The amino acid profiles show this increase when comparisons are made of values expressed as a percent on a dry matter basis (tables 4, 8 and 10). However, when the values are expressed as grams per 100 g of protein, it appears that there is very little difference between feedstock and stillage profiles. Allowing for a 5 percent error in the determination method, it appears that the stillages are lower in cystine and arginine than the feedstocks.

A comparison of the concentrating effect of individual nutrients from the feedstocks to the stillage (ie. protein content going from 10.3% in the feedstock to 25.3% in the freeze dried stillage gives a concentration effect of 2.5) showed no differences between the control and aflatoxin contaminated corn. An analysis of the nutrients in the stillage shows no difference between freeze drying and oven drying due to treatment, however, a difference did exist between drying methods in the amount of protein with the higher level present in the oven dried sample. There were no differences in the levels of any other nutrients due to drying method. The difference in protein levels may be explained by noting that a portion of the soluble carbohydrate fraction was removed prior to oven drying (tables 7 and 9). This indicates that a

portion of the carbohydrate dry matter was filtered out in the solubles fraction resulting in an increase in dry matter loss in the oven dried samples. This loss of dry matter appears to make the oven dried samples higher in protein when, in actuality, the proportion of the total dry matter is all that has increased. This is similar to changing the feedstock from an energy source to a protein source through the alcohol production process.

## Conclusion

The use of damaged grain can reduce the cost of fuel alcohol production. When mycotoxin contaminated wheat was used as a feedstock, no toxin was detected in the alcohol. The toxin was, however, concentrated in the by-product and would have to be dealt with before being used as a feed ingredient.

Analysis of the nutrient composition of the stillage showed no differences due to treatments. The nutrient quality of the by-product could, then, be determined from an analysis of the feedstocks. There were some differences in amino acid composition with decreases in glutamic acid and half-cystine and increases in alanine, methionine, and isoleucine.

The ability to develop optimum production conditions was limited as there were only two replications of each treatment. The decision to simulate "on farm" conditions meant that measurements of conversion and saccharification of starch during production was not as sophisticated as possible and optimum amounts of alcohol were not always obtained.

## Literature Cited

AOAC. 1975. Official Methods of Analysis (12th Ed.), Association of Official Analytical Chemists, Washington, D.C.

Berger, L.L. 1981. Nutritional Value of distillers feeds for livestock. Thirty-Sixth Kansas Formula Feed Conference, A-1.

Christensen, C.M. and D.B. Sauer. 1982. Microflora. In C.M. Christensen (Ed.) Storage of Cereal Grains and Their Products. Amer. Assoc. Cereal Chem., Inc., St. Paul, Minnesota.

Chu, F.S., C.C. Chang, S.H. Ashoor, and N. Prentice. 1975. Stability of aflatoxin $B_1$ and ochratoxin A in brewing. Applied Microbiol. 29: 313.

Dam, R., S.W. Tam, and L.D. Satterlee. 1977. Destruction of aflatoxins during fermentation and by-product isolation from artificially contaminated grains. Cereal Chem. 54: 705.

Edds, G.T. 1979. Aflatoxins. In Conference on Mycotoxins in Animal Feeds and Grains Related to Animal Health. P.B. Rep. PB-300-300. U.S. Dept. Commer., Off. Tech. Serv., Washington, D.C.

Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis. Agriculture Handbook 379. United States Department of Agriculture.

Inglett, G.E. 1970. Kernel structure, composition, and quality. In G.E. Inglett (Ed.) Corn: Culture, Processing, Products. Avi Publishing Co., Inc., Connecticut.

Klofenstein, T. and S.M. Abrams. 1981. Distillers by-products use - a review. Nebraska Cattle Day Rep. EC 81-218, p. 2.

Lillehoj, E.B., W.f. Maisch, and A. Lagoda. 1979. The fate of aflatoxin in naturally contaminated corn during ethanol fermentation. Can. J. Microbiol. 25: 911.

Liu, T.Y. and Y.H. Chang. 1971. Hydrolysis of proteins p-toluensulfonic acid. J. Biol. Chem. 246: 2842.

Maisch, W.F., M. Sobolov, and A.J. Petricola. 1979. Distilled beverages. In H.J. Peppler and D. Perlmau (Ed.) Microbial Technology. Vol. II. Academic Press, New York.

Mirocha, C.J. and C.M. Christensen. 1982. Micotoxins. In C.M. Christensen (Ed.) Storage of Cereal Grains and Their Products. Amer. Assoc. Cereal Chem., Inc., St. Paul, Minnesota.

Nip, W.K., F.C. Chang, F.S. Chu, and N. Prentice. 1975. Fate of ochratoxin A in brewing. Appl. Microbiol. 30: 1048.

Parker, R.B. Undated. Methodology for determining quality of silage. National Feed Ingredient Assoc., Des Moines, Iowa.

Parr Instrument Company. 1978. Instructions for the 1341 plain jacket oxygen bomb calorimeter. Manual No. 147, Moline, Illinois.

Pfost, H.B. and V.E. Headley. 1976. Methods of determining and expressing particle size. In H.B. Pfost (Ed.) Feed Manufacturing Technology, AFMA, Arlington, Virginia.

Robertson, J.B. and P.J. Van Soest. 1977. Dietary fiber estimation in concentrate feedstuffs. J. Anim. Sci. Vol. 34, Suppl. 1, p. 254.

Seitz, L.M., D.B. Sauer, R. Burroughs, H.E. Mohr, and J.D. Hubbard. 1979. Ergosterol as a measure of fungal growth. Phytopathology 69(11): 1202.

Seitz, L.M., D.B. Sauer, H.E. Mohr and D.F. Aldis. 1982. Fungal growth and dry matter loss during bin storage of high moisture corn. Cereal Chem. 59(1): 9.

Shotwell, D.L. 1977a. Aflatoxin in corn. J. An. Oil Chem. Soc. 54: 216A.

Shotwell, D.L., C.W. Hesseltine, E.E. Vandegraft, and M. Goulden. 1971. Survey of corn from different regions for aflatoxin, ochratoxin, and zearalenone. Cereal Sci. Today 16: 266.

Solar Energy Research Institute. 1980. Fuel From Farms - A Guide to Small-Scale Ethanol Production. SERI/SP-451-519. The National Technical Information Service, Springfield, Virginia.

Wilcox, R.A. 1972. Spoiled feedstuffs - harmless or dangerous. Extension Bulletin L-300, Cooperative Extension Service, Kansas State University, Manhattan, Kansas.

Table 2. PARTICLE SIZE OF FEEDSTOCKS USED IN ALCOHOL PRODUCTION FROM AFLATOXIN CONTAMINATED CORN

| Analysis | Treatment | |
|---|---|---|
| | Control | Aflatoxin |
| Mean particle diameter, | 800 | 784 |
| Geometric standard deviation | 2.11 | 1.97 |
| Particle number, particles/gm | 18201 | 12438 |
| Surface area, cm2/gm | 75.0 | 72.9 |

Table 3. COMPOSITION OF FEEDSTOCKS USED IN THE PRODUCTION OF ALCOHOL USING AFLATOXIN CONTAMINATED CORN[a]

| Analysis | Treatment | |
|---|---|---|
| | Control | Aflatoxin |
| Dry matter, % | 88.1 | 90.1 |
| Crude protein, % | 10.3 | 9.5 |
| Ash, % | 1.5 | 1.3 |
| Ether extract, % | 3.5 | 3.8 |
| Crude fiber, % | 2.7 | 2.6 |
| Neutral detergent fiber, % | 24.1 | 25.4 |
| Gross energy, Kcal/kg | 4509 | 4511 |
| Ergosterol, ppm | 1.90 | 2.36 |
| Aflatoxin, ppb | ND[b] | 426 |

[a] Values expressed on a dry matter basis

[b] None detected

Table 4. AMINO ACID COMPOSITION OF FEEDSTOCKS USED IN THE PRODUCTION OF ALCOHOL USING AFLATOXIN CONTAMINATED CORN.

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Aflatoxin | Control | Aflatoxin |
| | ----- % (dry basis) ----- | | ---g amino acid/100 g protein--- | |
| Aspartic Acid | .66 | .63 | 7.40 | 7.56 |
| Threonine | .30 | .29 | 3.34 | 3.42 |
| Serine | .48 | .47 | 5.44 | 5.58 |
| Glutamic Acid | 1.58 | 1.45 | 17.83 | 17.42 |
| Proline | .78 | .74 | 8.83 | 8.83 |
| Glycine | .35 | .35 | 3.98 | 4.16 |
| Alanine | .69 | .63 | 7.82 | 7.53 |
| Half Cystine | .13 | .13 | 1.41 | 1.52 |
| Valine | .28 | .27 | 3.12 | 3.18 |
| Methionine | .18 | .18 | 2.01 | 2.11 |
| Isoleucine | .19 | .18 | 2.10 | 2.14 |
| Leucine | 1.03 | .94 | 11.55 | 11.27 |
| Tyrosine | .40 | .39 | 4.48 | 4.69 |
| Phenylalanine | .45 | .43 | 5.07 | 5.15 |
| Histidine | .41 | .42 | 4.63 | 5.05 |
| Lysine | .22 | .21 | 2.43 | 2.48 |
| Arginine | .48 | .43 | 5.35 | 5.16 |

Table 5. ALCOHOL YIELD WHEN USING AFLATOXIN CONTAMINATED CORN AS A FEEDSTOCK

| Alcohol | Treatment | |
|---|---|---|
| | Control | Aflatoxin |
| Volume, ml | 383 | 700 |
| Specific gravity | .900 | .820 |
| Proof | 133 | 190 |
| Percent of theoretical[a] | 42 | 76 |
| Aflatoxin, ppb | ND[b] | ND |

[a] Calculated on a stoichiometric basis of 100% ethanol

[b] None detected

Table 6. PERCENT SOLIDS OF WHOLE STILLAGE FROM ALCOHOL PRODUCTION FROM AFLATOXIN CONTAMINATED CORN AS DETERMINED BY FREEZE DRYING AND OVEN DRYING METHODS

| Alcohol | Treatment | |
|---|---|---|
| | Control | Aflatoxin |
| Stillage-total weight, kg | 10.24 | 10.20 |
| Solids-freeze dried, % | 7.2 | 8.3 |
| Solids-filtered, oven dried, % | 15.5 | 13.6 |

Table 7. COMPOSITION OF FREEZE DRIED STILLAGE USING AFLATOXIN CONTAMINATED CORN FOR ALCOHOL PRODUCTION[a]

| Analysis | Treatment | |
|---|---|---|
| | Control | Aflatoxin |
| Dry matter, % | 95.3 | 92.4 |
| Crude protein, % | 25.3 | 27.7 |
| Ash, % | 7.5 | 6.4 |
| Ether extract, % | 10.2 | 9.9 |
| Crude fiber, % | 8.8 | 6.8 |
| Neutral detergent fiber, % | 37.7 | 37.8 |
| Gross energy, Kcal/kg | 4971 | 5320 |
| Soluble carbohydrates, %[b] | 9.5 | 7.6 |
| Ergosterol, ppm | 6.5 | 17.1 |
| Aflatoxin, ppb | ND[c] | 362 |

[a] Values expressed on a dry matter basis

[b] Values expressed as percent of the dry matter

[c] None detected

Table 8. AMINO ACID COMPOSITION OF FREEZE DRIED STILLAGE WHEN USING AFLATOXIN CONTAMINATED CORN FOR ALCOHOL PRODUCTION

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Aflatoxin | Control | Aflatoxin |
| | ----- % (dry basis) ----- | | ---g amino acid/100 g protein--- | |
| Aspartic Acid | 1.64 | 1.89 | 7.81 | 7.97 |
| Threonine | .74 | .90 | 3.53 | 3.87 |
| Serine | 1.26 | 1.38 | 5.96 | 5.76 |
| Glutamic Acid | 3.99 | 4.48 | 18.93 | 18.64 |
| Proline | 1.73 | 2.00 | 8.21 | 8.56 |
| Glycine | .90 | 1.02 | 4.29 | 4.28 |
| Alanine | 1.68 | 1.90 | 7.98 | 7.94 |
| Half Cystine | .24 | .21 | 1.13 | 1.23 |
| Valine | .68 | .77 | 3.24 | 3.34 |
| Methionine | .45 | .49 | 2.16 | 2.10 |
| Isoleucine | .47 | .53 | 2.23 | 2.25 |
| Leucine | 2.47 | 2.72 | 11.70 | 11.47 |
| Tyrosine | 1.04 | 1.12 | 4.95 | 4.76 |
| Phenylalanine | 1.07 | 1.17 | 5.08 | 5.08 |
| Histidine | .98 | 1.13 | 4.65 | 4.73 |
| Lysine | .52 | .53 | 2.46 | 2.52 |
| Arginine | .74 | .72 | 3.49 | 3.42 |

Table 9. COMPOSITION OF OVEN DRIED STILLAGE WHEN USING AFLATOXIN CONTAMINATED CORN FOR ALCOHOL PRODUCTION[a]

| Analysis | Treatment | |
|---|---|---|
| | Control | Aflatoxin |
| Dry matter, % | 95.4 | 95.1 |
| Crude protein, % | 31.8 | 31.2 |
| Ether extract, % | 13.0 | 11.4 |
| Gross Energy, Kcal/Kg | 5388 | 5463 |
| Soluble carbohydrates, %[b] | 4.4 | 4.6 |

[a] Values expressed on a dry matter basis.

[b] Values expressed as percent of the dry matter

Table 10. AMINO ACID COMPOSITION OF OVEN DRIED STILLAGE WHEN USING AFLATOXIN CONTAMINATED CORN FOR ALCOHOL PRODUCTION

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Aflatoxin | Control | Aflatoxin |
| | ----- % (dry basis) ----- | | ---g amino acid/100 g protein--- | |
| Aspartic Acid | 2.07 | 2.45 | 7.17 | 8.55 |
| Threonine | 1.04 | 1.07 | 3.59 | 3.78 |
| Serine | 1.61 | 1.67 | 5.58 | 5.85 |
| Glutamic Acid | 5.18 | 5.09 | 17.90 | 17.92 |
| Proline | 2.62 | 2.47 | 9.06 | 8.73 |
| Glycine | 1.10 | 1.12 | 3.82 | 3.95 |
| Alanine | 2.27 | 2.22 | 7.85 | 7.81 |
| Half Cystine | .41 | .53 | 1.43 | 1.82 |
| Valine | .96 | .93 | 3.32 | 3.25 |
| Methionine | .68 | .60 | 2.37 | 2.11 |
| Isoleucine | .68 | .58 | 2.37 | 2.06 |
| Leucine | 3.47 | 3.41 | 11.97 | 12.06 |
| Tyrosine | 1.38 | 1.24 | 4.79 | 4.37 |
| Phenylalanine | 1.53 | 1.26 | 5.29 | 4.46 |
| Histidine | 1.29 | 1.38 | 4.48 | 4.88 |
| Lysine | .63 | .66 | 2.19 | 2.33 |
| Arginine | 1.27 | 1.08 | 4.40 | 3.80 |

# QUALITY OF WHOLE STILLAGE WHEN DAMAGED WHEAT IS USED FOR THE PRODUCTION OF ALCOHOL

## Summary

Damaged wheat was used to investigate the quality of whole stillage from alcohol produced from damaged grain. Treatments included a control, a field sprouted white wheat, and deoxynivalenol contaminated samples obtained from the 1982 Kansas and Nebraska crops. Stillage samples were either freeze dried or were filtered and oven dried at 56 C. Grains and stillages were analyzed for crude protein, ether extract, crude fiber, ash, gross energy, neutral detergent fiber, and amino acid composition. Ergosterol levels were used to determine the fungal activity in the samples. Particle size of the feedstocks was also determined.

The deoxynivalenol analysis showed a level of 8.2 ppm in sample A and 6.2 ppm in sample B. An examination of the stillage from samples A and B showed that these levels were concentrated to 29.4 ppm and 14 ppm, respectively. No deoxynivalenol was detected in the alcohol from either sample.

Determination of the nutrient composition of the stillage showed no differences due to treatment except for the sprout damaged sample. Protein was concentrated more in the sprouted wheat stillage than in the control. Ether extract was concentrated more in both deoxynivalenol contaminated samples than in the control. There were differences noted between drying methods with oven drying giving higher values. This was due to a loss of dry matter during the filtering process. Amino acid analysis showed slight decreases in glutamic acid and half-cystine and increases in alanine, methionine, and isoleucine in the stillage.

## Introduction

In 1978 the United States produced nearly 1800 million bushels of wheat. Although better than 2000 million bushels were used or sold, there remained a recurring surplus of 925 million bushels (USDA, 1980). It is apparent that there is a need to develop alternative uses for wheat, one of which could be as a feedstock in ethanol production.

A wheat kernel is approximately 83% endosperm, 14.5% bran, and 2.5% germ. The chemical composition of wheat varies considerably with variety and growing conditions, however, a typical analysis of hard red winter wheat would be: dry matter 88%; 14.4% protein; 1.8% ether extract; 2.8% crude fiber; and 1.9% ash (NRC, 1982).

Sprout damage in wheat is a major problem in parts of the northwest U.S. and Canada. Sprouting in wheat, a result of wet weather following kernel maturation but prior to harvest, will directly affect the quality and quantity of milled flour (Zeleny, 1978). The extent of sprouting is an important criteria in the grading of wheat and sprouted wheat is considered damaged according to the Official Grain Standards of the United States.

Wheat may also be damaged by fungi either before or after harvest if conditions are adequate to encourage and support fungal growth. Unusually wet weather between the flowering and medium dough stage may result in shriveled, pink kernels, a condition commonly referred to as "scabby" wheat (Mirocha and Christensen, 1982). Scab is caused by the fungus Gibberella zeas (perfect stage of Fusarium graminearum) which may produce the mycotoxin deoxynivalenol (DON), or vomitoxin. Levels of DON between .4 and 1.8 ppm in feedstuffs have been shown to cause feed refusal while slightly higher levels have been reported to cause vomiting in swine (Vesonder and Hesseltine, 1981).

This study was designed to determine the nutritional value of the whole stillage resulting from ethanol production when sound, sprout damaged, and scabby wheat were used as feedstocks. The fate of DON during ethanol production was also determined.

## Materials and Methods

Sound wheat was obtained from the Kansas State University Department of Grain Science and Industry pilot feed mill. Field sprouted wheat, a white wheat determined by the Federal Grain Inspection Service to be 31.5% sprouted, was acquired from the USDA Grain Marketing Research Laboratory, Manhattan, Kansas. Samples of deoxynivalenol contaminated wheat were obtained from the 1982 Kansas and Nebraska crops.

Samples were ground through a hammermill having a 3.2 mm screen and stored in airtight containers (4 C). Duplicate samples were randomly processed through the pilot scale ethanol plant as described in Appendix B. A modification of the method of Scott et al. (1981) (using high-performance liquid chromatography rather than gas-liquid chromatography) was used to analyze for deoxynivalenol in feedstocks, stillages, and alcohols (Appendix E). Other analytical procedures carried out on feedstocks and stillages have been described earlier.

## Results and Discussion

The control and all treatments of wheat were ground through a 1 hp hammermill having a 3.2 mm screen. The particle size analysis (table 11) shows that the wheat contaminated with deoxynivalenol (DON) has a larger mean particle diameter, thus fewer particles per gram and less surface area than the control. This was likely due to a higher moisture content in the mold contaminated grain at the time of grinding.

An analysis of the nutrient composition of the feedstocks is presented in table 12. The protein level of the sprout damaged wheat was lower than in the control. The sprouted wheat used in this study was a soft white variety and the control was a hard red winter variety. Soft white wheats are normally lower in protein than hard red winter wheats. The ergosterol level, a measure of fungal invasion, was considerably higher in the wheat contaminated with deoxynivalenol, a metabolite produced by the fungus Fusarium graminearum. The analysis for the presence of DON showed none in the control, 8.2 ppm in the DON-A sample, and 6.2 ppm in the DON-B sample. Analysis of the alcohols (table 15) showed no detectable levels of DON from either sample. There was a concentration of DON in the freeze dried stillage in similar proportions to the concentration of the other nutrients.

Data on the resultant alcohol and stillage from fermentation of wheat samples are shown in tables 15 and 16. There is a large difference in solids content of the whole stillage between the freeze dried and oven dried samples. The stillage to be oven dried was filtered through cheesecloth, thus removing a portion of the liquid. In many operations some type of filtration or centrifugation is used to remove liquid and lower the cost of drying. Since the freeze

dried sample was not filtered, there is a lower percentage of solids than in the oven dried sample. The percent of theoretical was calculated stoichiometrically as described earlier. According to expected yield figures published by the Solar Energy Research Institute (1980), the percent of theoretical would be 65% for the control, 67% for the sprouted, 68% for the deoxynivalenol-A, and 67% for the deoxynivalenol-B. The proof of the alcohol indicates that there was a poor separation of water and alcohol in the reflux column except for the sprouted samples. Again this was due, in the early stages, to the small beads in the reflux column which caused the column to flood resulting in a poor separation. The change to larger beads seemed to correct the flooding problem and allowed for the collection of a higher proof alcohol sample.

The fermentation process tends to concentrate the nutrients remaining after the starch has been converted to alcohol. Starch usually composes approximately 2/3 of the kernel so the remaining nutrients are concentrated nearly three times. In this experiment, an estimate of 63% starch (db) for wheat (Solar Energy Research Institute, 1980) was used in calculating production parameters, thus the other nutrients should be concentrated nearly 2.7 times. The chemical composition of freeze dried (table 17) and oven dried (table 20) stillage shows the nutrients are concentrated from 2.1 to 3.5 times. The amino acid profiles also show this concentration effect when results are presented as a percent on a dry matter basis (tables 14, 19 and 22). When values are expressed as grams per 100 g of sample (tables 13, 18 and 21) it appears that the amino acid profile is not greatly altered during alcohol production. Allowing for a 5 percent inherent variability in the method, slight

reductions in glutamic acid and half-cystine appeared in the freeze dried stillage while increases were noted in alanine, methionine, and isoleucine.

A comparison of the concentrating effect of individual nutrients from feedstock to stillage showed differences in the effect for protein and ether extract (ie. the protein content going from 14.6 in the feedstock to 36.8 in the freeze dried stillage results in a concentration effect of 2.5).

The concentrating effect of protein in the sprouted wheat was higher than the control grain. Both DON samples exhibited a higher concentration effect for ether extract than the control. The first difference can be explained by looking at the amount of protein initially present in the feedstock. In the sprouted sample the white wheat has less protein, ash, fat, and fiber meaning there is more carbohydrate to be converted thus more concentration of the remaining nutrients. The ether extract content of the two DON contaminated samples is lower than in the control but the amount of carbohydrate to be converted is the same, thus the ether extract values should be lower than in the control. The results show that the levels are the same in the control and DON contaminated samples meaning the ether extract portions in the DON samples were concentrated more than the other nutrients. The gross energy values support the ether extract analysis as all three are essentially equal. It is possible that, during the fermentation, the yeast reacted with the mold infested sample resulting in an increase in detectable ether extract.

In addition, the larger particle size of the DON samples may also have contributed to this difference. More production runs would be needed to verify that the increase is real.

The analysis of nutrients in the stillage showed no differences due to treatment except for the sprout damaged sample. Again, this may be due to the lower values of the initial feedstock. There could also be some differences due to the physiological changes occuring in the kernel during the sprouting process which manifested themselves during the fermentation processes.

There were differences noted between drying methods with oven-drying giving higher values. As mentioned earlier, this can be explained by noting that the soluble carbohydrates fractions in the oven dried samples are less than in the freeze dried samples indicating a loss in dry matter, thus a proportional increase in the other nutrients.

## Conclusion

The use of damaged grain can reduce the cost of fuel alcohol production. When mycotoxin contaminated wheat was used as a feedstock, no toxin was detected in the alcohol. The toxin was, however, concentrated in the by-product and would have to be dealt with before being used as a feed ingredient.

Analysis of the nutrient composition of the stillage showed no differences due to treatments. The nutrient quality of the by-product could, then, be determined from an analysis of the feedstocks. There were some differences in amino acid composition with decreases in glutamic acid and half-cystine and increases in alanine, methionine, and isoleucine.

The ability to develop optimum production conditions was limited as there were only two replications of each treatment. The decision to simulate "on farm" conditions meant that measurements of conversion and saccharification of starch during production was not as sophisticated as possible and optimum amounts of alcohol were not always obtained.

# Literature Cited

Mirocha, C.J. and C.M. Christensen. 1982. Mycotoxins. In C.M. Christensen (Ed.) Storage of Cereal Grains and Their Products. Amer. Assoc. Cereal Chem., Inc., St. Paul, Minnesota.

NRC. 1982. United States - Canadian Tables of Feed Composition. National Academy Press, Washington, D.C.

Scott, P.M., P. Lau, and S.R. Kanhere. 1981. Gas Chromatography with electron capture and mass spectrometric detection of deoxynivalenol in wheat and other grains. J. Assoc. Off. Anal. Chem. 64(6): 1364.

Solar Energy Research Institute. 1980. Fuel From Farms - A Guide to Small-Scale Ethanol Production. SERI/SP-451-519. The National Technical Information Service, Springfield, Virginia.

USDA. 1980. Agricultural Statistics. United States Gov't. Printing Office, Washington, D.C.

USDA. 1978. Official Grain Standards of the United States (1978) U.S. Department of Agriculture, Washington, D.C.

Vesonder, R.F. and C.W. Hesseltine. 1981. Vomitoxin: natural occurrence on cereal grains and significance as a refusal and emetic factor to swine. Process Biochem. Dec./Jan., 1980/81.

Zeleny, L. 1978. Criteria of wheat quality, In Y. Pomeranz (Ed.) Wheat Chemistry and Technology. Amer. Assoc. Cereal Chem., St. Paul, Minnesota.

Table 11. PARTICLE SIZE OF FEEDSTOCKS USED IN ALCOHOL PRODUCTION FROM DAMAGED WHEAT

| Analysis | Treatment | | |
|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol |
| Mean particle diameter, | 820 | 739 | 1056 |
| Geometric standard deviation | 2.02 | 2.06 | 1.87 |
| Particle number, particles/gm | 12767 | 19247 | 3797 |
| Surface area, $cm^2/gm$ | 69.4 | 78.1 | 51.3 |

Table 12. COMPOSITION OF FEEDSTOCKS USED IN THE PRODUCTION OF ALCOHOL FROM DAMAGED WHEAT[a]

| Analysis | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| Dry matter, % | 88.9 | 87.1 | 88.5 | 89.9 |
| Crude protein, % | 14.6 | 11.0 | 15.5 | 16.6 |
| Ash, % | 1.9 | 1.5 | 1.9 | 2.1 |
| Ether extract, % | 1.7 | 1.5 | 1.2 | 1.0 |
| Crude fiber, % | 3.1 | 2.6 | 3.3 | 3.4 |
| Neutral detergent fiber, % | 18.3 | 14.9 | 34.7 | 37.8 |
| Gross energy, Kcal/kg | 4501 | 4496 | 4441 | 4346 |
| Ergosterol, ppm | 1.16 | NA[c] | 11.16 | 12.72 |
| Deoxynivalenol, ppm | ND[b] | NA[c] | 8.2 | 6.2 |

[a] Values expressed on a dry matter basis

[b] None detected

[c] Analysis not conducted on this sample

Table 13. AMINO ACID COMPOSITION OF FEEDSTOCKS USED IN THE PRODUCTION OF ALCOHOL FROM DAMAGED WHEAT

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| | g amino acid/100 g protein | | | |
| Aspartic Acid | 5.86 | 6.37 | 5.99 | 6.42 |
| Threonine | 2.99 | 2.75 | 2.83 | 2.95 |
| Serine | 5.22 | 5.53 | 4.99 | 5.23 |
| Glutamic Acid | 27.55 | 27.20 | 27.73 | 29.06 |
| Proline | 9.27 | 9.88 | 9.64 | 10.17 |
| Glycine | 4.35 | 4.51 | 4.23 | 4.33 |
| Alanine | 3.86 | 4.14 | 3.91 | 3.96 |
| Half Cystine | 1.66 | 1.75 | 1.47 | 1.72 |
| Valine | 3.12 | 3.27 | 3.09 | 3.22 |
| Methionine | 1.79 | 1.64 | 1.59 | 1.69 |
| Isoleucine | 2.37 | 2.28 | 2.16 | 2.19 |
| Leucine | 6.35 | 6.34 | 6.26 | .64 |
| Tyrosine | 3.64 | 3.66 | 3.44 | 3.57 |
| Phenylalanine | 4.66 | 5.04 | 5.24 | 5.50 |
| Histidine | 3.90 | 4.61 | 5.79 | 5.80 |
| Lysine | 2.67 | 2.45 | 3.15 | 3.46 |
| Arginine | 5.58 | 4.51 | 5.59 | 6.98 |

Table 14. AMINO ACID COMPOSITION OF FEEDSTOCKS USED IN THE PRODUCTION OF ALCOHOL FROM DAMAGED WHEAT

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| | % (dry basis) | | | |
| Aspartic Acid | .77 | .58 | .82 | .91 |
| Threonine | .40 | .25 | .39 | .42 |
| Serine | .69 | .50 | .69 | .74 |
| Glutamic Acid | 3.64 | 2.48 | 3.81 | 4.12 |
| Proline | 1.23 | .90 | 1.33 | 1.44 |
| Glycine | .57 | .41 | .58 | .61 |
| Alanine | .51 | .38 | .54 | .56 |
| Half Cystine | .22 | .16 | .20 | .24 |
| Valine | .41 | .30 | .42 | .46 |
| Methionine | .24 | .15 | .22 | .24 |
| Isoleucine | .31 | .21 | .30 | .31 |
| Leucine | .84 | .58 | .86 | .09 |
| Tyrosine | .48 | .33 | .47 | .51 |
| Phenylalanine | .62 | .46 | .72 | .78 |
| Histidine | .52 | .42 | .80 | .82 |
| Lysine | .35 | .22 | .43 | .49 |
| Arginine | .74 | .41 | .77 | .99 |

Table 15. ALCOHOL YIELD WHEN USING DAMAGED WHEAT AS A FEEDSTOCK

| Analysis | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| Volume, ml | 500 | 515 | 525 | 520 |
| Specific gravity | .865 | .823 | .900 | .900 |
| Proof | 159 | 192 | 134 | 133 |
| Percent of theoretical[a] | 63 | 66 | 66 | 64 |
| Deoxynivalenol, ppp | ND[b] | NA[c] | ND | ND |

[a] Calculated on a stoichiometric basis of 100% ethanol

[b] None detected

[c] Analysis not conducted on this sample

Table 16. PERCENT SOLIDS OF WHOLE STILLAGE FROM ALCOHOL PRODUCED FROM DAMAGED WHEAT AS DETERMINED BY FREEZE DRYING AND OVEN DRYING METHODS

| Analysis | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| Stillage-total weight, kg | 9.41 | 9.89 | 9.35 | 9.22 |
| Solids-freeze dried, % | 7.9 | 7.0 | 8.6 | 8.8 |
| Solids-filtered, oven dried,% | 14.7 | 14.2 | 15.4 | 15.8 |

Table 17. COMPOSITION OF FREEZE DRIED STILLAGE WHEN USING DAMAGED WHEAT FEEDSTOCKS FOR ALCOHOL PRODUCTION[a]

| Analysis | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| Dry matter, % | 94.6 | 92.5 | 95.1 | 96.5 |
| Crude protein, % | 36.8 | 31.9 | 36.0 | 35.9 |
| Ash, % | 5.8 | 4.2 | 6.6 | 5.2 |
| Ether extract, % | 4.8 | 4.8 | 4.7 | 4.5 |
| Crude fiber, % | 8.7 | 9.3 | 9.0 | 9.4 |
| Neutral detergent fiber, % | 34.3 | 34.4 | 36.1 | 42.8 |
| Gross energy, Kcal/kg | 4990 | 5100 | 4957 | 4917 |
| Soluble carbohydrates, %[b] | 8.6 | 10.8 | 8.9 | 11.7 |
| Ergosterol, ppm | 21.3 | NA[d] | 37.2 | 37.4 |
| Deoxynivalenol, ppm | ND[c] | NA | 29.4 | 14.0 |

[a] Values expressed on a dry matter basis

[b] Values expressed as percent of the dry matter

[c] None detected

[d] Analysis not conducted on this sample

Table 18. AMINO ACID COMPOSITION OF FREEZE DRIED STILLAGE WHEN USING DAMAGED WHEAT FEEDSTOCKS FOR ALCOHOL PRODUCTION

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| | g amino acid/100 g protein | | | |
| Aspartic Acid | 7.54 | 7.02 | 6.49 | 7.69 |
| Threonine | 3.55 | 3.64 | 3.45 | 3.55 |
| Serine | 5.74 | 5.62 | 5.36 | 5.62 |
| Glutamic Acid | 24.37 | 23.84 | 25.44 | 26.16 |
| Proline | 8.88 | 9.39 | 9.44 | 9.50 |
| Glycine | 4.47 | 4.91 | 4.51 | 4.62 |
| Alanine | 4.28 | 4.72 | 4.27 | 4.53 |
| Half Cystine | 2.01 | 1.33 | 1.26 | .99 |
| Valine | 3.87 | 3.56 | 3.25 | 3.19 |
| Methionine | 2.43 | 1.87 | 1.80 | 1.62 |
| Isoleucine | 2.67 | 2.50 | 2.53 | 2.33 |
| Leucine | 6.34 | 6.76 | 6.38 | 6.70 |
| Tyrosine | 4.20 | 4.02 | 4.00 | 3.71 |
| Phenylalanine | 4.24 | 4.20 | 4.80 | 4.40 |
| Histidine | 4.52 | 5.04 | 4.98 | 4.70 |
| Lysine | 2.94 | 3.10 | 3.35 | 2.83 |
| Arginine | 5.38 | 5.86 | 5.79 | 5.01 |

Table 19. AMINO ACID COMPOSITION OF FREEZE DRIED STILLAGE WHEN USING DAMAGED WHEAT FEEDSTOCKS FOR ALCOHOL PRODUCTION

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| | % (dry basis) | | | |
| Aspartic Acid | 2.45 | 1.94 | 2.02 | 2.42 |
| Threonine | 1.15 | 1.01 | 1.08 | 1.12 |
| Serine | 1.86 | 1.55 | 1.67 | 1.78 |
| Glutamic Acid | 7.88 | 6.60 | 7.95 | 8.29 |
| Proline | 2.87 | 2.60 | 2.94 | 3.00 |
| Glycine | 1.45 | 1.36 | 1.41 | 1.46 |
| Alanine | 1.38 | 1.31 | 1.33 | 1.43 |
| Half Cystine | .65 | .37 | .39 | .31 |
| Valine | 1.25 | .98 | 1.01 | 1.01 |
| Methionine | .79 | .52 | .57 | .51 |
| Isoleucine | .86 | .69 | .79 | .73 |
| Leucine | 2.05 | 1.87 | 1.99 | 2.11 |
| Tyrosine | 1.36 | 1.11 | 1.25 | 1.17 |
| Phenylalanine | 1.37 | 1.16 | 1.50 | 1.39 |
| Histidine | 1.46 | 1.39 | 1.56 | 1.48 |
| Lysine | .95 | .86 | 1.05 | .90 |
| Arginine | 1.73 | 1.62 | 1.81 | 1.58 |

Table 20. COMPOSITION OF OVEN DRIED STILLAGE WHEN USING DAMAGED WHEAT FEEDSTOCKS FOR ALCOHOL PRODUCTION[a]

| Analysis | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| Dry matter, % | 96.1 | 97.1 | 95.4 | 95.3 |
| Crude protein, % | 37.7 | 31.9 | 36.4 | 39.5 |
| Ether extract, % | 5.0 | 6.3 | 6.2 | 6.0 |
| Gross energy, Kcal/kg | 5125 | 5141 | 5162 | 5163 |
| Soluble carbohydrates, %[b] | 4.9 | 6.8 | 5.7 | 6.7 |

[a] Values expressed on a dry matter basis

[b] Values expressed as percent of the dry matter.

Table 21. AMINO ACID COMPOSITION OF OVEN DRIED STILLAGE WHEN USING DAMAGED WHEAT FEEDSTOCKS FOR ALCOHOL PRODUCTION

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| | g amino acid/100 g protein | | | |
| Aspartic Acid | 5.92 | 6.71 | 6.32 | 6.32 |
| Threonine | 2.98 | 3.43 | 3.30 | 3.08 |
| Serine | 5.33 | 5.28 | 5.22 | 5.28 |
| Glutamic Acid | 26.88 | 23.07 | 24.59 | 26.43 |
| Proline | 9.28 | 8.34 | 8.62 | 9.53 |
| Glycine | 4.29 | 4.70 | 4.23 | 4.35 |
| Alanine | 4.27 | 4.67 | 4.47 | 4.43 |
| Half Cystine | 1.55 | 1.71 | 1.69 | 1.33 |
| Valine | 3.45 | 3.71 | 3.60 | 3.34 |
| Methionine | 1.84 | 2.21 | 2.28 | 1.76 |
| Isoleucine | 2.46 | 2.75 | 2.84 | 2.45 |
| Leucine | 7.05 | 7.06 | 6.89 | 7.05 |
| Tyrosine | 3.76 | 4.25 | 4.28 | 3.79 |
| Phenylalanine | 5.37 | 5.59 | 5.80 | 5.52 |
| Histidine | 5.06 | 5.85 | 5.36 | 5.47 |
| Lysine | 3.10 | 3.55 | 3.61 | 3.13 |
| Arginine | 4.03 | 4.40 | 4.09 | 3.75 |

Table 22. AMINO ACID COMPOSITION OF OVEN DRIED STILLAGE WHEN USING DAMAGED WHEAT FEEDSTOCKS FOR ALCOHOL PRODUCTION

| Amino Acids | Treatment | | | |
|---|---|---|---|---|
| | Control | Sprouted | Deoxynivalenol-A | Deoxynivalenol-B |
| | % (dry basis) | | | |
| Aspartic Acid | 1.96 | 1.85 | 2.00 | 2.12 |
| Threonine | .99 | .95 | 1.04 | 1.03 |
| Serine | 1.77 | 1.45 | 1.65 | 1.77 |
| Glutamic Acid | 8.92 | 6.36 | 7.78 | 8.89 |
| Proline | 3.08 | 2.30 | 2.73 | 3.20 |
| Glycine | 1.42 | 1.29 | 1.34 | 1.46 |
| Alanine | 1.41 | 1.29 | 1.41 | 1.48 |
| Half Cystine | .52 | .47 | .53 | .44 |
| Valine | 1.14 | 1.02 | 1.14 | 1.12 |
| Methionine | .61 | .61 | .72 | .59 |
| Isoleucine | .81 | .75 | .90 | .82 |
| Leucine | 2.34 | 1.95 | 2.18 | 2.37 |
| Tyrosine | 1.25 | 1.17 | 1.35 | 1.27 |
| Phenylalanine | 1.78 | 1.54 | 1.84 | 1.86 |
| Histidine | 1.68 | 1.61 | 1.70 | 1.84 |
| Lysine | 1.03 | .97 | 1.14 | 1.05 |
| Arginine | 1.34 | 1.21 | 1.29 | 1.26 |

# QUALITY OF WHOLE STILLAGE WHEN GRAIN SORGHUM DAMAGED BY SELECTED STORAGE INSECTS AND NON-TOXIN PRODUCING FUNGI IS USED FOR ALCOHOL PRODUCTION

## Summary

Grain sorghum was used to study the quality of whole stillage from alcohol produced using damaged grain. Treatments were: control (no initial insect or fungal infestation), rice weevil, mixed (lesser grain borer, flat grain beetle, and Indian-meal moth), A. glaucus, and A. candidus. Stillage samples were either freeze dried or were filtered and oven dried at 56 C. Grains and stillages were analyzed for crude protein, ether extract, ash, crude fiber, neutral detergent fiber, gross energy, and amino acid composition. Ergosterol levels were used as an indication of fungal activity. Particle size of the feedstocks was also determined.

Amino acid analysis showed a decrease in glutamic acid and half-cystine in the stillage. Increases in the stillage were seen in methionine, isoleucince, histidine, lysine, and arginine.

Determination of the nutrient composition of the stillage showed some differences between treatments. The fungi, Indian-meal moth, and flat grain beetle feed mainly on the germ while the rice weevil consumes the endosperm. This results in an increase of protein and a decrease in ether extract and gross energy in the respective stillage. Protein and crude fiber were concentrated more in the mixed sample than in the control. Protein was concentrated more in the A. candidus sample than in the control.

There were also differences due to drying method with oven dried sample values being higher. This was due to a loss of dry matter during the filtering operation.

## Introduction

The United States accounts for more than one third of the world's sorghum production. The sorghum kernel contains about 82% endosperm, 83% of which is starch (Hosney et al., 1981).

Damage by insects or fungi can render grain unfit for human consumption. This grain is then subject to destruction or is discounted to the feed industry. The technology exists to utilize these grains in the production of ethanol.

The effect of storage insects or storage fungi on the physical and nutritional quality of grain sorghum was investigated by Dietz (1982). Internal infesting insect species such as the lesser grain borer (Rhizopertha dominica (F.)) and rice weevil (Sitophilus oryzae (L.)) feed primarily on the endosperm fraction, although the lesser grain borer may also consume the germ. Other insects such as the Indian-meal moth (Plodia interpunctella (Hubner)) and flat grain beetle (Cryptolestes pusillus (Schonherr)) feed predominantly on the germ fraction (Cotton and Wilbur, 1982).

Christensen and Kaufmann (1969) stated that some common grain-infesting insects carry fungal spores into the grain they attack and, as conditions become favorable, these fungi will develop. Storage fungi will affect grain by invading the germ and reducing viability, discoloring seeds, increasing respiration and heating, causing mustiness and caking and total decay (Christensen and Sauer, 1982). Storage fungi exist in equilibrium with specific relative humidities and, as the relative humidity increases, a natural selection of fungi follows. In nearly all cases of destruction by fungi, Christensen and

Sauer (1982) noted that the first fungus to appear was Aspergillus glaucus. Growth of A. glaucus, which requires a grain moisture content of 14.5 to 15%, results in a rise in grain moisture to the lower limits (15.5 to 16%) required by Aspergillus candidus.

The objective of this study was to examine the quality of the whole stillage resulting from the production of ethanol when grain sorghum damaged by molds or insects were used as feedstocks.

## Materials and Methods

Grain used in this study was obtained from research previously conducted in the Department of Grain Science and Industry at Kansas State University in which storage effects on physical and chemical characteristics of grain sorghum were examined (Dietz, 1982). Treatments of sorghum lots were: control (no initial insect or fungal infestation), rice weevil (initially infested at 1 adult/100 g of whole seed), and mixed (initially infested at 1 adult lesser grain borer/200 g of whole seed, 1 adult flat grain beetle/100 g of whole seed, and 1 Indian-meal moth egg/100 g of whole seed). Growth of A. glaucus and A. candidus was encouraged by conditioning lots of sorghum to 15.5% moisture content and 16.0% moisture content respectively. The grain was stored in galvanized steel bins (768 cm dia. and 124 cm high) for 120 days at 21 to 25 C and 70 to 75% relative humidity. Sorghum was sprouted in the laboratory (Appendix F) based on a method by Novellie (1961).

Analytical procedures conducted on feedstocks and stillages have been outlined earlier.

## Results and Discussion

All of the sorghum samples were ground through a 1 hp hammermill equipped with a 3.2 mm screen. The particle size analysis is shown in table 23. The mold infested, rice weevil infested, and mixed samples have a larger mean particle diameter than the control. This is likely due to the higher moisture content of these grains at the time of grinding giving them a more plastic consistency. No explanation as to why the A. glaucus sample exhibited a smaller mean particle diameter than the control as the moisture contents were similar to the A. candidus. Perhaps the A. glaucus sample was inadvertently allowed to dry down before grinding which would then give the response seen in the particle size analysis.

The nutrient composition of the sorghum feedstocks is presented in table 24. The decrease in the ether extract fraction of the mold infested and mixed samples may be attributed to the use of lipid fractions for metabolic energy by fungi and by the Indian-meal moth larvare. Other insects feed primarily on the endosperm portion of the grain thus an apparent increase in nutrient values may in fact be due to a decrease in the nitrogen free extract (Cotton and Wilbur, 1982). The amino acid compositions of the feedstocks are shown in tables 25 and 26.

Tables 27 and 28 list the production data for alcohol and stillage from fermentation of the sorghum samples. The reported percent of theoretical values were calculated on a stoichiometric basis explained by Maisch et al. (1979), however, if expected yields according to the Solar Energy Research Institute were used for the calculation (1980), the percent of theoretical yields would increase to 80% for the control,

93% for sprouted, 79% for A. glaucus, 67% for A. candidus, 71% for rice weevil, and 68% for the mixed sample. The proof and specific gravity of the alcohol indicates that a good separation was made in the reflux column. Part of the less than expected volume of alcohol can be attributed to the loss of starch in the feedstocks due to insect damage.

The difference in solids content between the freeze dried and oven dried stillage is due to the filtering of the stillage through cheesecloth, thus removing some of the liquid before drying. In many operations some type of filtration or centrifugation is used to remove liquid in order to reduce drying costs. Since the freeze dried sample was not filtered, there is a higher percentage of solids in the oven dried sample.

The fermentation process tends to concentrate nutrients in the stillage as the starch is converted to alcohol. As the starch makes up nearly 70 percent of the sorghum kernel, the percentages of protein, fat, fiber, and ash should increase nearly three times. The nutrient composition of freeze dried stillage (table 29) and oven-dried stillage (table 32) shows a concentration ranging from 2.4 to 4 times that in the feedstocks. Amino acid profiles presented as a percent on a dry matter basis (tables 26, 31, and 34) show this same concentration effect. When the profiles are expressed as grams of amino acid per 100 g of protein (tables 25, 30 and 33), there were few differences seen due to the fermentation process. Allowing for a 5 percent inherent error in the test procedure, it appeared that there was a slight decrease in glutamic acid and some samples also showed a decrease in half-cystine. There appeared to be an increase in the stillage of methionine, isoleucine, histidine, lysine, and arginine.

A comparison of the concentrating effect of individual nutrients from the feedstock to the stillage (i.e. the protein content going from 10.0% in the feedstock to 30.9 % in the freeze dried stillage results in a concentration effect of 3.1) showed some differences. For example, the mixed sample was different from the control in concentration of both protein and crude fiber. The A. candidus treatment also deviated from the control in the concentration of crude fiber.

The concentrating effect for both protein and crude fiber in the mixed and A. candidus treatments was lower than the concentrating effect in the control grain. The amounts of crude protein, crude fiber, and ash in the A. candidus and mixed feedstocks are larger than in the control. This is due to the attack on the germ and endosperm by insects which results in a higher percentage of protein, fiber, and ash in the sample. The exoskeleton of the insects will also contribute to the higher crude fiber value. The partial depletion of the endosperm fraction means there is less starch to be converted to alcohol, thus less concentration of the remaining nutrients in the stillage.

There were differences between treatments when nutrient levels in freeze dried and oven dried stillage were compared. The protein levels were higher in the rice weevil and A. glaucus samples than in the control. Rice weevils attack the endosperm fraction of the grain while the A. glaucus primarily utilizes the germ. The decrease in these portions results in a percentage increase of the remaining nutrients in the stillage. Ether extract and gross energy values were lower than the control for A. candidus, A. glaucus, and the mixed sample. Indian-meal moth and the flat grain beetle feed mainly on the germ as do the fungi. The ether extract in these samples is lower in the feed stocks and are, then, also lower in the stillage.

Differences were seen between drying methods with protein and fat levels being higher in the oven dried samples. Data from the determination of soluble carbohydrates on both freeze dried and oven dried stillage shows lower values for the oven dried samples indicating there was a loss of dry matter in these samples. Such a loss would have a tendency to increase the concentration of the protein and fat values.

## Conclusion

Two replications per treatment were used to study the quality of stillage from fuel alcohol production using damaged grain sorghum as feedstocks. Optimum production parameters were not able to be established thus maximum alcohol production was not always achieved.

The amino acid profiles showed an increase in the stillage of methionine, isoleucine, histidine, lysine, and arginine. A decrease was seen in glutamic acid and half-cystine.

A determination of the nutrient composition of the by-products showed differences due to treatment. Protein levels appeared to be higher than the control for rice weevil and A. glaucus samples while ether extract and gross energy values were lower than the control for the A. candidus, A. glaucus, and mixed samples. These differences can be explained by looking at the feedstocks and the type of damage that occurred to the grain before alcohol production.

The results indicate that the lower cost damaged grain sorghum could be used as a feedstock and the nutrient composition of the stillage could be predicted from an analysis of the feedstock.

# Literature Cited

Christensen, C.M. and H.H. Kaufmann. 1969. Grain storage: the role of fungi in quality loss. University of Minnesota Press, Minneapolis, Minnesota.

Christensen, C.M. and D.B. Sauer. 1982. Microflora. In C.M. Christensen (Ed.) Storage of Cereal Grains and Their Products. Amer. Assoc. Cereal Chem., Inc., St. Paul, Minnesota.

Cotton, R.T. and D.A. Wilbur. 1982. Insects. In C.M. Christensen (Ed.) Storage of Cereal Grains and Their Products. Amer. Assoc. Cereal Chem., Inc., St. Paul, Minnesota.

Dietz, J.C. 1982. The effect of stored-product insect and non-toxin producing storage fungi on the physical and nutritional quality of grain sorghum. Ph.D. Thesis. Kansas State University, Manhattan, Kansas.

Hoseney, R.C., E. Varriano-Marston, and D.A.V. Dendy. 1981. Sorghum and Millets. In Advances in Cereal Science and Technology, Vol. IV. Amer. Assoc. of Cereal Chem., St. Paul, Minnesota.

Maisch, W.F., M. Sobolov, and A.J. Petricola. 1979. Distilled beverages. In H.J. Peppler and D. Perlmau (Ed.) Microbial Technology. Vol. II. Academic Press, New York.

Novellie, L. 1962. Kaffircorn malting and brewing studies. XI. Effect of malting conditions on diastatic power of kaffircorn malt. J. Sci. Fd. Agric. 13: 115.

Table 23. PARTICLE SIZE OF FEEDSTOCKS USED IN ALCOHOL PRODUCTION FROM DAMAGED GRAIN SORGHUM[a]

| Analysis | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| Mean Particle diameter, | 662 | 600 | 544 | 795 | 839 | 897 |
| Geometric standard deviation | 2.38 | 2.24 | 1.63 | 1.92 | 1.89 | 1.74 |
| Particle number (particles/gm) | 75883 | 66252 | 13580 | 10368 | 7800 | 4166 |
| Surface area ($cm^2/gm$) | 97.8 | 102.9 | 92.1 | 69.4 | 64.9 | 57.9 |

[a] Mixed composed of lesser grain borer, flat grain beetle, and Indian-meal moth

Table 24. COMPOSITION OF FEEDSTOCKS WHEN DAMAGED GRAIN SORGHUM IS USED FOR ALCOHOL PRODUCTION[a]

| Analysis | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[c] |
| Dry matter, % | 86.6 | 95.1 | 87.8 | 88.0 | 88.3 | 88.4 |
| Crude protein, % | 10.0 | 9.5 | 11.2 | 12.6 | 10.9 | 14.7 |
| Ash, % | 1.5 | 1.6 | 2.1 | 2.6 | 1.9 | 2.3 |
| Ether extract, % | 3.0 | 2.9 | 2.5 | 2.4 | 3.3 | 2.5 |
| Crude fiber, % | 2.5 | 2.9 | 2.4 | 3.2 | 2.6 | 3.2 |
| Neutral detergent fiber, % | 18.2 | 18.2 | 24.8 | 29.2 | 20.9 | 18.4 |
| Gross energy, Kcal/kg | 4480 | 4462 | 4562 | 4489 | 4504 | 4526 |
| Ergosterol, ppm | 10.23 | NA[d] | 5.28 | 11.34 | NA | 6.92 |
| Visual damage, %[b] | NA | NA | 81.0 | 81.3 | 45.9 | 52.2 |

[a] Values expressed on a dry matter basis

[b] Percent damage found in 100 randomly selected kernels

[c] Mixed composed of lesser grain borer, flat grain beetle, and Indian-meal moth

[d] Analysis not conducted on this sample

Table 25. AMINO ACID COMPOSITION OF FEEDSTOCKS WHEN DAMAGED GRAIN SORGHUM IS USED FOR ALCOHOL PRODUCTION

| Amino Acid | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| | g amino acid/100 g protein | | | | | |
| Aspartic Acid | 7.34 | 9.10 | 7.56 | 8.02 | 7.73 | 7.69 |
| Threonine | 3.07 | 3.25 | 2.93 | 3.20 | 2.76 | 2.68 |
| Serine | 4.88 | 4.90 | 5.19 | 5.39 | 5.34 | 5.37 |
| Glutamic Acid | 19.53 | 17.34 | 18.99 | 17.84 | 19.34 | 18.80 |
| Proline | 8.28 | 8.61 | 8.29 | 7.54 | 8.19 | 8.01 |
| Glycine | 3.25 | 3.59 | 3.58 | 3.93 | 3.40 | 3.52 |
| Alanine | 8.76 | 8.53 | 9.13 | 8.05 | 9.23 | 9.21 |
| Half Cystine | 1.22 | 1.30 | 1.32 | 1.73 | 1.42 | 1.40 |
| Valine | 3.33 | 3.73 | 3.56 | 3.73 | 3.65 | 3.61 |
| Methionine | 1.73 | 1.69 | 1.55 | 2.07 | 1.63 | 1.58 |
| Isoleucine | 2.53 | 2.53 | 2.56 | 2.82 | 2.62 | 2.55 |
| Leucine | 12.22 | 11.18 | 11.94 | 10.67 | 12.14 | 12.05 |
| Tyrosine | 4.30 | 4.68 | 4.38 | 5.08 | 4.52 | 4.85 |
| Phenylalanine | 5.24 | 5.29 | 5.40 | 5.67 | 5.56 | 5.53 |
| Histidine | 4.47 | 4.44 | 4.77 | 4.71 | 4.14 | 4.47 |
| Lysine | 1.89 | 2.27 | 1.90 | 2.56 | 1.78 | 1.82 |
| Arginine | 4.29 | 4.02 | 3.70 | 4.17 | 3.40 | 3.46 |

[a] Mixture composed of lesser grain borer, flat grain beetle, and Indian-meal moth

Table 26. AMINO ACID COMPOSITION OF FEEDSTOCKS WHEN DAMAGED GRAIN SORGHUM IS USED FOR ALCOHOL PRODUCTION

| Amino Acid | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| | % (dry basis) | | | | | |
| Aspartic Acid | .66 | .79 | .74 | .92 | .82 | .80 |
| Threonine | .28 | .28 | .29 | .37 | .29 | .28 |
| Serine | .44 | .42 | .51 | .62 | .56 | .56 |
| Glutamic Acid | 1.76 | 1.50 | 1.86 | 2.04 | 2.04 | 1.96 |
| Proline | .74 | .75 | .81 | .86 | .86 | .84 |
| Glycine | .29 | .31 | .35 | .45 | .36 | .37 |
| Alanine | .79 | .74 | .89 | .92 | .97 | .96 |
| Half Cystine | .11 | .11 | .13 | .20 | .15 | .15 |
| Valine | .30 | .32 | .35 | .43 | .38 | .38 |
| Methionine | .16 | .15 | .15 | .24 | .17 | .16 |
| Isoleucine | .23 | .22 | .25 | .32 | .28 | .27 |
| Leucine | 1.10 | .97 | 1.17 | 1.22 | 1.28 | 1.26 |
| Tyrosine | .39 | .41 | .43 | .58 | .48 | .51 |
| Phenylalanine | .47 | .46 | .53 | .65 | .59 | .58 |
| Histidine | .40 | .39 | .47 | .54 | .44 | .47 |
| Lysine | .17 | .20 | .19 | .29 | .19 | .19 |
| Arginine | .39 | .35 | .36 | .48 | .36 | .36 |

[a] Mixture composed of lesser grain borer, flat grain beetle, and Indian-meal moth

Table 27. ALCOHOL YIELD FROM THE USE OF DAMAGED GRAIN SORGHUM FOR ALCOHOL PRODUCTION

| Analysis | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| Volume, ml | 620 | 720 | 608 | 520 | 545 | 523 |
| Specific gravity | .825 | .816 | .823 | .820 | .821 | .818 |
| Proof | 187 | 190 | 189 | 191 | 189 | 190 |
| Percent of theoretical[b] | 71 | 75 | 69 | 59 | 62 | 59 |

[a] Mixed composed of lesser grain borer, flat grain beetle, and Indian-meal moth

[b] Calculated on a stoichiometric basis of 100% ethanol

Table 28. PERCENT SOLIDS OF WHOLE STILLAGE FROM ALCOHOL PRODUCED FROM DAMAGED GRAIN SORGHUM AS DETERMINED BY FREEZE DRYING AND OVEN DRYING METHODS

| Analysis | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| Stillage-total weight, kg | 10.13 | 9.97 | 10.02 | 10.11 | 10.40 | 10.06 |
| Solids-freeze dried, % | 6.7 | 7.0 | 6.3 | 6.9 | 6.4 | 7.2 |
| Solids-filtered, oven dried, % | 12.9 | 13.0 | 13.9 | 13.4 | 12.8 | 13.9 |

[a] Mixed composed of lesser grain borer, flat grain beetle, and Indian-meal moth

Table 29. COMPOSITION OF FREEZE DRIED STILLAGE WHEN USING DAMAGED GRAIN SORGHUM FOR ALCOHOL PRODUCTION[a]

| Analysis | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[b] |
| Dry matter, % | 87.4 | 92.1 | 88.9 | 92.9 | 88.4 | 92.6 |
| Crude protein, % | 30.9 | 29.8 | 33.7 | 33.6 | 33.8 | 29.9 |
| Ash, % | 5.1 | 4.9 | 6.2 | 7.2 | 5.8 | 6.0 |
| Ether extract, % | 7.2 | 9.3 | 2.6 | 8.0 | 7.8 | 5.0 |
| Crude fiber, % | 8.6 | 9.5 | 8.3 | 8.9 | 9.0 | 8.9 |
| Neutral detergent fiber, % | 40.4 | 38.8 | 39.2 | 42.4 | 39.8 | 41.6 |
| Gross energy, Kcal/kg | 5627 | 5340 | 5475 | 5199 | 5549 | 5200 |
| Soluble carbohydrates, %[c] | 8.8 | 10.9 | 10.4 | 7.8 | 6.1 | 5.3 |
| Ergosterol, ppm | 20.3 | NA[d] | 9.5 | 24.6 | NA | NA |

[a] Values expressed on a dry matter basis

[b] Mixed composed of lesser grain borer, flat grain beetle, and Indian-meal moth

[c] Values expressed as percent of the dry matter

[d] Analysis not conducted on this sample

Table 30. AMINO ACID COMPOSITION OF FREEZE DRIED STILLAGE WHEN USING DAMAGED GRAIN SORGHUM FOR ALCOHOL PRODUCTION

| Amino Acid | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| | g amino acid/100 g protein | | | | | |
| Aspartic Acid | 7.72 | 8.89 | 7.63 | 7.63 | 7.85 | 7.81 |
| Threonine | 3.77 | 3.86 | 3.25 | 3.25 | 3.40 | 3.62 |
| Serine | 5.12 | 5.37 | 5.20 | 4.95 | 5.30 | 5.12 |
| Glutamic Acid | 15.86 | 16.12 | 18.39 | 17.49 | 20.00 | 16.72 |
| Proline | 7.31 | 8.73 | 7.99 | 7.62 | 8.25 | 7.71 |
| Glycine | 3.55 | 3.82 | 3.64 | 3.72 | 3.63 | 3.78 |
| Alanine | 8.76 | 8.53 | 9.13 | 8.05 | 9.23 | 9.21 |
| Half Cystine | 1.53 | .89 | 1.59 | 1.41 | .96 | 1.34 |
| Valine | 3.97 | 3.94 | 3.63 | 3.59 | 3.43 | 3.84 |
| Methionine | 2.41 | 1.98 | 1.96 | 1.82 | 1.58 | 2.14 |
| Isoleucine | 3.02 | 2.90 | 2.68 | 2.53 | 2.45 | 2.90 |
| Leucine | 11.53 | 11.69 | 10.78 | 10.20 | 12.01 | 10.73 |
| Tyrosine | 5.32 | 4.69 | 4.40 | 4.61 | 4.22 | 5.14 |
| Phenylalanine | 4.83 | 4.44 | 5.60 | 6.23 | 4.95 | 5.26 |
| Histidine | 5.24 | 4.56 | 5.47 | 6.07 | 4.09 | 5.46 |
| Lysine | 2.85 | 2.67 | 2.72 | 3.14 | 1.84 | 3.06 |
| Arginine | 4.84 | 3.88 | 4.00 | 5.67 | 3.94 | 4.81 |

[a] Mixture composed of lesser grain borer, flat grain beetle, and Indian-meal moth

Table 31. AMINO ACID COMPOSITION OF FREEZE DRIED STILLAGE WHEN USING DAMAGED GRAIN SORGHUM FOR ALCOHOL PRODUCTION

| Amino Acid | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| | % (dry basis) | | | | | |
| Aspartic Acid | 2.05 | 2.22 | 2.35 | 2.40 | 2.36 | 2.08 |
| Threonine | 1.00 | .97 | 1.08 | 1.03 | 1.02 | .97 |
| Serine | 1.36 | 1.34 | 1.60 | 1.56 | 1.59 | 1.37 |
| Glutamic Acid | 4.20 | 4.04 | 5.66 | 5.51 | 6.01 | 4.49 |
| Proline | 1.93 | 2.18 | 2.46 | 2.40 | 2.48 | 2.08 |
| Glycine | .94 | .96 | 1.12 | 1.17 | 1.09 | 1.01 |
| Alanine | 2.35 | 2.25 | 2.55 | 2.43 | 2.83 | 2.22 |
| Half Cystine | .41 | .22 | .49 | .44 | .29 | .38 |
| Valine | 1.05 | .99 | 1.12 | 1.13 | 1.03 | 1.03 |
| Methionine | .65 | .50 | .60 | .58 | .47 | .59 |
| Isoleucine | .81 | .72 | .83 | .80 | .74 | .79 |
| Leucine | 3.06 | 2.93 | 3.32 | 3.22 | 3.61 | 2.87 |
| Tyrosine | 1.42 | 1.17 | 1.36 | 1.45 | 1.26 | 1.39 |
| Phenylalanine | 1.29 | 1.11 | 1.73 | 1.96 | 1.48 | 1.44 |
| Histidine | 1.40 | 1.14 | 1.71 | 1.90 | 1.22 | 1.47 |
| Lysine | .76 | .67 | .84 | .98 | .55 | .83 |
| Arginine | 1.30 | .97 | 1.23 | 1.77 | 1.88 | 1.29 |

[a] Mixture composed of lesser grain borer, flat grain beetle, and Indian-meal moth

Table 32. COMPOSITION OF OVEN DRIED STILLAGE WHEN USING DAMAGED GRAIN SORGHUM FOR ALCOHOL PRODUCTION[a]

| Analysis | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[b] |
| Dry matter, % | 96.1 | 97.0 | 95.7 | 96.1 | 95.8 | 96.0 |
| Crude protein, % | 36.2 | 32.9 | 40.3 | 39.5 | 40.8 | 35.6 |
| Ether extract, % | 10.6 | 11.9 | 6.5 | 7.0 | 10.0 | 6.8 |
| Gross energy, Kcal/kg | 5552 | 5476 | 5552 | 5366 | 5560 | 5398 |
| Soluble carbohydrates, %[c] | 4.1 | 7.2 | 4.9 | 3.7 | 3.1 | 4.0 |

[a] Values expressed on a dry matter basis

[b] Mixed composed of lesser grain borer, flat grain beetle, and Indian-meal moth

[c] Values expressed as percent of the dry matter

Table 33. AMINO ACID COMPOSITION OF OVEN DRIED STILLAGE WHEN USING DAMAGED GRAIN SORGHUM FOR ALCOHOL PRODUCTION

| Amino Acid | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| | g amino acid/100 g protein | | | | | |
| Aspartic Acid | 7.08 | 7.71 | 7.51 | 7.76 | 7.57 | 7.58 |
| Threonine | 3.06 | 3.33 | 3.13 | 3.29 | 3.13 | 3.19 |
| Serine | 4.96 | 4.97 | 4.97 | 5.22 | 5.33 | 5.03 |
| Glutamic Acid | 19.66 | 18.01 | 19.33 | 18.38 | 19.64 | 18.59 |
| Proline | 8.51 | 8.32 | 8.32 | 7.69 | 7.73 | 7.60 |
| Glycine | 3.21 | 3.40 | 3.32 | 3.56 | 3.28 | 3.26 |
| Alanine | 9.12 | 8.81 | 8.98 | 8.77 | 9.16 | 9.14 |
| Half Cystine | 1.37 | 1.23 | 1.39 | 1.13 | 1.40 | 1.34 |
| Valine | 3.46 | 3.63 | 3.50 | 3.79 | 3.70 | 3.78 |
| Methionine | 1.63 | 1.72 | 1.55 | 1.70 | 1.67 | 1.68 |
| Isoleucine | 2.46 | 2.69 | 2.51 | 2.57 | 2.57 | 2.56 |
| Leucine | 12.49 | 11.64 | 12.26 | 12.14 | 12.82 | 12.74 |
| Tyrosine | 4.67 | 4.84 | 4.71 | 5.11 | 4.83 | 5.02 |
| Phenylalanine | 5.70 | 5.81 | 5.91 | 5.29 | 5.48 | 6.01 |
| Histidine | 4.47 | 4.84 | 4.59 | 5.42 | 4.33 | 5.05 |
| Lysine | 1.89 | 2.59 | 1.98 | 2.45 | 1.79 | 2.00 |
| Arginine | 3.43 | 3.70 | 3.37 | 3.47 | 3.13 | 3.09 |

[a] Mixture composed of lesser grain borer, flat grain beetle, and Indian-meal moth

Table 34. AMINO ACID COMPOSITION OF OVEN DRIED STILLAGE WHEN USING DAMAGED GRAIN SORGHUM FOR ALCOHOL PRODUCTION

| Amino Acid | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Sprouted | A. glaucus | A. candidus | Rice Weevil | Mixed[a] |
| | % (dry basis) | | | | | |
| Aspartic Acid | 2.30 | 2.35 | 2.67 | 2.58 | 2.67 | 2.37 |
| Threonine | .99 | 1.01 | 1.11 | 1.09 | 1.10 | .98 |
| Serine | 1.61 | 1.51 | 1.77 | 1.74 | 1.88 | 1.57 |
| Glutamic Acid | 6.39 | 5.48 | 6.88 | 6.12 | 6.93 | 5.81 |
| Proline | 2.77 | 2.53 | 2.96 | 2.57 | 2.73 | 2.37 |
| Glycine | 1.04 | 1.03 | 1.18 | 1.19 | 1.16 | 1.01 |
| Alanine | 2.96 | 2.68 | 3.19 | 2.92 | 3.23 | 2.89 |
| Half Cystine | .44 | .38 | .49 | .37 | .49 | .41 |
| Valine | 1.12 | 1.10 | 1.24 | 1.26 | 1.30 | 1.18 |
| Methionine | .53 | .52 | .55 | .57 | .59 | .52 |
| Isoleucine | .80 | .82 | .89 | .86 | .91 | .80 |
| Leucine | 4.06 | 3.55 | 4.36 | 4.05 | 4.53 | 3.98 |
| Tyrosine | 1.52 | 1.47 | 1.68 | 1.70 | 1.70 | 1.56 |
| Phenylalanine | 1.85 | 1.77 | 2.10 | 1.76 | 1.93 | 1.88 |
| Histidine | 1.45 | 1.47 | 1.63 | 1.80 | 1.53 | 1.57 |
| Lysine | .61 | .79 | .70 | .81 | .63 | .62 |
| Arginine | 1.12 | 1.13 | 1.20 | 1.15 | 1.11 | .96 |

[a] Mixture composed of lesser grain borer, flat grain beetle, and Indian-meal moth

# APPENDIX A

## PILOT SCALE ALCOHOL PLANT

1. A 22 liter glass flask and associated heating mantle equipped with three in-line spherical joints and two standard taper joints with adjustable thermowells for measuring pH and montering temperature. Teflon stirring rod runs through a water cooled bearing and a Teflon sealed stuffing box. (Reaction Assembly No. 6470, Ace Glass Inc., Vineland, N.J.).

2. Voltage controllers for heating mantle (Reaction Assembly No. 6470, Ace Glass Inc., Vineland, N.J.).

3. RTD temperature controller (Model 4201, Omega Engineering, Inc., Stanford, Conn.).

4. SCR DC motor controller (Model 6X165A, Dayton Electric Mfg., Co., Chicago, IL).

5. pH meter (Beckman Expandomatic SS-2, Beckman Instrument, Inc., Fullerton, CA).

6. DC gearmotor, 1/8 hp (Model 42381, Dayton Electric Mfg. Co., Chicago, IL).

7. Distilling column, 61 cm (Ace Glass Inc., Vineland, N.J.) packed with 15 mm (dia) glass beads.

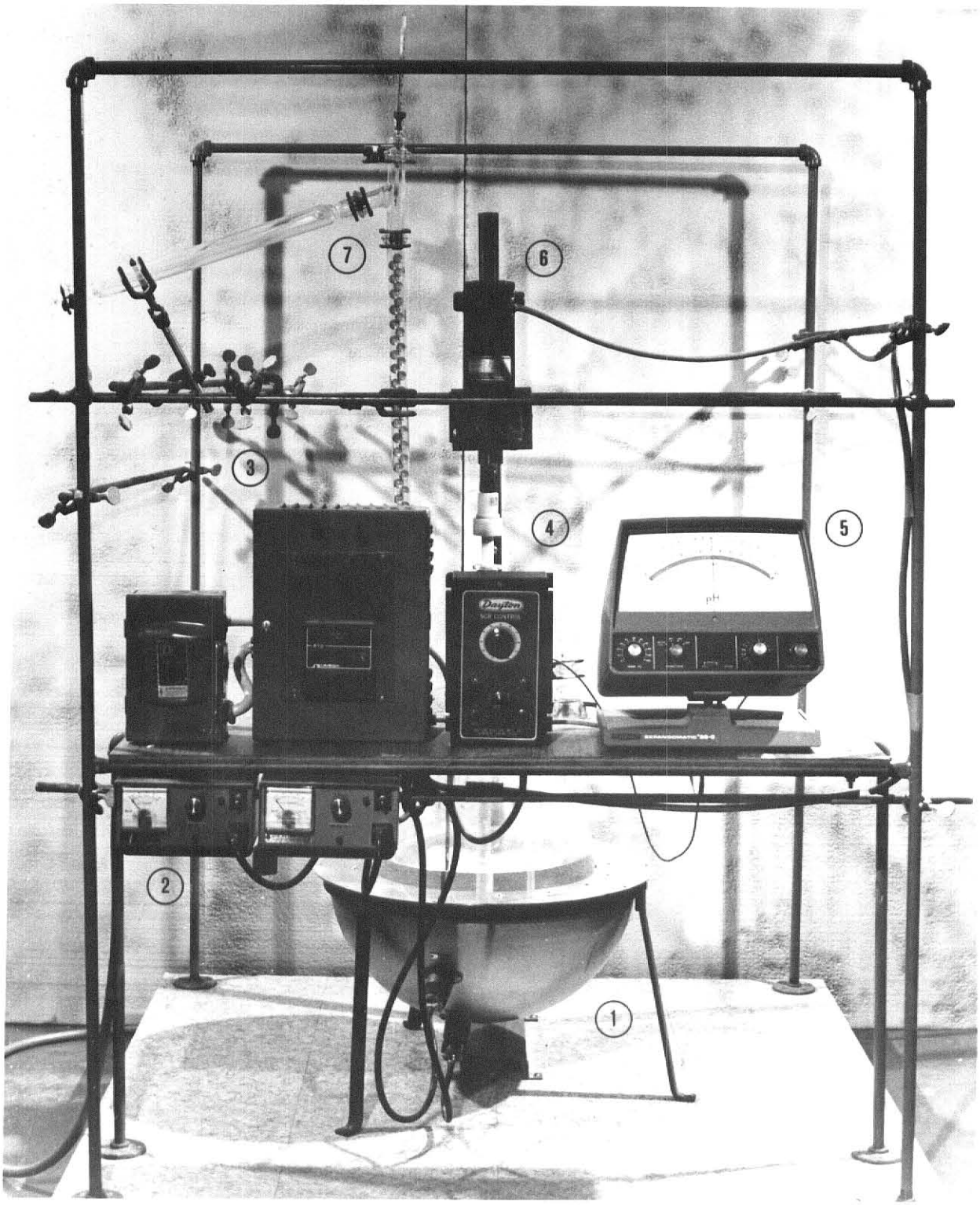


Figure 3. PILOT SCALE ALCOHOL PLANT - FRONT VIEW

1. A 22 liter glass flask with heating mantle.

2. Teflon coated steel reinforced stirring rod running through a water cooled bearing and an aluminum Teflon sealed stuffing box.

3. Distilling column packed with 15 mm (dia) glass beads.

4. DC gearmotor, 1/8 hp.

5. Condenser (400 mm length) and distillate take-off (Ace Glass Inc., Vineland, N.J.).

6. Termowell for measuring pH or temperature.

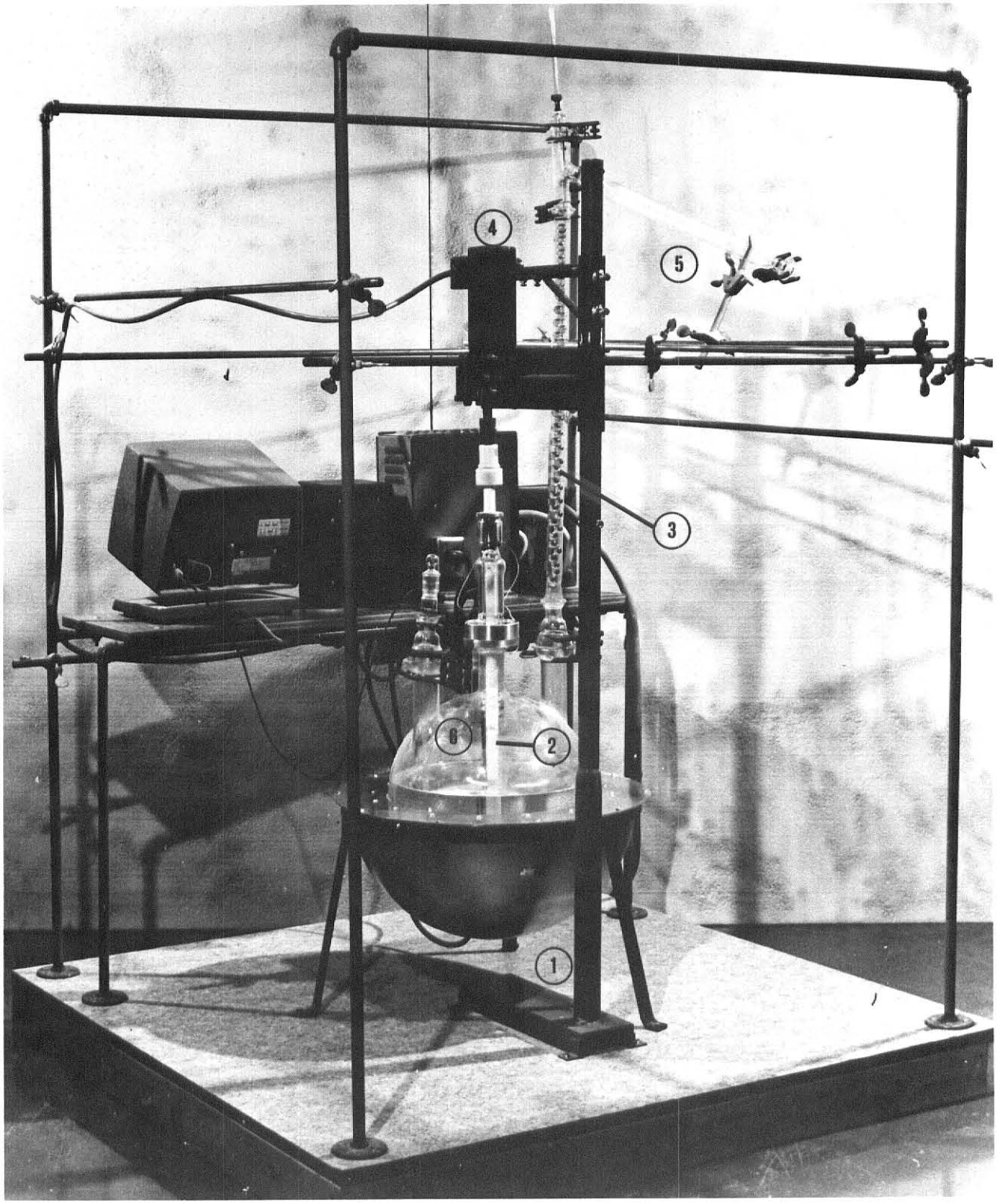


Figure 4. PILOT SCALE ALCOHOL PLANT - REAR VIEW

# APPENDIX B.

## ETHANOL PRODUCTION PROTOCOL

1. Feedstock ground using hammermill with 3.2 mm (1/8 in) screen (Burrows Electric Hammermill, 1 HP, 3450 RPM)[1].
2. Place 2000 ml deionized water in flask. Start stirring and continue to stir throughout procedure.
3. Add 2.0 kg feedstock. Add 1000 ml deionized water.
4. Add 200 ml 1.0 N calcium carbonate. Adjust pH to 6.2 if necessary with 2.5 N sodium hydroxide.
5. Increase temperature to 35 C.
6. Add alpha-amylase[2] at .15% dry starch basis (DSB) of feedstock.
7. Increase temperature to 98 C. If too thick a slurry forms as starch gelatinizes, stop heating and wait for enzyme to reduce viscosity. Resume stirring.
8. Hold at 98 C for 30 min., lower temperature to 90 C until liquifaction complete.
9. Lower temperature to 58 C. Add 2000 ml deionized water to aid in cooling.
10. Adjust pH to 4.2 with 7.2 N sulfuric acid.
11. Add glucoamylase[3] at 100 Diazyme Units/lb DSB of feedstock. Hold at 58 C until conversion complete.
12. Cool to 32 C. To aid in cooling add remainder of water needed to make a 15% slurry on a dry starch basis:

$$\text{ml water} = \left(\frac{\text{kg DSB} \times 1000}{.15}\right) - \text{ml water previously used}$$

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[1]Burrows Equipment Company, Evanston, Illinois

[2]Taka-Therm$^{R}$, Miles Laboratories, Inc., Elkhart, Indiana

[3]Diazyme$^{R}$ L-150, Miles Laboratories, Inc., Elkhart, Indiana

13. Mix 5 gm distiller active dry yeast (DADY)[1] into 200 ml deionized water at 32 C. Let stir 10 min. Add yeast mixture to mash, stir 5 min.
14. Transfer mash to 18.9 l container equipped with airtight lid and gas trap for release of $CO_2$. Mix contents, without removing lid, every few hours.
15. Allow fermentation to continue until $CO_2$ is no longer given off.
16. Transfer contents to flask. Distill off ethanol at 78-80 C.

[1]Red Star, Universal Foods Corporation, Milwaukee, Wisconsin

# APPENDIX C

## ERGOSTEROL DETERMINATION

1. Extract 25 gm sample with 100 ml methanol in a blender for 2 min. Rinse blender with an additional 50 ml methanol.
2. Centrifuge the mixture plus rinse for 10 min at 1000 to 1200 RPM. Pour off supernatant and resuspend pellet in 50 ml methanol. Centrifuge again and combine supernatants.
3. Mix supernatant with 20 gm KOH and 50 ml ethanol and reflux for 30 min.
4. Dilute cooled saponified mixture with 50 ml water.
5. Extract first with 150 ml of Skelly B then with a 50 ml portion of Skelly B. Combine Skelly B extracts.
6. Evaporate Skelly B extracts to dryness over a steam bath under a flow of nitrogen.
7. Dissolve residue in methylene chloride and transfer to a 2 dram vial using methylene chloride rinses.
8. Evaporate methylene chloride extracts to dryness as in step 6.
9. Dissolve residue in methanol and filter through a .5 m PTFE filter (Millipore Corp., No. SLS RO25WS).
10. Inject 10 ml sample into a high-pressure liquid chromatograph (HPLC).

    The HPLC system consisted of a reversed phase column ($C_{18}$, 5 m particle size, 150 x 3.9 mm from Waters Associates, Inc.) and water: methanol (95:5) mobile phase at .8 ml/min. A variable wavelength photometric detector was set at 282 nm. Ergosterol was eluted at 9 min.

# APPENDIX D

## AFLATOXIN DETERMINATION

Feedstocks:

1. Extract 50 gm sample with 100 ml methanol:water (75:25 v/v) in a blender for 2 min. Rinse blender with 50 ml methanol:water.
2. Centrifuge combined mixture at 3500 RPM for 10 min. Decant supernatant and resuspend pellet in 50 ml methanol:water. Centrifuge again and combine supernatants.
3. Place 40 ml methanol-water extract, 80 ml of 20% ammonium sulfate solution, and 40 ml hexane into a 250 ml separatory funnel. Shake for 1 min (vent frequently), then allow layers to separate.
4. Draw off upper layer (hexane) with a Pasteur pipet connected to a vacuum through a trap. Also remove the gummy proteinaceous material located between the two layers with the vacuum pipet.
5. Add 8 ml methylene chloride and shake (vent frequently). Allow layers to separate and draw off lower layer (methylene chloride) into a small vial. Repeat using 4 ml methylene chloride.
6. Evaporate combined extracts to dryness on a steam bath under a flow of nitrogen.
7. Add .5 ml of benzene:acetonitrite (98:2 v/v) to the vial and shake to dissolve residue.
8. Streak the entire amount on a thin layer chromatographic plate (Brinkman SIL G-25 HR). Develop the plates in chloroform:acetone (88:12 v/v). Allow plate to dry and place under a longwave ultraviolet lamp and mark the aflatoxin band.

9. Scrape the aflatoxin band into a small fritted glass filter funnel and wash with chloroform:acetone (8:2 v/v). Evaporate eluant to dryness on a steam bath with a stream of nitrogen directed into the vial.
10. Take up residue in 1.0 ml methanol and filter through a .5 m PTFE filter directly into a 2 ml serum vial. Place vial in autosampler of HPLC system.

HPLC system used was a Hewlett Packard 1084B equipped with autosampler, autoinjector, a 1040A variable wavelength detector, and integrator. The detector was set at 365 nm and 550 nm for sample and reference wavelengths respectively. The column was a 5 m - $C_{18}$ Resolve type (Waters Associates). The mobile phase was methanol:water (60:40 v/v) with a flow of .8 ml/min.

Alcohol:

1. Evaporate 250 ml of alcohol sample to dryness using a steam bath with a stream of nitrogen directed into the flask.
2. Take up residue in methylene chloride and transfer to a small vial. Dry down on a steam bath a with nitrogen stream directed into the vial.
3. Take up residue in .5 ml of benzene:acetonitrite (98:2 v/v).
4. Proceed with step 8 as outlined above.

Freeze dried samples:

1. Extract 25 gm sample with 150 ml methanol:water (75:25 v/v) in a blender for 2 min. Rinse blender with 50 ml methanol:water.
2. Centrifuge combined mixture at 3500 RPM for 10 min. Decant supernatant and resuspend pellet in 50 ml methanol:water. Centrifuge again and combine supernatants.

3. Place 40 ml methanol-water extract, 80 ml of 20% ammonium sulfate solution, and 40 ml hexane into a 250 ml separatory funnel. Shake for 1 min (vent frequently), then allow layers to separate.
4. Draw off upper layer (hexane) with a Pasteur pipet connected to a vacuum through a trap. Also remove the gummy proteinaceous material located between the two layers with the vacuum pipet.
5. Add 4 ml methylene chloride and shake (vent frequently). Allow layers to separate and draw off lower layer (methylene chloride) into a small vial. Repeat. Proceed with step 6 as outlined above.

# APPENDIX E

## DEOXYNIVALENOL (DON) DETERMINATION IN WHEAT

Freeze dried samples:

1. Extract a 25 g sample with 150 ml methanol:water (1:1) in a blender for 5 min. Rinse blender with 50 ml methanol:water (1:1).
2. Centrifuge the combined mixture at 5000 RPM for 5 to 10 min.
3. Sixty ml of supernatant was treated with 240 ml of ammonium sulfate (30%).
4. Filter using buchner funnel and a glass fiber filter (add celite to aid in filtering process).
5. Place 200 ml of filtrate into a separatory funnel and extract with 100 ml ethyl acetate. Repeat three times with 50 ml ethyl acetate.
6. Dry combined ethyl acetate extracts over anhydrous sodium sulfate.
7. Evaporate to dryness on a steambath with a stream of nitrogen directed into the container.
8. Dissolve residue in methylene chloride and transfer to silica gel column. Wash column with toluene:acetone (95:5) and discard wash. Elute DON with methylene chloride:methanol (95:5).
9. Evaporate eluate to dryness under nitrogen, dissolve residue in methylene chloride and transfer to a 2 dram vial. Evaporate to dryness under nitrogen.
10. Dissolve residue with 1.5 ml water:methanol (80:20). Solution was forced by syringe through a .5 m PTFE filter (Millipore Corp., Bedford, Ma, Cat. No. SLS RO25WS) directly into a 2 ml serum vial. Cap vial with an aluminum seal having a teflon-faced rubber septum.
11. Place in autosampler of HPLC system.

HPLC system was a Hewlett Packard model 1084B equipped with a variable wavelength detector, autosampler, autoinjector, and integrator. The detector was set at 224 and 240 nm for sample and reference wavelengths, respectively. The analytical column (150 x 4.5 mm) contained $C_{18}$ reversed-phase packing with 5 m particle size (Waters Associates, Milford, MA). A guard column ($C_{18}$, 5 m, 30 x 4.6 mm, Brownlee Lab., Santa Clara, CA) was positioned between the analytical column and the injector. Column temperature was 35°C. The mobile phase was water:methanol (80:20) with a flow of 0.8 ml/min. DON was eluted at 5.4 min. A 10 l portion of the sample was injected into the chromatograph.

Feedstocks:

1. Extract a 50 g sample with 150 ml methanol:water (1:1) in a blender for 5 min. Rinse blender with 50 ml methanol:water (1:1).
2. Proceed as outlined above.

Alcohol:

1. Evaporate 250 ml alcohol to dryness on a steam bath with a stream of nitrogen directed into the flask.
2. Proceed with step 8 outlined above.

# APPENDIX F

## SORGHUM SPROUTING PROCEDURE

1. Place 2.5 kg clean sorghum in 18.9 l bucket and rinse with water until rinse appears clear.
2. Cover sorghum with distilled water and place in incubator at 28 C. Allow to steep at this temperature for 20 hr. Rinse with distilled water 3 times during the steep period. Steep moisture should approach 40%.
3. Remove water, rinse sorghum with distilled water and place in incubator at 30 C to begin germenation process.
4. Germinate for approximately 48 hr or until acrospires are between 12 and 25 mm in length.
5. Spread sprouted grain on wire screen and dry at 56 C.

# APPENDIX G

## COMPOSITION OF DISTILLERS DRIED GRAINS WITH SOLUBLES FROM VARIOUS FEEDSTOCKS [a,b]

| Analysis | Feedstock | | |
|---|---|---|---|
| | Corn | Wheat | Sorghum |
| Dry matter | 7.5 | 7.5 | 11.0 |
| Crude protein | 29.2 | 34.6 | 35.3 |
| Ash, % | 5.0 | 4.4 | 4.7 |
| Ether extract, % | 11.2 | 6.8 | 9.0 |
| Crude fiber, % | 9.8 | 10.6 | 11.3 |

[a] Values expressed on a dry matter basis

[b] NRC, 1971

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# A STUDY ON THE QUALITY OF WHOLE STILLAGE WHEN DAMAGED GRAINS ARE USED AS FEEDSTOCKS FOR ALCOHOL PRODUCTION

by

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B.S., Grove City College, 1970

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Chemical analyses were used to investigate the quality of whole stillage produced from a pilot scale alcohol plant when damaged grains were used as feedstock material. The damaged grains included in this study were: aflatoxin contaminated corn; sprouted and deoxynivalenol contaminated wheat; sprouted, insect, and fungal damaged grain sorghum. The stillage samples were either freeze dried or were filtered and oven dried at 56 C. Analyses were conducted to determine moisture, crude protein, ash, ether extract, crude fiber, neutral detergent fiber, gross energy, and ergosterol. An analysis was made of feedstock particle size. Amounts of aflatoxin, deoxynivalenol and amino acids were determined in the feedstock, stillage and alcohol fractions.

Most nutrients were concentrated nearly three times in the stillage. When aflatoxin or deoxynivalenol contaminated grains were used, no toxins were detected in the alcohol. Toxins were, however, concentrated in the by-product. Ergosterol levels were higher in the aflatoxin contaminated corn, deoxynivalenol contaminated wheat, and fungal damaged grain sorghum than in the control. Ergosterol levels were also concentrated in the stillage.

Few differences were noted between feedstock and stillage profiles when amino acid compositions were determined on a gram per 100 g of protein basis. A determination of nutrients in the stillage showed no differences due to treatment for corn or wheat stillage. Increases in protein and decreases in ether extract and gross energy were noted in grain sorghum stillage.