USE OF *CANDIDA UTILIS* FOR ALTERING PROTEIN CONTENT OF DRY-MILLED GRAIN SORGHUM FRACTIONS

by

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CHAPTER I

INTRODUCTION

Considerable interest is being shown at the present time in increasing the protein supply in foods. One area of research connected with this problem is involved with using yeast for this purpose.

Simultaneously, interest is being shown in ways to more effectively utilize the large amount of grain sorghum available as a human food source. This is important because the grain sorghum plant is adapted to suitable growth in so many regions of the world and is considered a staple food in several countries. For example, fermented sorghum mush called "atoles" is popular in Latin American countries, and a comparable product in the African continent is consumed in large quantities. The major shortcoming of these foods appear to be that the quantity and quality of protein obtained in a serving is inadequate for the daily requirements of human beings.

Research efforts are being made internationally to improve the nutritive qualities of such grain sorghum preparations. Accordingly, the purpose of this study was to use yeast preparations in preparing a potentially high protein food from a certain fraction of grain sorghum kernels.

CHAPTER II

REVIEW OF LITERATURE

During World War I, Delbruck and his coworkers at Berlin's Institute for the Fermentation Industry recognized the fact that Torulopsis utilis could be used to produce protein for food and feed supplements (32). According to Pyke (52), the commercial potential of propagating food yeast was realized by German workers in Berlin at the Institute für Gärungsgewerbe during the World War I period. Prior to and during World War II, a critical shortage of protein stimulated the growth and development of this food and fodder yeast industry. During this period yeast became a partial substitute for meat (3, 73).

The German pulp industry was producing a plentiful supply of fermentable sugars from hardwood hydrolyzates which, due to the pentose assimilating ability of Torulopsis utilis (Torula utilis or Candida utilis), became the substrate for yeast propagation (12, 52, 66). By 1943 the plant at Tornesch, under an arrangement with the German Ministry of Nutrition, was producing 20,000 long tons of yeast annually for both human and animal consumption (66).

During this same period in the United States,

brewers' yeast was being recovered for use in animal feeds. A smaller portion of the yeast was debittered and fortified with B vitamins and used in foods and pharmaceuticals (6). Hence, food yeast was originally considered a by-product of the brewing industry, but food yeast today has a certain advantage over the brewers' yeast in that it grows more readily and has a more pleasing flavor (26, 66).

Production of food yeast in the United States was initiated by the Sulphite Pulp Manufacturers' Research League, Inc., in 1939 (32). This League established a pilot operation to investigate the possibility of using T. utilis in waste hydrolyzates from the pulp industries. This research was directed as a pollution control measure and would not have been financially practical had the protein and vitamin demand been less critical. As a result of this research, the first plant in the United States to commercially produce fodder yeast was the Lake States Yeast Corporation at Rhinelander, Wisconsin, in 1948.

T. utilis, often called Torula yeast, is a major food or nutrient yeast and is called fodder yeast when used for the manufacturing of protein for animal feeds (11, 12). Food yeasts, in general, are good sources of the B complex vitamins, proteins, fats, and minerals. Depending upon the conditions of propagation, T. utilis averages over 50% protein in the dry matter (Kjeldahl N x 6.25), is

high in the B complex vitamins, and is high in certain minerals (9, 12, 33, 35, 38, 73).

The "technological suitability" of yeasts has enabled them to be the forerunner in the microbial world for the production of high quality protein (4, 32). Pyke (52) described Torula utilis as "robust" in character. This yeast strain is capable of assimilating such carbon-containing compounds as sugars (glucose, mannose, arabinose, xylose, fructose, galactose, and sucrose); acids (malic, glyceric, fumaric, lactic, acetic, pyruvic, tartaric, aspartic, and other acids); alcohols (glycerol, ethanol, methanol, n-butanol, n-hexanol, and other alcohols); and other compounds such as propionaldehyde and acetaldehyde. Torula yeast assimilates nitrogen-containing compounds such as urea, ammoniacal salts, ammonium hydroxide, pyrimidine, peptones, asparagine, and other amino acids (4, 11, 27, 30, 34, 36, 47, 68, 72). As well as naturally occurring substrates, this yeast can be grown successfully on synthetic substrates (37). T. utilis utilizes most substrates in high yields, is easily acclimated to most carbon and nitrogen substrates, is easily separated from the impoverished slurry since cells are of suitable size and uniformity, is agreeable in odor, is resistent to bacterial infection, and has a generative time of eighty-four minutes under optimum conditions (4, 47).

The search for more protein (especially during critical protein shortages) and problems associated with waste disposal have turned scientists to studies of food yeasts, especially in the areas where low-grade protein, potential protein from wastes, and cheap carbohydrate material is plentiful. Such investigations have ended with such substrates for yeast propagation as wood hydrolyzates, potato starch waste, citrus press liquor, barley, dairy by-products, petroleum, and many others (13, 16, 18, 25, 26, 53, 69). Most of these waste materials have a large B. O. D. value (biochemical oxygen demand) which must be reduced according to legislative decrees before disposal of effluent wastes may be effected (32, 34, 43, 52).

Numerous food yeasts have been studied for their adaptability for growth in various waste materials and in other similar substrates. Dunn (11) has extensively reviewed the literature concerning this subject and has listed some of the microorganisms and raw materials studied. Wiley and associates (73) compared the growth and composition of T. utilis on various substrates with the same yeast grown of wood effluents. Graham et al. (18) and Porges et al. (50) experimented with T. utilis and T. cremoris using cheese whey as a propagation medium. T. utilis was found unsatisfactory because of its inability to utilize lactose. Harris et al. (23) grew both T. utilis and

S. cereviseae on wood hydrolyzates and found S. cereviseae was inferior in this medium because of inhibitory materials present. Harris and associates (24) later reported increased yields of S. cereviseae after additional toxic substances were removed. Kurth (34) found T. utilis, Mycotorula lipolytica, and Hanisenula suaveolens comparable in the utilization of sugars and organic acids present in wood stillage waste. Peterson et al. (47) reported comparable sugar utilization and yeast yields using T. utilis, Hanisenula suaveolens, and Candida tropicalis. Peterson and associates also used bakers' yeast but found it unsuitable for growth on wood hydrolyzates since it could only utilize the hexoses present. Of the yeasts studied, Peterson selected T. utilis for subsequent experiments because of its established nutritive value.

For feed protein production, most workers have preferred T. utilis and other food yeast to S. cereviseae, commonly referred to as brewers' or bakers' yeast (34, 35, 47). The latter removes only the hexoses in alcohol production and leaves the pentoses unfermented (34). Therefore, even after the alcohol has been removed by distillation, the stillage waste still has a considerable B. O. D. value. Alcoholic fermentation of waste wood sugar by brewers' yeast removes only 63-82% of the sugars present (26).

Three methods have been used, directly or indi-

rectly, to render the carbohydrates of various raw materials available for yeast growth. The cellulose and hemicellulose of wood and other similar raw materials are broken down either by acid saccharification (15, 21, 62, 66) or by anaerobic microbial degradation (19). A third method involves enzyme saccharification of complex starch material (16).

Wastes from the wood processing industries (sulfite pulping and fermentation) have been found to be acceptable media for T. utilis propagation. These materials represent a readily available carbon source which is necessary for proper yeast production and energy expenditures (19, 36, 51, 72). With these substrates, incremental amounts of nitrogen and phosphorus (and in some instances, potassium) are required (24, 32). However, Carter and Phillips (9) have reported Torula yeast will grow in the absence of any organic nitrogen source.

Before wood waste can be used for T. utilis propagation, it was found that extensive pre-treatment is usually necessary. Toxic materials must be removed by clarification techniques; the hydrogen-ion concentration must be neutralized to the desired pH; the precipitates must be removed; the sugar concentration must be adjusted; and supplemental nutrients must be added (24, 32, 34, 47).

Wood waste residues, especially wood hydrolyzates

from sulfite pulp mills, contain considerable amounts of toxic materials (sulfur dioxide and complexes of sulfur dioxide) which are inhibitory to yeast growth (32). These toxic materials are removed by clarification procedures which usually involve neutralization and subsequent heat treatment. The precipitates are removed by filtration. Following filtration, the sugar concentration is adjusted. Various pre-treatment procedures have been studied and are presented by Peterson et al. (47) and others (24, 32, 34).

The sugar concentration appears to be a critical factor in the production of Torula yeast from wood hydrolyzates. Peterson et al. (47), using batch propagation, reported reducing sugar concentrations of 2% gave yeast yields which were less than 50% of those obtainable on concentrations of 1% reducing sugars. Peterson and coworkers believed this loss in efficiency was partially due to exceeding the toxicity threshold of yeast. They also reported that solution concentrations less than 1% reducing sugars were of no significant advantage over 1% concentrations. Harris and associates (24) reported maximum yeast growth when 6% sugar concentrations were fed into a continuous propagator. Harris et al. (26) earlier reported sugar concentrations of 1% were more satisfactory in the conventional batch propagators. In continuous propagation the initial sugar concentration must be held at a minimum until

the yeast become acclimated to the medium whereas, in batch propagations, the sugar concentration remains critical throughout the fermentation. Initial sugar concentrations of 0.2 to 2.5% reducing sugars have been used in batch and continuous production and increases in concentrations to 5 to 6% reducing sugars are feasible in continuous propagation after a short inductive period (24, 26, 32, 34, 47).

Following the adjustment of sugar concentration, the wort is fortified with sources of nitrogen and phosphorus. Wood slurries are notably deficient of these two elements (26, 34, 47). In some instances potassium was also a recommended supplement (24). Harris et al. (24) found wood hydrolyzates to contain sufficient amounts of magnesium for yeast growth. The source and concentration of nutrients have varied from installation to installation primarily because of availability and economics. The following nutrients and amounts (per gram of reducing sugar) were most commonly used: 0.03 to 0.032 grams nitrogen as urea, 0.05 grams phosphorus as monobasic potassium phosphate, and 1.1 grams of potassium chloride (24, 34, 47).

Peterson et al. (47) found more nitrogen recovered in the yeast (based on nitrogen supplied) using urea as the nitrogen source, than that recovered when ammonium salts were used. Harris and his coworkers (24) also found a greater nitrogen recovery using urea but when excess amounts

were added, the nitrogen recovered in the yeast cells was lower. During the course of his experiment, Harris found 3.2 pounds of urea nitrogen per 100 pounds of sugar achieved the maximum recovery of nitrogen in the yeast. The yeast yield was not affected by phosphate levels as long as there was sufficient quantities present. However, the yeast will absorb phosphorus if excess amounts are added (47, 73).

The hydrogen-ion concentration of the wort is generally adjusted during the extensive pre-treatment process. Various reagents, such as ammonium hydroxide, calcium carbonate, calcium hydroxide, and lime have been used to adjust the hydrogen-ion concentration to an optimum of pH 4.5 to 5.5 before inoculation (24, 25, 26, 32, 47). Kurth (34) found ammonium hydroxide formed an amorphorus precipitate when used to neutralize wood stillage waste. Peterson et al. (47) satisfactorily used ammonium hydroxide simultaneously as a basic reagent and also as a source of nitrogen for wood hydrolyzate worts.

Small amounts of organic acids in wood liquor worts are utilized during yeast growth. Therefore, an increase in pH is realized. Consequently, care must be exercised to maintain optimum pH during incubation (24, 26). Harris et al. (26) reported bacterial contamination of the wood hydrolyzate wort at pH 6 to 6.6, but this dissipated when

the pH was adjusted to 5.

Generally, T. utilis utilizes the fermentable sugars present within a twenty-four hour incubation period at 25 to 35 C (26, 34). Using batch propagation, Peterson et al. (47) reported approximately 90% of the reducing sugars in wood hydrolyzates were utilized in 16 hours at 30 C with a standard inoculum (1 gram of dry cells per liter of medium). After an initial laboratory inoculation of T. utilis, fermentation, using continuous propagation, is completed in approximately 4 hours at 37 C (32). Harris et al. (24) achieved maximum yeast growth in 3 to 4 hours at 29 to 30 C using a laboratory continuous fermentor. Peterson et al. (47) reported high incubation temperatures (35 C) favored yeast growth but alcohol was produced.

Kurth and Cheldelin (35) stated that time periods longer than that required for maximum yeast growth usually resulted in rapid autolysis and reduction of yeast yields. Peterson and associates (47) also observed loss in yeast if not harvested immediately following fermentation. In this same report, Peterson and coworkers further demonstrated that inoculum size had no obvious effect on yeast yields.

Harris et al. (25) used T. utilis as an alcohol producer from waste wood hydrolyzates. In this respect, the yeast was found to be more desirable than brewers'

yeast since it propagated more readily and removed as much as 80-83% of the fermentable sugars. Since maximum carbohydrate utilization and yeast yield were desired, aerating procedures were employed by Harris et al. (24) and Peterson et al. (47) to achieve this goal. By aerating, they found that T. utilis utilized as much as 86-94% of the reducing sugars present. Yeast yields approximating 50 grams dry yeast per 100 grams of sugar consumed have been consistently reported (24, 26, 34, 35).

Adequate aeration is essential for efficient conversion of carbohydrates to yeast protein (37). Aeration of the propagation wort serves as an agitator which aids in the suspension and distribution of yeast cells to prevent fermentation in isolated areas, removes toxic end products such as accumulated carbon dioxide, dissolves in the medium to become available for yeast respiration, and stimulates vegetative growth (3, 51, 52). de Becze and Liebmann (3) reported that only the oxygen dissolved in the liquid is available for yeast and that this amount is small (about 0.0009% at 20 C).

In this respect, Kurth (34) reported that the fineness of the air bubbles is more important than the total volume of air passed through the fermentor. Kurth and Cheldelin (35) obtained optimum yields of T. utilis in 18 to 24 hours. However, they felt this minimum in-

cubation period could be reduced by using better aerating techniques.

Harris and associates (24) demonstrated the effect of air and yeast cell number when grown in concentrated wood sugar solutions under continuous operation. It was found when the air volume was held constant at 0.5 cubic feet per minute and when the sugar concentration was increased to 8%, alcohol was produced. It was believed that the air-to-yeast cell ratio was considerably lower in the concentrated wort than in the dilute wort. Similarily, Peterson *et al.* (47) noticed a loss in efficiency, using batch propagations, when the reducing sugar concentration was increased from 1% to 1.25% reducing sugars.

Harris *et al.* (24) found that addition of oxygen to the air supply had about the same effect as increasing the volume of air passed through the propagator. It was found, however, that although sugar utilization by the yeast was enhanced there was no effect on yeast yields.

Air volumes have varied from operation to operation (17, 22, 32, 47). The air requirements and rates depend upon the fermentor used, design of the aerating system, and many other variables. The subject of propagator designs, principles, and systems has been adequately presented by de Becze and Liebmann (3), Hixon and Goden (31), and other authorities (2, 55).

The principal outlet for Torula yeast is as a feed supplement. The Lake States Yeast Corporation commercially produces large quantities of Torula yeast for the poultry industry, and lesser amounts for food purposes (32). Using Torula yeast produced by this plant, Cambell and Ringrose (8) compared its nutritive value with that of brewers' yeast as a feed supplement for growing chicks and laying pullets. It was concluded that Torula protein was comparable to brewers' yeast as a poultry feed supplement.

They found, however, that both yeasts were deficient in some unknown nutritive fraction required by chicks and laying pullets. Klose and Fevold (33) stated that Torula yeast could be used to supplement 80% of the animal protein in poultry rations without loss in feed efficiency. Poor gains made by chicks on a high yeast diet were attributed to methionine deficiencies of the yeast protein.

Harris et al. (22) conducted feeding experiments with rats using Torula yeast harvested from wood sugars. The rats showed a gain in body weight of only 60% of gains achieved when milk casein supplied the protein. The rats receiving yeast protein also exhibited loss of hair which was indicative of the absence of an essential amino acid. Weight gains comparable to gains on casein diets and hair restoration occurred when the yeast protein diet was supplemented with methionine. Klose and Fevold (33), feeding similar diets to

rats, also reported weight losses and denudation. Both deficiencies were corrected when the rats received a supplemented diet of methionine. It was also demonstrated that feed supplementation with cystine partially corrected the methionine imbalance. Klose and Fevold also reported that high concentrations of yeast protein ingested by chicks and rats over a limited period of time appeared to be non-toxic.

Food yeasts are good sources of the water soluble vitamins. Ringrose (54) found the riboflavin content of Torula yeast sufficient for growing chicks and for the hatchability of eggs from pullets. Wiley and coworkers (73) produced yeast from sulfite liquor which assayed high in riboflavin and pantothenic acid. Kurth and Cheldelin (35) reported similar observations when T. utilis was grown on waste wood sugar.

The thiamine content of yeast is directly related to the thiamine content of the medium. With proper aeration, almost all thiamine present in the medium is absorbed by the yeast cells (68). Kurth and Cheldelin (35) reported yeast thiamine contents of 6.2 micrograms per gram of dry matter when grown in wood sugar having a thiamine concentration of 2.7 micrograms per gram of sugar. Substrates high in thiamine content produce yeast with high thiamine values (73).

Wiley et al. (73) reported consistently high con-

centration levels of the three important vitamins--biotin, niacin, and folic acid. Other vitamins found in Torula yeast which are nutritively significant are pyridoxine hydrochloride and p-aminobenzoic acid (35, 52, 54, 73).

The waste from cannery dehydration plants processing citrus fruits has presented these industries with waste disposal problems. This waste, which consists of the peel, seeds, and rag, has been concentrated and used for animal feeding. This method, however, results in an objectionable bitter flavor due to the presence of naringin. Other methods of disposal--trucking to remote areas, running waste into deep wells, or flooding waste lands--were likewise found unsatisfactory for public health reasons (43, 69).

Investigators found that citrus press liquor requires only minor pretreatment before inoculation with T. utilis. Nolte and associates (43) prepared the press juice by screening, boiling for five minutes, filtering, adjusting the sugar concentration, and fortifying with the necessary nutrients.

The soluble solids in press liquor is approximately 8-10% (Brix) of which, 66% is sugar (69). Nolte and associates (43) found sugar concentrations over 1.8% inhibitory for the propagation of T. utilis. It was also found that yeast yields were much higher at a more dilute sugar solution (1%) of press liquor. Veldhuis (69) recom-

mended 2.5 Brix or less for efficient yeast production. At this concentration Veldhuis reported a 94% sugar consumption by T. utilis using batch propagating techniques.

The composition of press juice varies from season to season but on an average contains 2.4% sucrose and 4.23% total reducing sugars. Nolte and coworkers (43) reported yields of 37-47.7% dry yeast (based on total sugars present) in press juice containing 1.8% sugars and yields of 44.3-48% dry yeast with juice containing 1.0% sugars by batch propagation. Veldhuis (69) reported yields of 70% dry yeast (based on total sugars present) in 1.5 Brix press liquor and yields of 29% dry yeast with juice of 6.6 Brix. Lewis et al. (38) reported yields of 55 grams of dry yeast per 100 grams of sugar present.

Although press liquor contains nitrogen and phosphorus, these quantities were inadequate for yeast growth (65). Lewis et al. (38) used ammonia as the nitrogen source. Veldhuis (69) added ammonium sulfate and trisodium phosphate to correct the nitrogen and phosphorus deficiencies, respectively. Nolte et al. (43) used the same compounds at the rate of 6%, based on total sugars present. Throughout the course of propagation, Nolte and coworkers found it necessary to add additional ammonium sulfate since the initial fortification did not supply the necessary nitrogen for propagation. Additional ammonium sulfate was added at the rate

of 1.75% (based on total sugars present) at the end of the first hour and for the following three hours. If the entire quantity were added in one fortification, excessive foaming occurred during aeration, propagation was depressed, and the yeast yields were low. Owens et al. (65) report 0.19 pound of ammonium sulfate, 0.045 pound of anhydrous ammonia, and 0.045 pound of 75% phosphoric acid are necessary nutrients for each pound of yeast produced.

Other nutrients, such as potassium and magnesium sulfates, were tried by Nolte and associates (43) to enhance yeast growth. It was found that these two compounds, alone or in combination, had no significant advantage.

Although the average pH of press juice is 5.7, Nolte et al. (43) reported using sodium carbonate to maintain the pH between 4.4 and 6.5. Sodium carbonate was not generally required until after the completion of two hours incubation. Veldhuis (69) maintained the pH at 4 by using varying ratios of ammonium sulfate and sodium phosphate. Owens and associates (65) controlled the pH between 4 and 4.5 by varying the ratio of ammonium sulfate to ammonia.

Nolte and coworkers (43) incubated the citrus press liquor between 29 to 30 C with "violent" aeration. Yeast growth was complete in the 1% (total sugars) slurry after 8 hours incubation using a batch fermentor. A 4% (by volume) of previously acclimated yeast was added to the

batch as starter. Yeast containing 52 to 55% protein (N x 6.25) were harvested from the medium. Lewis, Stubbs, and Noble (38) grew T. utilis in fruit juice media in laboratory fermentors and obtained 10-fold increases in yeast (based on initial inoculum size) within 6 to 8 hours. The sugar concentration was maintained at 0.5%, and 1.5 liters of air per minute per liter of media were supplied. Veldhuis (69) incubated 2.5 Brix press liquor with fine air bubbling through the wort at a rate of 0.19 cubic feet per minute per gallon of liquor. With a detention time of 2.5 to 3 hours, 100% sugar utilization and yeast yields of 63% (based on total sugars present) were realized.

The riboflavin content of yeast grown on citrus peel juice (48.2 micrograms per gram of dry yeast) is lower than when grown in fir wood hydrolyzates (80.8 micrograms per gram of dry yeast) according to microbiological assays made by Wiley et al. (73). The thiamine content of Torula yeast grown on citrus peel (13.7 micrograms per dry gram) is considerably higher than yeast from wood hydrolyzates (6.9 micrograms per dry gram). Lewis et al. (38) demonstrated that the thiamine content could be increased in yeast grown in fruit juices by removing the air supply and encouraging a fermentative metabolism in thiamine fortified worts. Thiamine is depleted from the media under aerating conditions (45).

The pantothenic acid and niacin contents of yeast grown in citrus press are slightly lower than the contents of yeast grown in wood sugar but the biotin content is higher (38, 73). However, the content of pyridoxine is comparable.

Tsilenis and Hedrick (62) investigated the possibility of growing food yeasts in olive residue hydrolyzates. T. utilis, Pichia membranaefaciens, S. carlsbergensis, Candida krusei, and Geotrichum species were eventually selected for this purpose. T. utilis was found to be the most effective yeast for propagation in olive hydrolyzates and favored a sugar concentration between 1.5 to 2% reducing sugars.

Urea did not effectively serve as a nitrogen source for T. utilis when grown in olive hydrolyzates. A combination of malt extract (1 gram per 100 ml hydrolyzate) and yeast extract (1 gram per 100 ml hydrolyzate) resulted in a much higher cell population after 48 hours of incubation in shaker flasks.

T. utilis utilized approximately 80% of the reducing sugars present in olive hydrolyzates within 48 hours when incubated at 28 C. The hydrogen-ion concentration of the liquor was pH 5.2. Under these conditions, Tsilenis and Hedrick reported 50 to 65% dry yeast yields based on total sugars consumed. The protein content of

the dried yeast was approximately 50%. This is about a 4.5 to 5.5% conversion of dry olive hydrolyzates to protein (62). Tsilenis and Hedrick predicted 90 thousand tons of dry yeast could be produced from the 2 million tons of olive residue produced annually in the Mediterranean.

Protein waste water--liquor remaining after protein precipitation and separation--from the potato processing industries is an almost complete substrate for T. utilis propagation (53, 67). This waste contains approximately 1.2% solids analyzing 37% protein, 12% sugar, and 0.8% starch (53). Dawson and associates (67) reported that almost all of the original sugar extracted during the processing of sweet potatoes remained in the wash waste. The sugar content varied from 0.75-1%.

Dawson et al. (67) found the 0.1% soluble nitrogenous compounds in sweet potato waste insufficient in supplying the nitrogen required for yeast growth. The nitrogen present is readily assimilated by the Torula yeast but only supplies one-third to one-half the nitrogen requirement. The ammonium hydroxide needed to maintain the pH of the waste at 3.8 to 6.5 supplied the necessary nitrogen for growth. Dawson and coworkers found all other essential nutrients present in sufficient quantities. Reiser (53) added phosphorus, potassium, and calcium but found no advantage with this fortification. Reiser recovered 50%

of the original solids as yeast product without the addition of any supplemental nutrients.

Yields of 40-50% dry yeast (based on sugars present) were obtained by Dawson and associates (67) within 8 hours of incubation by using batch propagation methods with adequate aeration. During propagation, the sugar concentration was reduced to approximately 0.05%. Reiser (53) reported yeast product yields of 40% (based on total solids supplied) when potato waste with a pH of 5 was incubated at 30-32 C and when air was supplied at the rate of 1.2 volumes per solution volume per minute.

Limited chick feeding experiments were conducted by Reiser (53) using yeast material harvested from potato waste. The yeast product contained approximately 55% protein and, according to Reiser, was nutritionally equivalent to a blend of fish, meat, and soybean meal as a supplement in poultry rations.

Due to the high purine content of yeasts, large quantities may have deleterious effects (9, 60). However, feeding human subjects special diets using yeast protein as the principal protein source, Smith et al. (59), Pierce (48), and Still and Koch (60) found no significant increase of uric acid nitrogen in the urine. However, when the yeast diets were supplemented with meat protein, Still and Koch (60) reported increases in uric acid excretion. After

several weeks of feeding, either the high or low purine supplemented yeast diets, Still and Koch reported no increases in the uric acid content in the blood of their subjects. Smith *et al.* (59) and Hawk *et al.* (29) reported laxative effects on some of their subjects after yeast ingestion.

The demand for animal feed protein stimulated interest in the conversion of readily available carbohydrate material into yeast protein. Accordingly, Montana investigators used barley as a carbon source, and fertilizer-grade nitrogen for the propagation of *T. utilis* (16).

Goering and Houle (16) saccharified the carbohydrates present in a barley mash with 0.5% bacterial amylase and 10% distillers' malt (based on total grain present) preparation. The mash was cooked, filtered, and sterilized. The sugar concentration was then adjusted to 1% reducing sugars. Using a continuous pilot-plant Waldhof fermentor, maximum sugar utilization was achieved when wort containing 4% reducing sugars was fed at the rate of 1.5 liters per hour. At this sugar concentration, approximately 99% of the reducing sugars present were utilized.

Goering and Houle also investigated the sugar utilizing preferences of *T. utilis*. It was found that pentoses were preferentially utilized from the wort which, as indicated by paper chromatograms, contained higher oligo-

saccharides, glucose, maltose, and isomaltose in addition to the pentoses. A marked reduction in the oligosaccharides and isomaltose after fermentation indicated saccharifying ability of T. utilis. The most surprising result for Goering and Houle was the identical concentration of glucose and maltose in the spent beer, although the initial concentration of maltose was approximately ten times the glucose concentration. These workers found that the addition of 3% urea, 1% primary calcium phosphate, and 1% potassium chloride to the wort (based on sugars present) gave maximum yeast yields.

Continuous propagation was conducted at 30 C and pH 4.4-4.7 with approximately 7.5 liters of air supplied per minute. Yeast yields of 61-78% based on sugars present were obtained using these conditions and 75-99% of the sugars present were utilized.

Concerning the nutritive value of yeasts and of cereal grains, Wiley and associates (73) reported that high thiamine contents of food yeast may be achieved by growing yeast in substrates high in thiamine, such as grain worts used by brewers.

Owens et al. (65), discussing T. utilis in general, stated,

> . . .it is deficient in methionine, one of the essential amino acids, which, however, is present in cereal proteins. A ration containing this yeast with some cereal would provide all the essential amino acids.

Rosenberg (57) and Pond et al. (49) found cereal grains to be deficient in the essential amino acids, lysine and threonine. For proper cereal supplementation Rosenberg (57) suggested the first limiting amino acid, lysine, should be supplemented in an amount that would obtain a balance with the second limiting amino acid. Pond, Hillier, and Benton (49) calculated grain sorghum to be deficient in methionine. However, no growth response was observed when methionine was used to supplement grain sorghum fed to rats. It was concluded that the cystine level of milo was sufficient to meet the sulfur-containing amino acid requirements. Pond et al. achieved rat growth rates equivalent to a purified diet containing 11% casein when a milo ration was supplemented with 0.5% L-lysine and and 0.2% DL-threonine. Riboflavin, nicotinic acid, pantothenic acid, and biotin are present in grain sorghum in larger amounts than in yellow corn (41).

Before World War II, grain sorghum was used largely for feeding purposes. Today it is used in many areas of the world, such as India and Europe, as a basic food source. Corn crop failures and shortages of certain foods and materials after the war stimulated grain sorghum production in the United States (64). Grain sorghum, being similiar to corn in composition, has been since used as a partial substitute for corn and corn derivatives in certain areas

(41, 64, 71, 74).

Grain sorghum is now used in the fermentation, dry-milling, and wet-milling industries (41). Three sorghum fractions are produced during the dry-milling process--brewers' grits, industrial flour and concomitant, or germ.

The approximate composition of the germ fraction is 10.4% protein, 5.7% fat, 66.7% starch, and 4.1% ash (40). Its principal use is presently as a feed for livestock.

Harden (20) believed this fraction to be good for protein supplementation in foods for human consumption. Skinner (58) has already demonstrated the biological value of grain sorghum protein when properly supplemented.

CHAPTER III

EXPERIMENTAL PROCEDURE

Procedures for converting carbohydrate material to products containing considerable quantities of yeast protein (16, 25, 32, 34, 47, 53, 73) were modified for use in treating the "germ fraction" from dry-milled grain sorghum in an attempt to increase the quantity of protein in that fraction. A commercial source of Torulopsis utilis obtained from the American Type Culture Collection (1) served as the microorganism in this study and an enzyme preparation, Diazyme 160, was purchased from Miles Laboratories, Inc. (42) for use in starch conversion to monosaccharides.

The so-called "grain sorghum germ fraction" used as the substrate was obtained from a local dry-milling concern (28). This fraction contained 11.4% protein, 10-13% moisture, 5.3% fat, 3.4% ash, and 66-70% starch (by difference) (40). The kernel components contained in this fraction have been described by Marshall (40) and Harden (20).

At the outset considerable work was involved in developing suitable equipment and laboratory procedures for the experiment. This preliminary work consisted

mainly of 1) developing a suitable propagator for growing the yeast, 2) establishing proper methods for hydrolyzing the grain sorghum substrate for yeast utilization, and 3) determining proper laboratory procedures for evaluating the results. The propagator designed for the experiment consisted of a series of one gallon glass jars capped with lids equipped with three holes through which tubing was inserted for the purposes of serving as an air exit, sampling valve, and air supply. A diagramatic sketch is shown in Figure 1. The air, supplied by a portable air compressor (Champion Pneumatic, Model 20 B 7H), was saturated by passing through a container of water at 40 C (see saturator in Figure 1). The air rate was controlled by regulating the air outlet valve on the compressor storage tank. Air flow rate was not measured but air was passed through the propagator at a rate to insure adequate agitation without mechanical carryover. The propagation jars containing the prepared samples were held in an incubator at 31-33 C during the propagation period. Usually the experimental samples were incubated under these conditions of temperature and air flow for a period of 18-24 hours.

In preliminary work 51 sets of data were collected to establish proper operational procedures (e.g., air volume, air control, sample size, inoculum size, inoculum

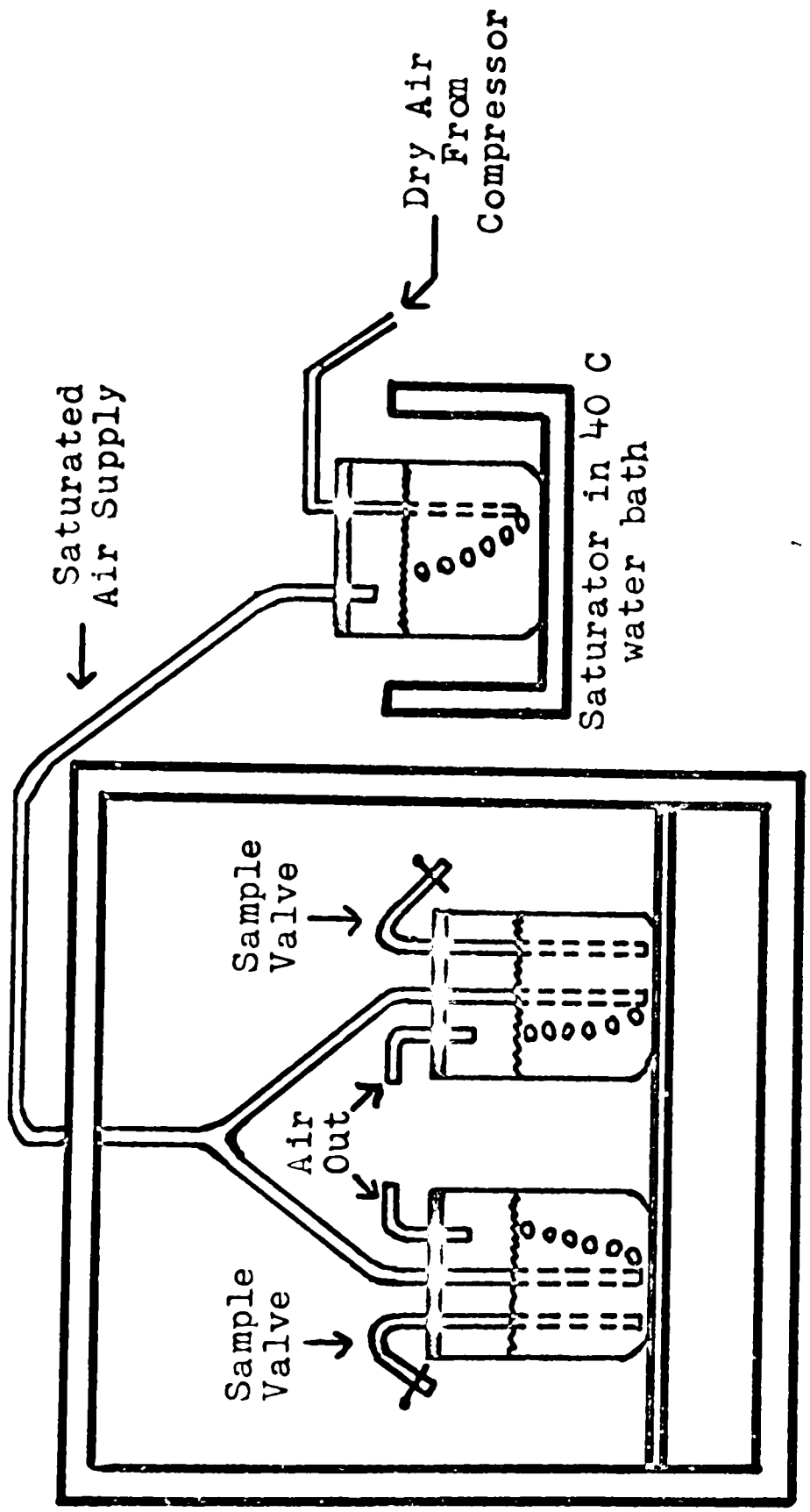

Fig. 1--System Used in Propagating T. utilis
on Grain Sorghum Germ Fraction

maintenance, and laboratory procedures to be used in evaluating experimental conditions and results). Based on this preliminary research, the various phases of procedure were developed and are described in the following paragraphs.

Inoculum Maintenance

The starter inoculum was prepared by transferring yeast cells from the original stock culture of T. utilis to malt agar slants which were incubated for 48 hours at 32 C. These yeast cells were then transferred to test tubes containing clear hydrolyzates of the germ fraction. The hydrolyzate was prepared by pouring 600 ml of boiling water over 250 grams of germ fraction contained in a 1,000 ml Erlenmeyer flask. A teaspoon of Dow Corning antifoam and 50 ml of concentrated hydrochloric acid were then added to the flask and mixed. The flask with a condenser was placed on a hot plate, and the mixture refluxed for 2.5 to 3 hours. At this time the flask was removed and allowed to cool to room temperature. The hydrolyzate was then filtered through No. 1 filter paper. The clear filtrate was diluted with 1,500 ml of water. Approximately 30-40 ml of concentrated sodium hydroxide was added to adjust the pH to 4.0-5.5. A precipitate was formed upon addition of the sodium hydroxide which was subsequently removed by filtration through No. 1 filter-

paper. The clear, dark filtrate was then stored under refrigeration for future use in 1) acclimating the yeast to the grain sorghum sugars and 2) maintaining a ready supply of yeast cells. The test tubes were incubated at room temperature while shaking on a shaking apparatus (Burrell Wrist-Action Shaker) at approximately 125 strokes per minute. After 24 hours of incubation, the tubes were removed from the shaker and the contents were added to 250 ml centrifuge tubes. The yeast suspension was centrifuged, using an International centrifuge, type SB, size 1, at 3,000 r.p.m. for 10 minutes. The liquid was decanted and the yeast cells were collected and used to reinoculate fresh filtrate preparations. This procedure was continued over a period with the container capacity and hydrolyzate volume becoming progressively larger.

Yeast cells collected in excess of that required for immediate experimental use were washed, drained of excess water, air dried, and stored under refrigeration. These dried cells remained viable throughout the course of this study and required only a short inductive period before maximum yeast activity was realized. Cells not dried or used immediately as inoculum were washed and stored at pH 3.5-4.5 in the refrigerator. This yeast cream remained viable for approximately one week. If not used within this period, acclimation was nullified.

The modified washing procedure was adopted from Kurth et al. (34) and was as follows:

1. Suspend the yeast precipitate in 25-50 ml of 0.35% HCl (1 ml conc HCl per 100 ml water).
2. Centrifuge for 5 minutes at 3,000 r.p.m.
3. Decant acid solution.
4. Resuspend yeast in 25-50 ml of 1% sodium carbonate solution.
5. Centrifuge for 5 minutes at 3,000 r.p.m.
6. Decant basic solution.
7. Add 25-50 ml distilled water and adjust pH to 3.5-4.5.
8. Store in refrigerator.

Preparation of Grain Sorghum Fraction Slurry

Protein and total solids data were collected from a representative sample of germ fraction. An approximate 10% slurry of germ fraction was prepared in bulk cream cans and pasteurized between 65.5-71.1 C for 2 hours in a buttermilk culture cabinet. The temperature was maintained just below the gelatinization temperature range of grain sorghum starch which is generally between 67.5-75 C (36). The can was then placed in a refrigerated vault and sample aliquots were taken as needed.

Laboratory Tests Utilized

The laboratory testsused for describing the various components of experimental slurries and for describing the end products obtained after incubation were 1) a modified Kjeldahl for total protein determination, 2) a modified non-protein nitrogen determination, and 3) a quantitative total solids determination.

The modified Kjeldahl procedure was as follows:

1. Weigh a 15-30 gm sample (7-8% total solids) of slurry suspension into a Kjeldahl flask.

2. Add one teaspoon of digest mixture (3.2 parts copper sulfate and 96.8 parts anhydrous sodium sulfate).

3. Add 25-30 ml concentrated sulfuric acid.

4. Digest on standard Kjeldahl digesting unit 15 to 20 minutes after the solution turns a clear green.

5. Cool the flask and contents to room temperature and add 200 ml of water.

6. Allow to cool and add a small quantity of pumice powder or boiling chip.

7. Add concentrated sodium hydroxide until characteristic blue layer forms.

8. Connect to Kjeldahl distilling apparatus, which has a previously placed receiver flask containing 50 ml of 4% boric acid with indicator. (boric acid indicator: 2.5 ml of 0.1% bromocresol green solution and

1.5 ml of 0.1% methyl red solution dissolved in 95% alcohol per liter of acid).

9. Distill and collect 150 ml of distillate.

10. Titrate excess base with standard hydrochloric acid solution and record figures.

11. Calculate percent protein using factor of 6.25 times percent nitrogen.

The non-protein nitrogen determination was a modified procedure by Ling (39) for non-protein nitrogen determinations of milk. The procedure consisted of the following steps:

1. Weigh a 25-40 gm sample (7-8% total solids) of slurry suspension into a 100-110 ml Volumetric flask.

2. Add 25 ml of 15% trichloroacetic acid.

3. Mix, allow to stand for 5 minutes, and add distilled water to the 100 ml dilution mark.

4. Allow precipitate to settle and filter through No. 40 filter paper.

5. Pipette 25 ml of the filtrate into a Kjeldahl flask and proceed in accordance with previously described Kjeldahl nitrogen determination.

6. Compute percent nitrogen with proper consideration of the dilutions.

Total solids percentages were determined by weighing samples into aluminum solids dishes and placing in an

oven at 100-105 C for 24 hours.

Sample Preparation and Data Collection

For the purpose of obtaining quantitative data for evaluating experimental methods, the following procedure was adopted:

1. Collect and record empty weight data on glass propagators and add 500-600 gm of previously prepared germ slurry.

2. Collect and record total weight of propagator plus slurry.

3. Add Diazyme 160 at the rate of 0.63 gm dry enzyme per 50 gm grain sorghum solids. Diazyme 160 is a commercial preparation by Miles Laboratories containing the enzyme amyloglucosidase obtained from the mold Aspergillus niger and converts starch completely to glucose (10, 42, 46, 63). Optimum pH range is 3.35-4.4 and optimum temperature is 60 C.

4. Add yeast inoculum at an approximate rate of 10% wet cells, based on total slurry weight. (Total solids and protein determinations were made periodically on the yeast cream).

5. Add dibasic ammonium phosphate--$(NH_4)_2HPO_4$--at rate of 1.0% (based on total slurry weight).

6. Mix the slurry thoroughly to solubilize the added ingredients.

7. Take an aliquot for testing.

8. Collect and record weight data on propagator plus final slurry.

9. Place propagator in incubator at 31-33 C and connect saturated air supply.

10. Determine total solids, protein, and non-protein nitrogen in accordance with previously described procedures.

11. Remove propagator after 18-24 hours incubation and rinse solids collected on sides with distilled water, and collect propagator weight data.

12. Determine total solids, protein, and non-protein nitrogen concentrations.

Experimental Design

Data were collected for six sets of control groups and eleven sets of experimental samples in which percentages of protein, non-protein nitrogen, and total solids were obtained. The control groups consisted of 1) two sets containing the prepared germ fraction slurry only; 2) two sets containing the prepared germ fraction slurry and Diazyme 160; 3) two sets containing the prepared germ fraction slurry and ammonium phosphate; 4) four sets containing the prepared germ fraction slurry, Diazyme 160, and ammonium phosphate; 5) two sets containing the prepared germ fraction slurry and yeast;

and 6) five sets containing the prepared germ fraction slurry, Diazyme 160, and yeast. The experimental samples consisted of eleven sets containing the prepared germ fraction slurry, Diazyme 160, ammonium phosphate, and yeast.

Interpretation of Data

Utilizing the data regarding slurry weights, total solids, and percentages of total nitrogen and non-protein nitrogen, quantitative amounts of protein and total solids were computed on the basis of 100 grams of grain sorghum solids. A comparison was made of the quantities of these components before incubation and the quantities present in the slurries after incubation.

Although representative samples of incubated slurries were oven dried, ground into a fine powder, and placed in storage, no data concerning physical characteristics, organoleptic qualities, solubilities, or bacterial contamination were routinely recorded. These dried samples were surrendered to another party for further analysis.

CHAPTER IV

RESULTS AND DISCUSSION

The experimental work was divided into three phases: 1) to develop propagating techniques and analytical procedures, 2) to accumulate control data concerning the effect of various components added to slurry preparations, and 3) to evaluate data obtained from actual experimental runs.

With respect to the first portion of the study, 51 sets of data concerning material balances involving total solids, protein, and non-protein nitrogen contents before and after incubation were tabulated. The actual results obtained were not recorded in this thesis since the work was exploratory in nature. However, the results of these experiments indicated 1) that propagating techniques described earlier in the Experimental Procedure section, Chapter III, were satisfactory in promoting growth and sugar utilization, 2) that enzyme preparations added with the inoculum were superior to acid hydrolysis in providing monosaccharides for yeast growth, probably because of toxic effects of high concentrations of sugars (26, 47), 3) that quantitative material balances could be made by using analytical techniques chosen for the experiment, and 4) a product could be obtained in a dry state which was higher in

total protein than the original grain sorghum substrate.

With respect to phase two of this experiment, the data concerning control samples are shown in Tables 1 and 2. It may be noted from these tables that results were converted to a 100 grams grain sorghum solids basis. This means that, although the sample preparations may have contained anywhere from 25-75 grams grain sorghum solids, the control data related to components added and nitrogen and total solids recovery were converted to a 100 gram basis and placed into the tables.

It may also be noted that Table 1 contains data concerning the control groups before they were incubated, and Table 2 contains data on the same groups after incubation. The controls were chosen in such a way as to reflect the effect of each ingredient and combination of ingredients on the experimental protein gains. For example, control 1 consisted of a slurry containing only water and grain sorghum fraction. It will be noted that from the accompanying data, the protein nitrogen and non-protein nitrogen before incubation averaged 1.72 and 0.50 grams per 100 grams of grain sorghum solids, respectively. It may be noted from Table 2 that these two components did not change appreciably after the controls had been incubated. Concerning this same group, 100 grams of total solids were in the control before incubation, whereas, practically no total solids were lost during

TABLE 1

DATA CONCERNING TEST ON CONTROL SAMPLES BEFORE INCUBATION
(Figures Based on 100 Grams of Grain Sorghum Solids)

Control Group[a]	$(NH_4)_2HPO_4$ Added grams (1)	Yeast Mtl. Added[b] grams (2)	Total Nitrogen grams (3)	Non-Protein Nitrogen grams (4)	Protein Nitrogen grams (5)	Total Solids grams (6)	Protein in Dry Matter % (7)
1	---	---	2.24	0.50	1.72	100.0	10.8
2	---	---	2.27	0.48	1.77	100.6	11.0
3	11.32	---	4.36	2.66	1.70	111.2	9.6
4	11.61	---	4.39	2.69	1.70	111.7	9.5
5	---	46.7	2.94	0.51	2.43	110.7	13.7
6	---	41.8	3.01	0.64	2.37	113.5	13.0

[a] 1 - Contains grain sorghum fraction only (ave. of two tests).
2 - Contains grain sorghum fraction plus enzyme (ave. of two tests).
3 - Contains grain sorghum fraction plus ammonium phosphate (ave. of two tests).
4 - Contains grain sorghum fraction, ammonium phosphate, plus enzyme (ave. of four tests).
5 - Contains grain sorghum fraction plus yeast (ave. of two tests).
6 - Contains grain sorghum fraction, yeast, plus enzyme (ave. of five tests).

[b] See Table 5 for data concerning yeast inoculum.

TABLE 2

DATA CONCERNING TEST ON CONTROL SAMPLES AFTER INCUBATION
(Figures Based on 100 Grams of Grain Sorghum Solids)

Control Group[a]	Total Nitrogen grams (1)	Non-Protein Nitrogen grams (2)	Protein Nitrogen grams (3)	Total Solids grams (4)	Protein in Dry Matter % (5)	Gain in Protein	
						Grams per 100 Grams Grain Solids (6)	Percent of Orig. Dry Matter (7)
1	2.27	0.50	1.77	98.9	11.2	0.31	0.31
2	2.22	0.40	1.82	80.5	17.2	0.31	0.31
3	4.13	2.40	1.73	110.7	9.8	0.18	0.16
4	4.39	2.58	1.81	77.4	14.6	0.68	0.61
5	3.03	0.45	2.58	105.1	17.7	0.92	0.90
6	3.01	0.38	2.63	59.0	27.9	1.62	1.43

[a]See Table 1 for description of sample groups and Table 5 for data concerning yeast inoculum.

incubation as reflected by the fact that 98.9 grams of total solids were recovered. However, this loss in total solids caused an increase in the percentage of protein in the dry matter from 10.8 to 11.2%.

Control group 2, containing grain sorghum fraction plus enzyme, showed a similar amount of total nitrogen and non-protein nitrogen (columns 3 and 4 of Table 1) to control group 1 before incubation. However, after this group of controls had been incubated (column 4 of Table 2), a considerable amount of total solids was lost (100.6 grams before vs. 80.5 grams after incubation). Simultaneously, the total nitrogen, non-protein nitrogen, and protein nitrogen did not change appreciably. This indicates that the combined effect of enzyme action and propagating procedures caused a loss of total solids. Since the nitrogen content remained relatively constant, it was assumed the loss was due to conversion of some of the carbohydrate material to carbon dioxide and water. Because of the loss in total solids, the percent protein in the dry matter increased from 11% before to 17.2% after incubation with an apparent gain of 0.31 grams of protein per 100 grams of grain solids.

Control group 3, containing grain sorghum fraction plus ammonium phosphate, obviously showed an increase in total nitrogen when compared to control groups 1 and 2

(column 3 of Table 1 and column 1 of Table 2) with an accompanying increase in non-protein nitrogen (column 4 of Table 1 and column 2 of Table 2). The increase of approximately 2.0 grams in non-protein nitrogen compared favorably with the actual grams of nitrogen added in the form of dibasic ammonium phosphate. It may be noted from a comparison of column 5 of Table 1 and column 3 of Table 2, that the protein nitrogen (obtained by difference of total nitrogen and non-protein nitrogen) compared favorably with similar data concerning control groups 1 and 2. It will also be noted from column 6 of Table 1 and column 4 of Table 2, that no appreciable loss of total solids was incurred during the incubation period. Furthermore, as would be expected, the percentage of protein in dry matter in the final product (9.8) was practically the same as the percentage in dry matter before incubation (9.6). The gain in protein of 0.18 grams per 100 grams grain solids (column 6 of Table 2) was considered insignificant in view of the fact that small gains were also encountered when grain sorghum alone was subjected to the propagating procedure.

Control group 4 contained grain sorghum fraction plus ammonium phosphate and enzyme. It compared favorably with control group 3 with respect to total nitrogen, non-protein nitrogen, and protein nitrogen, but the total solids retained in the slurry (column 4 of Table 2) after incubation

decreased considerably. This was expected since control group 2, which contained grain sorghum plus enzyme, also showed a significant decrease in the percent total solids. Because of this decrease in grams of total solids, the percent protein in the dry matter increased from 9.5% before incubation to 14.6% after incubation. Columns 6 and 7 of Table 2 indicate a somewhat larger gain in protein than one would expect. But again, this amount of gain will prove to be rather insignificant when the data concerning experimental samples are discussed later. Another gratifying result reflected by the data is that total nitrogen, non-protein nitrogen, and total solids of control group 3 were comparable to data accompanying control 4, thereby showing the propagating procedure and analytical technique were satisfactory.

Control groups 5 and 6 both contained grain sorghum fraction and yeast but to group 6 was added enzyme. It may be noted from Table 1 that the quantitative data regarding total solids, total nitrogen, and non-protein nitrogen for the two groups were comparable, from Table 2 that there was a slight decrease in non-protein nitrogen accompanied by a comparable increase in protein nitrogen, and from Table 2, that the grams total solids recovered were much less in control group 6 than in control group 5. Also, comparison of column 6 of Table 1 with column 4 of Table 2,

reveals yeast action alone, during propagation, caused a slight decrease in total solids content of the slurry (110.7 vs. 105.1). This was expected since there probably was some yeast action on the carbohydrate material even without the aid of enzyme to provide monosaccharides. A comparison of control group 5 to both control groups 4 and 1, with respect to gain in protein (column 6 of Table 2), shows that yeast action alone caused a considerable increase in protein over that of the controls (0.92 vs. 0.68 and 0.31, respectively).

It is interesting to note that the increase in protein reflected by control group 6 surpassed the gain associated with control group 5 by approximately 57% (1.62 vs. 0.92), column 6 of Table 2. This indicated that enzyme action made available more carbohydrate material for yeast utilization. Since this gain in protein was accompanied by a loss in grams of total solids, the percentage of protein in dry matter naturally increased and the amount of increase is reflected in the fact that before incubation the slurry contained 13% protein in the dry matter while after incubation the figure was 27.9%. The gain in protein was also described in column 7 of Table 2 as amounting to 1.3% of the original dry matter.

Data associated with results obtained from experimental samples (those containing grain sorghum fraction,

yeast, enzyme, and added ammonium phosphate) are shown in Tables 3 and 4. Again, these data were reported on a basis of 100 grams grain sorghum solids. Table 3 contains data concerned with the samples before incubation and Table 4 contains information on those samples after incubation. Column 1 of Table 3 shows that the grams of ammonium phosphate added varied from 16.6 to 10.2 grams per 100 grams of grain sorghum solids. This amounts to an approximate range of 2-3.2 grams of nitrogen added from this source per 100 grams of grain sorghum solids and is comparable to nitrogen amounts recommended by other workers (24, 34, 47). Although this point was not studied, it is believed that gains in protein would not have diminished considerably had this amount of ammonium phosphate been reduced. This premise is based on the fact that the non-protein nitrogen in the final products (column 2 of Table 4) were well above the non-protein nitrogen content of control groups discussed earlier. It seems plausible that since all of the non-protein nitrogen added was not utilized, it would not have been necessary to add so much ammonium phosphate. This observation should be of interest in the event subsequent studies are made on this subject.

Column 2 of Table 3 shows the amount of yeast material added to the slurries per 100 grams of grain sorghum solids. As is shown in Table 5, the percentages of total solids varied considerably in the yeast inoculum

TABLE 3

DATA CONCERNING TEST ON EXPERIMENTAL SAMPLES BEFORE INCUBATION
(Figures Based on 100 Grams of Grain Sorghum Solids)

Sample Number	$(NH_4)_2HPO_4$ Added grams (1)	Yeast Mtl. Added[a] grams (2)	Total Nitrogen[b] grams (3)	Non-Protein Nitrogen grams (4)	Protein Nitrogen grams (5)	Total Solids grams (6)	Protein in Dry Matter % (7)
1	16.6	40.1	5.50	3.63	1.87	129.6	9.0
2	17.9	38.7	5.50	3.60	1.90	130.3	9.1
3	15.2	26.3	5.57	3.65	1.92	123.6	9.7
4	15.2	26.5	5.57	3.53	2.04	123.7	10.3
5	11.6	30.0	5.29	3.04	2.25	121.3	11.6
6	11.6	30.1	5.16	3.04	2.12	121.4	10.9
7	11.7	23.4	5.35	3.03	2.32	119.3	12.1
8	11.7	23.2	5.22	3.03	2.19	119.3	11.5
9	12.7	26.7	5.41	3.15	2.26	121.2	11.7
10	12.7	26.8	5.55	3.15	2.40	121.3	12.3
11	10.2	18.9	4.85	2.92	1.93	117.3	10.3
Ave.	13.4	28.2	5.36	3.25	2.11	122.6	10.8

[a]See Table 5 for data concerning yeast inoculum.

[b]Both the initial and final Kjeldahl data were used in computing total nitrogen.

TABLE 4

DATA CONCERNING TEST ON EXPERIMENTAL SAMPLES AFTER INCUBATION
(Figures Based on 100 Grams of Grain Sorghum Solids)

Sample Number	Total Nitrogen grams (1)	Non-Protein Nitrogen grams (2)	Protein Nitrogen grams (3)	Total Solids grams (4)	Protein in Dry Matter % (5)	Gain in Protein		Ratio of Final to Initial Protein Content (8)
						Grams per 100 Grams Grain Solids (6)	Percent of Orig. Dry Matter (7)	
1	5.50	2.70	2.90	80.6	21.1	6.4	4.9	1.55
2	5.50	2.59	3.12	80.2	24.3	7.6	5.8	1.64
3	5.57	2.40	3.17	84.6	23.4	7.8	6.3	1.65
4	5.57	2.56	3.01	83.7	22.5	6.1	4.9	1.48
5	5.29	2.47	2.92	72.3	25.2	4.2	3.5	1.30
6	5.16	2.36	2.78	66.7	26.0	4.1	3.4	1.31
7	5.35	2.32	2.98	69.2	26.9	4.1	3.4	1.28
8	5.22	2.28	3.04	72.3	26.2	5.3	4.4	1.39
9	5.41	2.18	3.23	76.2	26.5	6.1	5.0	1.43
10	5.55	2.34	3.21	73.1	27.3	5.1	4.2	1.34
11	4.85	1.90	2.95	56.2	32.7	6.4	5.4	1.53
Ave.	5.36	2.37	3.03	74.1	25.6	5.7	4.7	1.45

TABLE 5

COMPOSITION OF YEAST INOCULUM USED IN THE VARIOUS EXPERIMENTS

Sample Number[a]	Total Solids %	Total Nitrogen in Dry Matter %	Non-Protein Nitrogen in Dry Matter %	Protein in Dry Matter %
Control 5	32.1	7.5	0.5	43.7
Control 6	32.2	5.9	1.2	29.4
Samples 1 thru 4	14.0	6.5	3.1	21.3
Samples 5 thru 7	32.2	7.9	1.7	38.8
Samples 8 thru 10	31.0	8.6	2.0	41.2
Sample 11	34.0	7.4	1.2	38.8

[a]See Tables 1 thru 4.

preparations and that the non-protein nitrogen in the dry matter varied from 0.5 to 3.1. This is attributed to the fact that the yeast inoculum material, being obtained by centrifugation of grain sorghum filtrate, probably contained some grain sorghum precipitates in the solids. Also, it is possible that the various samples of yeast materials varied in the amount of yeast growth. No effort was made to enumerate the number of yeast cells in the inoculum nor determine the amount of total protein and/or non-protein nitrogen contributed by the yeast cells themselves. When comparing the total nitrogen contents of the samples before incubation and after incubation (column 3 of Table 3 and column 1 of Table 4), it will be noted that the two sets of data are identical. Actually, during testing, some small differences were noted in this area but for the sake of reflecting protein gains clearly the total nitrogen content of the samples after incubation were tabulated in Table 4.

The amount of non-protein nitrogen decreased during incubation as reflected by a comparison of column 4 in Table 3 and column 2 in Table 4. The average non-protein nitrogen content decreased from 3.25 grams per 100 grams of grain solids before incubation to 2.37 after incubation for an average loss of 0.88 grams. Accompanying the decrease in non-protein nitrogen was an increase in protein nitrogen

from an average of 2.11 to 3.03 grams per 100 grams of grain solids and an average gain of 0.92 grams.

The grams of total solids decreased during propagation as reflected by a comparison of column 6 in Table 3 and column 4 in Table 4. This was expected since control groups containing enzyme showed a similar decrease in this respect. The average decrease from 122.6 grams to 74.1 grams of total solids shows a percentage decrease, based on original total solids, of approximately 38%. Accompanying this decrease was an increase in protein nitrogen content from 2.11 to 3.03%. This amounted to an average gain of 5.7 grams of protein per 100 grams of grain sorghum solids (column 6 of Table 4).

Expressed in another way, the percent protein in dry matter increased from an average of 10.8% (column 7 of Table 3) to an average of 25.6% (column 5 of Table 4). The gain in protein, in terms of percent of original dry matter, ranged from 3.4 to 5.8 for an average of 4.7% (column 7 of Table 4). The ratio of final to initial protein content as shown in column 8 of Table 4, ranged from 1.28 to 1.65 for an average of 1.45. This indicates that a gain in total protein of approximately 45% was accomplished by the propagation procedures investigated and studied.

Although this percent increase in protein content is comparable to those of other workers, difficulty would be experienced in making exact comparisons, since most of

the work reported is expressed in terms of yeast yields per unit of sugar utilized. Since it was deemed unnecessary to determine quantitatively the amounts of sugar and carbohydrate materials remaining in the slurries (although these determinations were made in preliminary studies), one can only estimate comparisons of net yields. However, the relatively large increase in protein content found in this study was considered grounds for continued study on the final product with respect to organoleptic and nutritive qualities should international interest continue to be directed toward this area.

CHAPTER V

SUMMARY AND CONCLUSIONS

For many people throughout the world, grain sorghum preparations serve as a principal protein source in their diets. Since grain sorghum and grain sorghum derivatives have considerable nutritional shortcomings, interest for improving its food value is a present endeavor by many international concerns. Accordingly, it was the purpose of this study to use yeast preparations in preparing a potentially high protein food from a dry-milled fraction of grain sorghum kernels.

The grain sorghum material used in the study was a "germ" fraction obtained by a commercial concern when processing grain sorghum kernels by a dry-milling procedure to obtain three major fractions called grits, flour, and germ. The germ fraction consisted of the outer portion of the kernel and contained approximately 11.4% protein, 10-13% moisture, 5.3% fat, 3.4% ash, and 66-70% starch (by difference).

Prior to the actual experimental work 51 sets of data were accumulated to develop a satisfactory procedure for propagating yeast on the fraction. During this period, it was decided that slurries containing grain sorghum

fractions should be pasteurized between 65.5-71.1 C for 2 hours prior to the addition of other ingredients. The yeast used was Torulopsis utilis, obtained from the American Type Culture Collection and a commercial preparation of enzyme called Diazyme 160, purchased from Miles Laboratories, Inc., was added to break the starch portion of the fraction into simple sugars for yeast utilization. Additionally, a nitrogen and phosphorus source of dibasic ammonium phosphate was added to all slurries to provide the necessary elements for satisfactory yeast growth. An apparatus was constructed to provide a source of saturated air, and the propagating media were incubated at 31-33 C.

Essentially, the procedure adopted consisted of 1) preparing a slurry of grain sorghum germ fraction of approximately 10% by weight; 2) pasteurizing this slurry; 3) adding a strain of Torula yeast, a commercial enzyme preparation, and supplemental nutrients; 4) collecting compositional data concerning protein nitrogen, non-protein nitrogen, and total solids on the slurry and yeast material; 5) incubating at 31-33 C with a supply of saturated air for 18-24 hours; and 6) collecting the final compositional data on the sorghum slurry after incubation. In addition to collecting data on the experimental slurries, control data were obtained on each slurry component and combination of components.

Final data indicated that the modified Kjeldahl procedures for determining total nitrogen and non-protein nitrogen were satisfactory in that duplicate results could be obtained and satisfactory quantitative protein and total solids balances could be maintained on samples before and after incubation. The addition of ammonium phosphate to the slurries promoted yeast growth beyond that found when the slurries were not fortified and the addition of enzyme to mixtures greatly enhanced yeast nitrogen assimilation over that experienced when enzyme was not used. In connection with the enzyme, it was found that its activity promoted the loss of solids during incubation, and this was attributed to a possible breakdown of a portion of the carbohydrates to carbon dioxide and water. This phenomenon occurred in control groups with enzyme as well as experimental samples.

Considerable amounts of protein were manufactured by the experimental procedure as reflected by the fact that an average gain of 5.7 grams of protein per 100 grams of original grain sorghum solids was realized. This amounted to approximately a 45% increase in final total protein over that in the initial grain sorghum product.

In general, when starting with 100 grams of grain sorghum and added ingredient solids, approximately 60 grams of final dry matter resulted. This means that a consid-

erable amount of carbohydrate material was expended as energy and/or converted to yeast protein during the process. The average percent protein in dry matter before propagation was 10.8 whereas the content after incubation was 25.6.

Samples of final slurries were dried and stored under refrigeration for further study. No attempt was made to evaluate the product organoleptically or nutritionally. Instead, the samples were given to another group of researchers who plan to initiate evaluation studies in this area.

It may be concluded that yeast, similar to the type used in this study, can produce protein from water slurries containing dry-milled grain sorghum germ fraction, when supplemented with small amounts of dibasic ammonium phosphate and when an enzyme such as Diazyme 160 is used for starch conversion. Also, when a procedure such as the one described is followed, one may expect an increase of approximately 45% in total protein over that in the original fraction.

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