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The effect of variation in mashing procedures upon the alcoholic fermentation of corn

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NOTE TO USERS

THE EFFECT OF VARIATION IN MASHING PROCEDURES UPON THE ALCOHOLIC FERMENTATION OF CORN

by

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I. INTRODUCTION

Ethanol is regarded as one of the most important substances used by man. Indeed, it has been said that, next to water, ethanol is the most useful chemical compound known, because of its countless industrial and medicinal applications. The production of this alcohol by the fermentation of carbohydrates was probably one of the earliest chemical processes to be discovered and put to use. Although for centuries the process was used only for the production of beverages, it has now developed into a highly important chemical industry.

Recently the production of ethanol has assumed still greater significance, for ethanol is of tremendous importance in the nation's war effort. Huge quantities are used in the manufacture of smokeless powder. Of still greater importance is the fact that the United States government is depending upon ethanol to a large extent as a raw material for the production of synthetic rubber. These new demands have called for an enormous increase in production. In 1941, this country produced about 125,000,000 gallons of industrial alcohol. Of this quantity approximately 85 per cent was produced by fermentation, and the remainder was synthesized from ethylene. Less than 10 per cent of the total was obtained by the fermentation of grains, the chief raw material being blackstrap molasses imported from the West Indies. The War Production Board has estimated the ethanol requirements for 1943 to be 534,500,000 gallons, or more than four times the record 1941 figure. Since the supply of ethylene is limited, almost the entire increase must be met by the fermentation industry.

In order to meet production goals drastic changes have been necessary in the industry. Several new plants have been built or are in the process of construction. Distilleries have been ordered to stop the production of whiskey and are now producing industrial alcohol at full capacity. The situation has been greatly complicated by the fact that the supply of raw material normally used, molasses, has been cut off because of the shortage of shipping facilities. As a result, it has been necessary to convert the ethanol plants to the use of grain as the starting material. This has imposed a considerable hardship upon the industry, for most producers had neither the necessary equipment nor experience in the use of grains. To be sure, the whiskey distillers have always used grains; however, their principal aim has always been to improve the quality of their product rather than to achieve the highest possible efficiency in the plant. Consequently the production of grain alcohol has remained an art rather than a science.

Since yeast cannot ferment starches directly, it is necessary to saccharify the grain to produce fermentable sugars. The fermentation industry in the United States has always used barley malt for this purpose. Malt contains an enzymic complex known as amylase, which brings about this conversion. Other biological materials are known to contain similar enzymes, but have never been used commercially in the fermentation industry. It is, of course, desirable to get the highest possible conversion of starch to sugar, and consequently maximum yields of ethanol. It is this phase of the process which is least understood. A considerable portion of the original carbohydrate is usually not utilized by the yeast.

It was with these problems in mind that the present investigation seeking improved methods of preparing corn mashes for fermentation was undertaken.

II. HISTORICAL

The preparation of alcoholic beverages from starchy materials is an ancient art. For centuries malt and mold preparations have been used to convert the starch of grains to fermentable sugars, though nothing was known of their mode of action until comparatively modern times. Not until the latter part of the eighteenth century was there any scientific investigation of the enzymes involved.

The enzyme amylase was first discovered in malt by Irvine in 1785, according to Pringsheim (1932). Kirchhoff (1815) found that the enzyme liquefied starch paste with the production of fermentable sugars. He stated that this transformation of starch to sugars is a vital step in the alcoholic fermentation of starchy materials.

Payen and Persoz (1833) prepared an extract of malt, and precipitated from it, by the addition of ethanol, a material which was capable of digesting starch. This material could be dried and preserved without losing its activity. They called it "diastase," a term which is frequently used synonymously with "amylase."

Further work by Dubrunfaut (1847), O'Sullivan (1872), and Schulze (1874) established that the action of malt amylase gave rise to a sugar which was not glucose. The sugar was given the name "maltose."

Maercker (1878) suggested that amylase is not a simple enzyme, but consists of two different enzymes. This was confirmed by Ohlsson (1922) who reported that two distinct enzymes could be separated from malt

extract. They were separated on the basis of their different stability toward heat at different pH levels. The first fraction breaks starch down into dextrins, and was called "dextrinogenase" (α-amylase). The second fraction, "saccharogenase" (β-amylase), converts starch and dextrins to maltose. More recently Blom, Bak, and Braae (1937) have also expressed the view that α-amylase is responsible for the liquefying action, whereas the action of β-amylase is purely saccharogenic.

The end product of the action of amylase upon starch is maltose. The reaction does not go to completion, however, as only 70 to 80 per cent of the theoretical quantity of maltose is ordinarily obtained. Various explanations for this fact have been offered, the most popular being that the reaction is an equilibrium, and that the sugar already formed interferes with further saccharification. However, Pringsheim and Schmalz (1923) have postulated a different theory. They reported that saccharification proceeds only to a certain point, at which a "limiting dextrin" remains intact. This dextrin was shown to be a trihexosan. It may be hydrolyzed by amylase when the latter is acted upon by an activator, or so-called "complement" of amylase. Under these conditions amylase is empowered to carry out quantitative saccharification beyond the usual limiting degradation. Such an activator was found in fresh yeast that had been liquefied with toluene. Amylases and their activators are found together in nature, but in varying proportions. Pringsheim and Beiser (1924) showed that the complement was present in malt. The investigations on complement have been confirmed by other workers, including Kuhn (1925), Sjöberg (1925), and Hoop and van Laer (1925).

Within recent years a great amount of work has been done upon the amylases, dealing largely with their purification and composition, and their action upon pure starch preparations. Since this thesis concerns the preparation of corn mashes for the ethanol fermentation, this review will be limited to topics which have a bearing on that problem. The above survey gives a background for understanding the nature of the action of amylolytic agents upon grain mashes.

Molds, as well as malt, are known to have the power of saccharifying starches, and have been used for that purpose by the peoples of the Orient for many years. In China, a preparation known as Chinese yeast cake is widely used. Calmette (1892) was the first to make a scientific study of this so-called yeast. He found that a certain species of mold always predominated in the mixture of microorganisms which was present. This mold, now known as Mucor rouxii, had the ability to saccharify starch, and also to convert the sugar to alcohol slowly. Calmette concluded that the amylolytic activity of the Chinese yeast cake was due to this organism.

Not long after the work of Calmette, the French investigators Collette and Boidin (1897) developed a process using Mucor rouxii as a saccharifying agent for the ethanol fermentation. Their process was called the "Amylo" process, and has been used rather extensively in the countries of southern Europe. The original method was described as follows: the starchy material was boiled, and liquefied by a little malt, acid, or mold. It was then boiled again, cooled, and a pure culture of the mold was added. Saccharification and fermentation of the starch occurred, the latter being

hastened by the addition of yeast. It was stated that the process gave much better yields of ethanol than did the customary method using malt. The process has since been improved by the use of steam pressure for cooking the mash, and of aeration during the first 24 hours to speed the growth of the mold.

Owen (1933) has suggested that processes in which molds are grown directly in the mash for the saccharification of the starch possess the following advantages: (1) economy due to a saving in malt; (2) decreases in losses due to infection introduced with the malt; (3) increased yields of alcohol; and (4) higher purity of alcohol formed in the mash.

Neubauer (1933) pointed out that there are also disadvantages in the Amylo process. Special installations and more power are necessary. In addition, an expert personnel and a complete bacteriological laboratory are required.

Boidin (1933) described some improvements which had been made in the process. A more vigorous species of mold, Mucor Delemar, was used for the saccharification. A very active yeast culture was added to the mash along with the mold inoculum, which was able to carry on fermentation at a relatively high temperature. The time of fermentation was reduced to 2.5 to 3 days.

In Japan, a fungal preparation known as "koji" is used. It is prepared by growing molds on steamed rice or other starchy materials. The koji is then used to saccharify grain starches for the preparation of sake (rice beer) and other fermented products. Korschelt (1878) called the fungus involved Eurotium. It has since been renamed Aspergillus oryzae.

The use of Aspergillus oryzae in occidental countries may be traced back to Takamine, a Japanese investigator. Takamine advocated the use of the enzymes of this mold in the distilling industry, and obtained several patents for the manufacture of enzymic materials. Takamine (1914) has reviewed his work in this field. He found that the mold grew well on wheat bran in a pneumatic drum, the mass being tumbled by rotation while being subjected to a current of moistened air. The material was then dried, and used in the same manner as malt. This preparation was known as "Taka-koji." Hiram Walker and Sons, Inc., made large scale tests with this material in a distillery in Canada. Ortved (1912) reported that the tests were entirely successful; that higher ethanol yields were obtained, and that the Taka-koji could be prepared much more cheaply than malt. However, distillers of potable alcohol have never adopted the process because of the belief that slight off-flavors or odors might result.

Recently the investigation of the use of mold amylase preparations to replace malt was taken up by Underkofler, Fulmer, and Schoene (1939). They grew two strains of Aspergillus oryzae on wheat bran in a rotating drum. The resulting material, mold-bran, was quite satisfactory for saccharifying corn mashes. The alcohol yields were found to be about 12 per cent better than with malt, under the same conditions.

Schoene, Fulmer, and Underkofler (1940) investigated various methods of preparing grain mashes for fermentation. Preliminary liquefaction of the mashes was accomplished by premalting and by cooking with dilute acids. The premalting technique was found to be satisfactory for 20 per cent mashes, but not for heavier ones. Mold-bran and soybean meal

were not as satisfactory as malt for this purpose. Mashes cooked for 30 minutes at 20 pounds per square inch steam pressure in the presence of hydrochloric acid were quite smooth and free of lumps. The 20 per cent mashes were adequately thinned by 0.02 to 0.03 normal acid, while 60 per cent mashes required 0.12 normal acid. All mashes were adjusted to pH 5.0 with ammonium hydroxide before the saccharifying agent was added. For saccharification, malt, mold-bran, and soybean meal were employed, alone and in combinations. Mold-bran was found to be most effective, while soybean meal was of little value. Combinations were apparently no more effective than mold-bran alone. An investigation was also made of the effectiveness of hydrochloric acid for complete saccharification of the starch, rather than mere liquefaction. About 90 per cent conversion of starch to sugar was apparently obtained, but ethanol yields from the fermentation were considerably lower than those from mashes saccharified by amylase, thus confirming earlier work by Severson (1937). However, the addition of mold-bran to acid-saccharified mashes resulted in normal ethanol yields; malt was ineffective for this purpose.

Banzon (1940) investigated the use of mold-bran as a saccharifying agent for the production of ethanol from cassava. The ethanol yields were found to be considerably better than when malt was used. The best results were obtained when the mold-bran was introduced into the mash at 30° C. The customary malting period at elevated temperatures could thus be eliminated, resulting in a saving of time, equipment, and power.

Goering (1941) investigated the use of mineral acids and of mold-bran as saccharifying agents for grain mashes and pure starch. He found

that ethanol yields were best when acid saccharification was supplemented by mold-bran. Acid hydrolyzed starch fermented much more completely than did acid hydrolyzed corn. This seemed to indicate that the acid hydrolysis of whole corn produced a substance which reduced ethanol yields.

Underkofler, Goering, and Bucksloo (1941) prepared mold-bran using several different species of mold, and compared the effectiveness of these preparations with that of malt. Saccharifying power as measured by the Lintner method indicated that malt was much richer in amylase; however, in fermentation tests the use of mold-bran always resulted in the higher ethanol yields. Apparently the true saccharifying power of mold-bran cannot be determined by the Lintner method.

Beresford and Christensen (1941) studied improved mashing procedures for the fermentation of both potatoes and grains. They reported that when cooked mashes were cooled to saccharification temperature (55° to 60° C.), "some hitherto unsuspected irreversible physical-chemical changes occurred in the starch." These changes were first discovered through measurement of mash viscosities after saccharification, and were confirmed by fermentation studies. It was reported that the irreversible changes could be prevented by cooling the mash immediately from 100° to 55° C., by mixing it rapidly with the appropriate quantity of cold water. Mashes treated in this manner fermented with the production of higher yields of ethanol, and required only about one half the usual quantity of mold-bran for saccharification. Another method was also found to be quite effective, namely, two-stage saccharification. The cooked mash at 100° C.

was mixed with sufficient cold water to bring the temperature to 80° C. One fourth of the total requirement of saccharifying agent was added at this temperature, and the mash could then be cooled slowly to 55° C. without the irreversible changes taking place. This method was used in a pilot plant with good results.

Hao (1942) made comparative tests on the saccharifying ability of mold-bran preparations produced by 27 strains of molds. Yields of ethanol of at least 90 per cent of theory were obtained from 20 per cent corn mash saccharified with mold preparations from 23 of the strains tested. Various strains of Aspergillus oryzae appeared to be most satisfactory, although certain species of Mucor and Rhizopus were also quite effective. The molds were cultivated on wheat bran in aluminum pots equipped for aeration. Growth of the molds in the pots was more rapid and uniform, and the amylolytic activities were greater than when grown in a rotating drum. Hao also made some brief tests on methods of thinning mashes for saccharification by mold bran. He found that premalting or premolding gave satisfactory results. Also, the addition of malt or mold-bran to the mash at 75° C. resulted in good ethanol yields, the mold-bran being slightly the better. The best method was found to be treatment with hydrochloric acid. The mash was cooked with 0.04 normal acid for 30 minutes, at a steam pressure of 20 pounds per square inch. After cooling, it was adjusted to a pH of 4.5 to 5.0 by the addition of concentrated sodium hydroxide. The mash was quite thin and easily saccharified by mold-bran. Ethanol yields as high as 95 per cent of theory were reported.

Gallagher, Bilford, Stark, and Kolachov (1942) have reported the development of a method for rapid saccharification of mashes, for use in a continuous process. Over 70 per cent of the cooked starch was converted to maltose by malt in one minute at 62.8° C. The ethanol yields obtained by the fermentation of such mashes were approximately 2 per cent higher than those obtained from mashes held for 30 to 60 minutes at 62.8° C. The mashes were sufficiently thin for pumping in a distillery.

Underkofler (1942) has reviewed the topic of microbial amylases and their application to the ethanol fermentation. The various applications of mold and bacterial amylases were discussed. It was stated that when malt and mold-bran are used under optimum conditions for each the alcohol yields obtained are essentially the same, based on total dry matter going into the process, with apparently a slight advantage for mold-bran. Mold-bran may be considerably better than malt, or vice versa, depending on the conditions used. The possible use of bacterial amylase in distillery practice was also discussed.

III. EXPERIMENTAL

A. Materials

1. Corn

The corn used in these investigations was the best quality corn obtainable on the local market. Two lots of the grain were used. The first lot was purchased already ground. It was thoroughly mixed, sampled, and stored in well-stoppered bottles. A test tube containing about 1 or 2 ml. of carbon disulfide was placed in each bottle to prevent the development of insects. This corn contained 58.3 per cent starch, or an equivalent of 64.8 per cent glucose. The method of analysis was the Official Diastase Method with Subsequent Acid Hydrolysis, of the Association of Official Agricultural Chemists (1940). The moisture content was 10.7 per cent. This corn will hereinafter be designated as Lot No. 1.

The second quantity of corn, Lot No. 2, was purchased in the form of shelled corn, and was ground to a fine meal in a burr mill. It was mixed, sampled, and bottled in the same manner as Lot No. 1. Upon analysis it was found to contain 62.4 per cent starch, or a glucose equivalent of 69.4 per cent. It contained 7.65 per cent moisture.

The two lots of corn were also analyzed by direct acid hydrolysis, also an official method of the Association of Official Agricultural Chemists. The glucose equivalents according to this method were 72.4 per

cent for Lot No. 1 and 72.2 per cent for Lot No. 2. These values were considerably higher than those determined by the diastase method. It is known that direct acid hydrolysis breaks down pentosans as well as starch, and therefore higher results must be expected by this method. Inasmuch as the corn was to be saccharified by diastase for fermentation by yeast, the values obtained by the diastase method of analysis were used as a basis for calculating theoretical yields of ethanol.

2. Barley malt

The barley malt was obtained from the Fleischmann Malting Company. It was ground to a meal and stored in a stoppered bottle. It has a Lintner value of 113.1. The moisture content was 7.56 per cent.

3. Mold-bran

The mold-bran used in this investigation was prepared by growing a strain of Aspergillus oryzae on wheat bran according to the method of Hao, Fulmer, and Underkofler (1943). It was air dried and passed through a Wiley mill, then stored in tightly stoppered bottles. The moisture content was 8.08 per cent.

4. Malt extract

The malt extract used to prepare beer wort for yeast cultures was Blue Ribbon Malt Extract. This product is made by the Pabst Brewing Company, Peoria Heights, Illinois.

B. Methods of Procedure

1. Microbiological procedures

a. Yeast culturing. The yeast cultures were carried on 10 per cent beer wort. To prepare the beer wort, 100 grams of malt extract were dissolved in hot water, and the volume was made up to one liter. The solution was brought to a boil, and the insoluble material was allowed to settle. The supernatant liquid was then placed in Erlenmeyer flasks: 30 ml. of the wort were used in each 50-ml. flask for carrying the cultures, and 350 ml. in each 500-ml. flask for cultures employed for inoculating experimental mashes. The flasks were plugged with cotton and sterilized for 30 minutes under a steam pressure of 15 pounds.

The yeast cultures used in this investigation were strains of Saccharomyces cerevisiae designated in this laboratory as yeast number 43 and yeast number 51. Each culture was transferred to beer wort in a 50-ml. flask and incubated at 30° C. for 24 hours. By means of sterile pipettes 1 to 3 ml. of these cultures were transferred to other flasks, and the subcultures incubated for the same length of time and at the same temperature. Transfers were made daily in this manner throughout the course of the investigation to keep the cultures in a vigorous condition.

The inoculum for experimental mashes was prepared by inoculating 350 ml. of beer wort with 5 to 8 ml. of a vigorous yeast culture and incubating at 30° C. for 24 hours.

b. Preparation of mold-bran. The mold-bran was prepared by the

method developed by Hao, Fulmer, and Underkofler (1943). The method was as follows: 750 grams of wheat bran were thoroughly moistened with 750 ml. of 0.3 normal hydrochloric acid. The moist bran was packed into an aluminum pot perforated in the bottom and equipped for aeration from the top. The pot was then placed in the autoclave and heated for 30 minutes at a steam pressure of 15 pounds. The bran was unpacked and spread out on a paper to cool to 30° C. It was then inoculated with a well-sporulated culture of Aspergillus oryzae designated in this laboratory as Number 38.

The inoculum was previously prepared in flasks by mixing 25 grams of wheat bran with 25 ml. of 0.3 normal hydrochloric acid in each 500-ml. Erlenmeyer flask. These flasks were plugged with cotton and sterilized in an autoclave for 30 minutes at 20 pounds steam pressure. After cooling to room temperature, the bran mixture in the flasks was inoculated from a stock culture of Aspergillus oryzae. The stock cultures were maintained on agar slants in a refrigerator. The cultures on the bran were incubated at 30° C. until well sporulated. These cultures were used to inoculate the bran in the aluminum pots, the inoculum being thoroughly mixed into the bran by carefully working the material with the hands.

After inoculation the bran was repacked into the pot, a thermometer inserted in the lid, and the pot placed in an incubator at 30° C., where it remained until the temperature rose to 35° C. The pot was then removed from the incubator and the contents aerated by alternately applying air pressure and suction. After 34 hours the growth was complete. The mold-bran was unpacked from the pot and spread out on paper to sporulate and to dry. When dry it was ground through a Wiley mill and was stored in tightly stoppered bottles.

c. Fermentation of experimental mashes. The experimental mashes contained 50 grams of ground corn in 225 ml. of water, corresponding to a 22.2 per cent mash. These were prepared according to the various mashing procedures described elsewhere in this thesis. The thinned mashes were always placed in 500-ml. Erlenmeyer flasks and were then saccharified by malt or mold-bran. The flasks were plugged with cotton and allowed to stand for a designated length of time, at a designated temperature to effect saccharification. The temperature was then adjusted to 30° C., and 20 ml. of a 24-hour yeast culture were added to each flask. The inoculum was mixed into the mashes by swirling the flasks, and all flasks were placed in an incubator at 30° C. Fermentation was allowed to continue for 80 to 90 hours. At the end of that period the mashes were analyzed for ethanol.

All fermentations were run in duplicate. The results listed in the tables are the averages of the two values obtained.

2. Analytical procedures

a. Determination of the starch content of the corn. The corn was analyzed for starch content by two methods, both of which were conducted in accordance with the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (1940). The first was the Official Diastase Method, followed by Acid Hydrolysis. The reducing substances formed in the hydrolysis were estimated according to a modification of the Shaffer and Somogyi method developed by Underkofler, Guymon, Rayman, and Fulmer (1943). The reagents were standardized by means of a sample of pure glucose.

The second method was by the Direct Acid Hydrolysis Procedure. Reducing sugars were estimated by the same procedure as was used in the diastatic analysis.

b. Determination of moisture. Moisture determinations were made by means of a "Moisture Teller," an apparatus manufactured by the Harry W. Dietert Company, Detroit, Michigan. The method consisted of subjecting a 5 gram sample to a stream of air at 240° F. for 15 minutes, and determining the loss in weight.

c. Determination of ethanol. The entire volume of fermented mash was placed in a Kjeldahl flask, together with approximately 0.5 gram of calcium carbonate and 2 or 3 drops of castor oil. The calcium carbonate was added to neutralize the acids present, and the oil to prevent excessive foaming. The mash was distilled, and the first 100 ml. of the distillate were collected in a 100-ml. volumetric flask.

The distillate was placed in a thermostat at 25° C. and allowed to attain that temperature. The specific gravity was then determined by means of a Chainomatic Westphal balance. The ethanol concentration in grams per 100 ml. of solution was read from an appropriate table.

d. Calculation of ethanol yield. In a typical experiment the mash contained the following: 50 grams of corn with a starch content of 62.4 per cent equivalent to 69.4 per cent glucose; one gram of malt; 2 grams of mold-bran; and 20 ml. of beer wort, introduced with the inoculum. The entire fermented mash was distilled, and the first 100 ml. of distillate were collected. The specific gravity ($25^{\circ}/25^{\circ}$) of the distillate was 0.9721, which corresponded to 17.43 grams of ethanol per 100 ml. of distillate.

Since ethanol was produced not only from the corn but also from the other constituents of the mash, it was necessary to apply corrections for the latter. Correction factors for malt, mold-bran, and inoculum were determined separately. The corrections were made as follows:

20 ml. of inoculum	=	0.59 gram ethanol
1 gram of malt	=	0.33 gram ethanol
2 grams of mold bran	=	0.24 gram ethanol
Total	=	1.16 grams ethanol

17.43 - 1.16 = 16.27 grams ethanol from corn

From the equation

$$C_6H_{12}O_6 \longrightarrow 2CO_2 + 2C_2H_5OH,$$

180 grams of glucose should yield 92 grams of ethanol. Thus 50 grams of corn should yield (50)(0.694)(92/180) = 17.74 grams of ethanol. The ethanol yield was therefore (16.27/17.74)(100) = 91.7 per cent of theory.

3. Viscosity measurements

Viscosities of mashes were measured following the period of saccharification, and preceding the addition of inoculum. All measurements were made at 30° C.

The apparatus used consisted of a 100-ml. pipette, from which the constricted tip had been removed. The pipette was clamped in a vertical position on a ring stand. The corn mash under examination was drawn into the pipette up to the graduation mark, and the drainage time was measured with the aid of a stop watch. The drainage time observed was a function of the viscosity of the mash. It was also a function of the density,

but as all mashes were the same concentration the density was assumed to be constant. The drainage time for distilled water at 30° C. was found to be 4.01 seconds. The drainage time for a given mash, divided by 4.01, was therefore expressed as the "specific" viscosity of the mash.

4. pH measurements

All pH measurements were made with the aid of a Cameron pH Meter. This instrument employs a glass electrode and operates on batteries. It was purchased from the Wilkens-Anderson Company of Chicago, Illinois.

C. Experimental Results

1. Investigation of quick cooling as a method for thinning corn mashes

a. General procedure. Beresford and Christensen (1941) have reported that cooked starch mashes are more fermentable if they are cooled rapidly from the cooking temperature to the saccharification temperature, than if they are allowed to cool slowly. They found that cooked mashes underwent some irreversible physical-chemical change when cooled slowly. This change affected both viscosities and fermentability. The effect was almost entirely eliminated if the cooked mashes were taken directly from the cooker at a temperature of 100° C., and mixed immediately, in a suitable mixer, with sufficient cold water, containing the saccharifying agent, to lower the temperature to 55° C. The rapid cooling resulted in an increase in ethanol yields, and decreased the amount of saccharifying agent required. The effect was the same for tubers and for all of

the common grains, but was more pronounced in the case of tubers.

In the present investigation the mashes were prepared for fermentation as follows: 50 grams of ground corn were placed in a 250-ml. beaker, and 100 ml. of hot water (about 90° C.) were added. The mixture was stirred vigorously to prevent lumping, and the corn gelatinized immediately. The beaker of mash was covered and cooked in an autoclave for one hour at a steam pressure of 15 pounds. The cooked mash was removed from the autoclave at 100° C. and quickly transferred to a Whiz Mixer, which contained 100 ml. of cold water and the desired amount of saccharifying agent. The diluted mash was mixed for two minutes, and then transferred to a 500-ml. Erlenmeyer flask. The mixer was rinsed with 25 ml. of water at 55° C., and the rinsings were added to the mash in the flask. The temperature of the mash was about 55° C. at this point.

The mash was allowed to stand at either 55° C. or 30° C. for a specified length of time, in order that saccharification might take place. All mashes were then brought to 30° C., inoculated with yeast culture, and incubated at 30° C. After about 85 hours they were analyzed for ethanol.

Since it was not possible to make a quantitative transfer of the cooked corn from the beakers to the mixer, a correction had to be made for the amount lost. The residue in the beaker was found to be about 0.2 gram when dried. Correction was made by preparing the mash from 50.2 grams of corn instead of 50 grams.

b. <u>The effect of quick cooling upon ethanol yields</u>. In order to determine whether there are advantages in quick cooling of corn mashes,

it was necessary to compare ethanol yields from quickly cooled mashes with yields from mashes which had been allowed to cool slowly.

One series of mashes was prepared by the quick-cooling procedure previously described using malt as the saccharifying agent. Another series was gelatinized and cooked in the same manner, but the beakers of mash were cooled to 55° C. by allowing them to cool slowly in the autoclave until the temperature was between 60° and 70° C., then placing them in a malting bath at 55° C. When the mashes reached this temperature, they were mixed with the requisite amounts of malt and 100 ml. of water at 55° C., and held at this temperature for one hour before inoculation. In all other respects both series were treated in the same manner.

The yeast culture used for both series was Saccharomyces cerevisiae number 43. For both series malt was used for saccharification, and the saccharification period was one hour at 55° C. The results are shown in Table 1.

The data in Table 1 show that quick cooling of mashes after cooking leads to slightly higher ethanol yields. The differences were small at some concentrations of malt, but were significant when taken as a whole. The advantageous effect of quick cooling was especially noticeable when only 4 per cent malt was used. This malt concentration was sufficient to give maximum yields when the mashes were cooled rapidly, whereas 6 per cent malt was required for maximum yields when the mashes were cooled slowly.

Table 1

Ethanol Yields from Slowly-Cooled and Quickly-Cooled Mashes

Malt, g. per 100 g. corn*	Ethanol yields, % of theory	
	Slowly-cooled mashes	Quickly-cooled mashes
2	----	76.0
4	79.8	84.3
6	82.8	84.6
8	82.3	84.0
10	81.8	84.7
12	83.5	84.1

*Corn from Lot No. 1.

c. The effect of variation of the length of time of saccharification upon ethanol yields. It is a well-known fact that malt amylase is much more active at 55-65° C. than at the fermentation temperature. It is therefore common practice to hold the mash at "malting temperature" for a period of time in order to promote saccharification. The following experiments were carried out to determine how long the quickly-cooled mashes should be held at 55° C. in order to secure maximum ethanol yields. The first series was held at this temperature only for the 2 minutes during which the mashes remained in the mixer. The warm mashes were then poured into flasks and cooled immediately to 30° C. in cold water. The other series were held at the saccharification temperature for 1, 2, and 3 hours. Yeast culture number 43 was used in all cases. The results are shown in Table 2.

An examination of the data of Table 2 reveals that quickly-cooled mashes do not require long saccharification periods at high temperatures under the conditions used. A period of only 2 minutes at 55° C. gave the best ethanol yields. A period of 3 hours was better than 1 or 2 hours. It is interesting to note that in three of the four series shown in Table 2, a malt concentration of only 4 per cent was sufficient. In commercial practice it is customary to use 10 to 12 per cent.

d. The use of mold-bran for saccharifying quickly-cooled mashes. Beresford and Christensen (1941) have reported that rapidly cooled mashes may be saccharified about equally as well by green malt and mold-bran, if each is used at its own optimum concentration. This series of experiments was designed to demonstrate the optimum conditions for saccharification by mold-bran.

Table 2

Ethanol Yields from Quickly-Cooled Mashes Saccharified by Malt

Malt, g. per 100 g. corn*	Ethanol yields, % of theory, when saccharified at 55° C. for			
	2 minutes	1 hour	2 hours	3 hours
2	79.3	76.0	75.4	78.5
4	85.6	84.3	84.0	86.4
6	85.5	84.6	83.0	83.8
8	85.7	84.0	83.7	86.2
10	87.1	84.7	84.1	84.3
12	87.2	84.1	83.0	86.2

*Corn from Lot No. 1.

The mashes were prepared by the quick-cooling procedure, and the mold-bran was stirred in at 55° C. The concentration of mold-bran ranged from 1 to 6 per cent of the total weight of corn used. The yeast culture used was Saccharomyces cerevisiae number 43. The alcohol yields obtained are presented in Table 3.

It is evident from the data of Table 3 that long periods of saccharification at 55° C. were not beneficial to the action of mold-bran. A period of 2 minutes at 55° C., using 3 per cent mold-bran, gave a yield of 83.3 per cent, whereas the highest yield obtained was only 84.5 per cent, with a 3 hour saccharification period and using 6 per cent mold-bran. A period of one hour was definitely poorer than 2 minutes. A 3 hour period was the best when the highest concentrations of mold-bran were used, but very poor with low concentrations.

A comparison of Tables 2 and 3 reveals the fact that malt was more effective than mold bran in the saccharification of quickly-cooled corn mashes for fermentation by yeast. This is of interest in view of the fact that Underkofler (1942) has recently pointed out that mold-bran is superior to malt under various other conditions. It is entirely possible that a temperature of 55° C., even though for only 2 minutes, may partially inactivate the mold-bran, as Banzon (1940) and Hao (1942) have shown that best results are obtained when the mold-bran is mixed into the mash at 30° C. However, it would not be practical to cool the mashes in the present type of experiment to 30° C. before adding the mold-bran, as the mashes would then be too thick to handle. Mashes containing less than 2.5 per cent mold-bran were extremely thick even though they were mixed at 55° C.

Table 3

Ethanol Yields from Quickly-Cooled Mashes Saccharified by Mold-Bran

Mold-bran, g. per 100 g. corn*	Ethanol yields, % of theory, when saccharified at 55° C. for			
	2 minutes	1 hour	2 hours	3 hours
1.0	71.6	61.7	70.0	63.5
1.5	80.1	74.3	79.3	74.7
2.0	82.0	76.4	83.3	78.8
2.5	82.7	76.6	83.1	79.5
3.0	83.3	78.7	82.7	83.3
3.5	83.3	80.2	84.4	83.8
4.0	81.8	80.8	84.2	84.2
6.0	82.7	80.9	82.7	84.5

*Corn from Lot No. 1.

e. The use of different yeast cultures in the fermentation of quickly-cooled mashes. Since different strains of yeast vary widely in their ability to produce alcohol from saccharified grains, it was thought advisable to test a yeast culture other than strain number 43. For this work a strain of Saccharomyces cerevisiae known as number 51 in this laboratory was chosen. The previous experiments using malt and mold-bran and varying the saccharification period were repeated, using culture number 51. The results are tabulated in Tables 4 and 5.

Ethanol yields were not greatly altered by changing to yeast number 51. However, certain differences in the two strains are apparent. Table 4 shows that culture number 51 produced most ethanol from mashes which had been saccharified at 55° C. for one hour, and that the optimum malt concentration was 6 per cent. It has already been noted that strain number 43 produced maximum yields from mashes saccharified for 2 minutes or for 3 hours, with an optimum malt concentration of 4 per cent. The former strain gave slightly higher yields, but required more malt.

The results shown in Table 5 indicate that yeast number 51 did not ferment the mashes saccharified by mold-bran very satisfactorily, although the maximum yields were slightly higher than those obtained with yeast number 43. Unlike the latter culture, number 51 was benefited by saccharification of the mashes at 55° C., as evidenced by the gradual increase of yields with saccharification time. It may be concluded that mold-bran is inferior to malt for the saccharification of mashes thinned only by rapid cooling.

Since culture number 51 gave slightly better ethanol yields from

Table 4

Ethanol Yields from Quickly-Cooled Mashes Saccharified by Malt and Fermented by Yeast Strain Number 51

Malt, g. per 100 g. corn*	Ethanol yields, % of theory, when saccharified at 55° for			
	2 minutes	1 hour	2 hours	3 hours
2	80.8	81.8	75.7	79.2
4	85.3	87.1	82.6	83.6
6	86.1	87.7	86.6	84.0
8	84.7	86.8	83.5	83.2
10	83.7	86.7	83.3	83.4
12	84.5	87.6	84.1	85.4

*Corn from Lot No. 1.

Table 5

Ethanol Yields from Quick-Cooled Mashes Saccharified by Mold-Bran and Fermented by Yeast Strain Number 51

Mold-bran, g. per 100 g. corn*	Ethanol yields, % of theory, when saccharified at 55° C. for			
	2 minutes	1 hour	2 hours	3 hours
1.0	65.7	69.5	74.9	72.6
1.5	65.9	76.7	81.8	81.5
2.0	71.9	82.8	82.2	83.8
2.5	73.4	80.5	82.8	84.7
3.0	77.8	83.2	84.0	85.3
3.5	79.3	83.9	82.9	84.6
4.0	80.1	81.6	84.3	85.3
6.0	83.2	82.4	83.8	85.2

*Corn from Lot No. 1.

mashes saccharified by either malt or mold-bran than did number 43, the former strain was used in all subsequent fermentations.

2. Two-stage mashing of corn mashes for fermentation by yeast

a. General procedure. It has been reported by Beresford and Christensen (1941) that potato mashes were readily thinned by adding a portion of the saccharifying agent at elevated temperatures. The potato mashes were quickly cooled by adding them to sufficient water in a mixer to reduce the temperature to 80° C. The cooling water contained one fourth of the total requirement of saccharifying agent. The viscosities of the mashes were reduced by this treatment, and the mashes were then cooled slowly to 55° C. before the remainder of the saccharifying agent was added.

Experiments on the two-stage mashing of corn mashes were carried out by methods similar to those of Beresford and Christensen. Fifty and two tenths grams of ground corn were placed in a 400-ml. beaker and gelatinized by the addition of 200 ml. of hot water. The beaker of mash was covered, and cooked in an autoclave for one hour at a steam pressure of 15 pounds. It was removed from the autoclave at 100° C., and the mash was quickly transferred to a Whiz Mixer. Mixing was started at once, and the temperature immediately dropped to about 85° C. and continued to fall slowly. As soon as the mash reached the desired temperature, the requisite amount of malt or mold-bran for the first stage of saccharification was added, and mixing was continued for one minute. The mash was then cooled to 55° C. by immersing the bowl of the mixer in cold water. The remainder of the saccharifying agent was added and mixed with the

mash for two minutes. The contents of the mixer were then poured into a 500-ml. Erlenmeyer flask. The mixer was rinsed with 25 ml. of water, and the rinsings were added to the mash. The flask was plugged with cotton and allowed to stand for one hour at either 55° C. or 30° C., to permit saccharification to proceed. The inoculum was added at 30° C., and the mash was allowed to ferment at that temperature for about 85 hours. It was then analyzed for ethanol.

The first stage of two-stage mashing is referred to as "thinning"; the second stage is true saccharification. The purpose of the first stage is to thin the mash so that it may be easily handled and more easily attacked by amylase during saccharification. The enzymes of the thinning agent may be expected to be very largely destroyed by the heat, but must remain active long enough to partially break down the starch molecules.

b. The use of mold-bran for thinning. In spite of the fact that mold-bran is quite heat-sensitive, there was a possibility that it would retain its activity long enough at elevated temperatures to effect thinning of the mash. Preliminary tests indicated that concentrations of mold-bran as high as 2 per cent, when added to mashes at 80° C., did not thin them perceptibly. A series of mashes was then prepared in which 3 per cent mold-bran was used for thinning at 75° C., and 3 to 10 per cent mold-bran was used for saccharification. The mashes were mixed with the initial charge of mold-bran in the mechanical mixer, and poured while hot into Erlenmeyer flasks. The flasks were cooled directly to 30° C. in cold water. This treatment caused the mashes to solidify, indicating that the mold-bran had had little or no effect. The saccharifying charge

of mold-bran was then added and stirred as thoroughly as possible into the gel-like mashes with a heavy stirring rod. The flasks were maintained at 30° C. for one hour. The mashes had then thinned considerably, but were still so thick that viscosity measurements could not be made with the pipette apparatus at hand. They were inoculated and fermented in the usual manner. The ethanol yields are listed in Table 6.

The ethanol yields were surprisingly good, in spite of the original high viscosities of the mashes. The highest ethanol yield was 90.4 per cent of theory, at a mold-bran concentration of 6 per cent. However, the process must be considered impractical because of the difficulties involved in handling the viscous mashes.

c. The use of malt for thinning. In this series of investigations 1, 2, and 3 per cent malt were used for thinning. The thinning temperature employed was 80° C. Following the thinning process, the mashes were cooled to 55° C., and mixed with from 2 to 6 per cent mold-bran for 2 minutes in the mixer. They were then poured into Erlenmeyer flasks and cooled to 30° C. After one hour yeast inoculum was added.

It was found that malt added at 80° C. was quite effective in thinning the mashes. This was plainly evident from the fact that the motor of the electric mixer speeded up perceptibly within 15 seconds after the dry malt had been added to the mash being mixed. The mashes were quite thin and poured readily, especially when thinned with 2 or 3 per cent malt. Ethanol yields are presented in Table 7.

The use of one per cent malt for thinning resulted in fairly good ethanol yields. Yields were higher than in the previously described

Table 6

Ethanol Yields from Mashes Thinned by 3% Mold-Bran at 75° C.

Mold-bran for saccharification, g. per 100 g. corn*	Ethanol yields, % of theory
3	85.3
4	89.1
5	89.7
6	90.4
8	89.7
10	89.3

*Corn from Lot No. 2.

Table 7

Ethanol Yields from Mashes Thinned by Malt at 80° C.

Mold-bran for saccharification, g. per 100 g. corn*	Ethanol yields, % of theory, using		
	1% malt	2% malt	3% malt
2.0	79.4	81.4	83.5
2.5	85.6	86.2	87.1
3.0	83.7	87.0	85.1
3.5	83.8	88.3	86.1
4.0	88.4	89.1	89.8
5.0	87.2	91.5	90.2
6.0	89.4	91.4	91.1

*Corn from Lot No. 1.

quick-cooling investigations, the highest being 89.4 per cent, at a concentration of 6 per cent mold-bran. Two per cent malt was even more effective and yields were higher in every case. A yield of 87.0 per cent was obtained at a mold-bran concentration of only 3 per cent, while the yield was 91.5 per cent at a concentration of 5 per cent mold-bran. The yield at 3 per cent mold-bran may be considered satisfactory for such a small amount of saccharifying material. However, it must be borne in mind that an additional 2 per cent of malt had been used for thinning. The 91.5 per cent yield is far superior to any obtained in the previous quick-cooling experiments. Three per cent malt proved to be no better for thinning than was 2 per cent, in fact was apparently not quite as good since ethanol yields were slightly lower.

The high fluidity of the mashes and the high ethanol yields indicate that two-stage mashing might be used to good advantage. Two per cent malt was used for the first stage in most of the following experiments, since this had been found to result in maximum yields.

d. Effect of the second-stage mixing temperature upon mashes saccharified by mold-bran. It was considered possible that the two-minute mixing period at 55° C. might be eliminated without ill effect, as the mashes were sufficiently thin for handling at 30° C. A series of mashes was prepared and thinned with 2 per cent malt as before. However, instead of cooling the mashes to 55° C. in the mixer, they were poured while hot into the fermentation flasks, and cooled to 30° C. in cold water. The proper amount of mold-bran was then added to each flask and mixed with the mash by swirling the flask. The inoculum was added

after one hour. A similar series was prepared in which 3 per cent malt was employed for thinning. This was deemed advisable inasmuch as a mash mixed at 30° C. is likely to have a higher initial viscosity than one mixed at 55° C.; a greater amount of thinning agent might therefore be beneficial. A third series was prepared in which 2 per cent malt was used for thinning, and in which the mold-bran was mixed into the mashes at 55° C. This third series repeated one of those listed in Table 7, except that the corn was from a different lot. In all three series the thinning temperature was 80° C.

The results, presented in Table 8, show that mixing at 55° C. was not only unnecessary, but was undesirable. Mixing at 30° C. resulted in yields about 2 or 3 per cent higher, when all other factors remained the same. A yield of 91.1 per cent was obtained, when only 4 per cent mold-bran was employed. Increasing the proportion of mold-bran did not appreciably increase the alcohol yields.

Of the two series mixed at 30° C., the one thinned with 2 per cent malt gave about 2 per cent better ethanol yields than that thinned with 3 per cent malt. This agrees well with the data on similar series in Table 7. Obviously, cooling the mashes to 30° C. before adding the mold-bran did not make necessary the use of more malt in the thinning stage.

e. The effect of the thinning temperature upon ethanol yields. The temperature at which the thinning charge of saccharifying agent is added is of considerable importance. The rate of enzyme action is certain to be increased by an increase in temperature; therefore, it is desirable to add the malt at as high a temperature as possible. On the other hand,

Table 8

Effect of the Temperature of Second-Stage Mixing upon Ethanol Yields

Mold-bran for saccharification, g. per 100 g. corn*	Ethanol yields, % of theory		
	Mixed at 55° Thinned by 2% malt	Mixed at 30° Thinned by 2% malt	Mixed at 30° Thinned by 3% malt
3.0	87.0	----	84.5
3.5	88.4	87.2	86.7
4.0	88.3	91.1	88.4
4.5	89.1	89.4	89.4
5.0	88.6	91.5	89.4
6.0	88.4	91.1	89.4

*Corn from Lot No. 2.

the rate of enzyme destruction by heat is also increased by an increase in temperature, and must eventually, at some temperature, become great enough to prevent any thinning action whatever. Therefore, there must exist an optimum temperature for thinning. Beresford and Christensen (1941) have reported that the optimum for potato mashes is between 75° and 80° C. In order to determine the optimum for corn, three series of fermentations were employed, in which the mashes were thinned at 70°, 75°, and 85° C. respectively. In all cases mold-bran was mixed into the mashes at 30° C. The ethanol yields are listed in Table 9. The series thinned at 80° C., as presented in Table 8, is repeated in Table 9 for purposes of comparison. All series were thinned by 2 per cent malt. Best ethanol yields were obtained from mashes thinned at 75° C. The best yield was 91.5 per cent of theory, at 5 per cent mold-bran. However, in this series all yields were 90 per cent or better except those from mashes containing only 3 per cent mold-bran. In the case of mashes containing as much as 4 per cent mold-bren, yields were almost identical with those obtained from similar mashes thinned at 80° C. When 85° C. was used as the thinning temperature, the mashes were not thinned appreciably. They were extremely viscous when cooled to 30° C. Also, the ethanol yields were decidedly poorer, the best being 88.2 per cent. The mashes thinned at 70° C. gave rather good yields, but definitely not as good as those thinned at 75° C. A yield of 90.5 per cent was obtained at a mold-bran concentration of 6 per cent, but in all other cases yields were between 88.0 and 89.8 per cent.

Table 9

Influence of the Temperature of the First Stage Mixing in Two-Stage Mashing

Mold-bran for saccharification, g. per 100 g. corn*	Ethanol yield, % of theory, when thinned at			
	70°	75°	80°	85°
3.0	88.0	88.4	----	----
3.5	89.5	90.0	87.2	84.8
4.0	89.9	90.5	91.1	87.2
4.5	89.8	91.1	89.4	88.2
5.0	89.6	91.5	91.5	87.3
6.0	90.5	91.1	91.1	86.4

*Corn from Lot No. 2.

The above data point to the conclusion that the optimum temperature for thinning corn mashes with malt is between 75° and 80° C.; these two temperatures are about equally satisfactory. The effectiveness of the thinning action does decrease sharply at 85° C., however. At thinning temperatures lower than the optimum, ethanol yields decrease rather gradually.

f. The use of malt for saccharification. In this investigation malt was used in both stages of the two-stage mashing. Two per cent malt was used for thinning at 80° C., and 2 to 12 per cent for the second stage, at 55° C. In one series the flasks of mash were malted at 55° C. for one hour. In the second series they were cooled to 30° C. immediately after the two-minute mixing period at 55°. The ethanol yields are presented in Table 10.

The ethanol yields did not compare favorably with those obtained when mold-bran was used. An examination of Tables 9 and 10 reveals that the use of malt resulted in yields about 2 to 10 per cent lower than when mold-bran was used. The maximum yield, using malt, was 88.2 per cent of theory, at a malt concentration of 8 per cent for the second-stage saccharification. It has already been pointed out that the use of mold-bran resulted in maximum yields when the mold-bran concentration was 4 to 5 per cent. Thus the use of malt not only resulted in poorer ethanol yields, but necessitated the use of much larger proportions of the saccharifying agent.

A period of 2 minutes saccharification at 55° C. was found to be preferable to a period of one hour at that temperature. Ethanol yields

Table 10

Ethanol Yields from Mashes Thinned by Malt at 80° C., and Saccharified by Malt

Malt for saccharification, g. per 100 g. corn*	Ethanol yield, % of theory, when saccharified at 55° for	
	One hour	Two minutes
2	78.0	75.6
4	86.3	75.8
6	86.1	87.2
8	86.2	88.2
10	85.9	87.7
12	84.8	86.4

*Corn from Lot No. 2.

were about 2 per cent higher in the former series. However, it should be pointed out that malting for a full hour decreased by one half the quantity of malt required to give maximum yields; only 4 per cent malt gave the best yields.

3. Mineral acids as thinning agents for corn mashes

a. General procedure. Schoene, Fulmer, and Underkofler (1940) have shown that the preliminary liquefaction of a 20 per cent corn mash may be accomplished by cooking it with 0.02 to 0.03 normal hydrochloric acid. Mashes treated in this manner were smooth and liquid upon saccharification and highest yields of ethanol were obtained when the cooked mashes were adjusted to pH 5.0 before saccharification. Hao (1942) used 0.04 normal hydrochloric acid to liquefy 20 per cent corn mashes.

In the present investigation two modifications of the acid-thinning procedure were used. The first method was similar to that used by the above-mentioned investigators. Fifty grams of ground corn were placed in a 500-ml. Erlenmeyer flask and 225 ml. of 0.04 normal acid were added at about 90° C. The mixture was stirred vigorously and the corn gelatinized promptly. The flask was covered, and heated in an autoclave for one hour at 15 pounds steam pressure. It was then allowed to cool to the desired saccharification temperature, either 55° C. or 30° C. The mash was adjusted to pH 5.0 by the addition of sodium carbonate solution or solid calcium carbonate. When a series of mashes was to be adjusted to this pH at 55° C., one flask was cooled to 30° C. and the amount of neutralizing agent necessary to make the adjustment was care-

fully measured. The same amount was then added to each of the other flasks. After the pH of the mash had been adjusted, the saccharifying agent was added. The flask was plugged with cotton and was allowed to stand at the chosen saccharifying temperature for one hour. The temperature of the mash was then brought to 30° C., and 20 ml. of yeast culture were added. The mash was incubated at 30° C. for about 85 hours, and was then analyzed for ethanol.

In the second type of acid-thinning experiment, rapid cooling of the cooked mash was employed. The mash was prepared as follows: 50 grams of corn were placed in a 250-ml. beaker, and 100 ml. of 0.08 normal acid at about 90° C. were added, with rapid stirring. The corn gelatinized immediately. The beaker was covered and heated in an autoclave at a steam pressure of 15 pounds for one hour. It was removed from the autoclave at 100° C., and the mash was quickly transferred to a Whiz Mixer which contained 100 ml. of cold water and a sufficient amount of a neutralizing agent to bring the pH to 5.0. It was necessary to have determined the correct amount of neutralizing agent previously. The mash was mixed for about one minute, and the temperature was found to be about 55° C. From this point on the procedure varied somewhat, according to the type of experiment. The saccharifying agent was added, either at 55° C. or at 30° C., and saccharification was allowed to proceed for one hour. All variations in procedure have been noted in the description of each experiment. The mash was inoculated, incubated, and analyzed in exactly the same manner as were the slowly cooled mashes.

b. The fermentation of slowly cooled mashes. In this experiment

both hydrochloric and sulfuric acids were used. Mold-bran was employed for saccharification. All pH adjustments were made with a concentrated solution of sodium carbonate. There was no saccharification period at 55° C., the mold-bran being added at 30° C. The mashes proved to be quite thin and free of lumps. The ethanol yields are given in Table 11.

The ethanol yields were quite good, as compared with those obtained using other thinning methods. Hydrochloric acid was the better of the two acids, resulting in a yield of 92.8 per cent at a mold-bran concentration of 4 per cent. In the case of sulfuric acid, the maximum yield was 90.8 per cent at a 4 per cent mold-bran concentration. In general, yields were about 2 per cent higher when hydrochloric acid was used. These findings are in accord with those of Goering (1941), who reported that hydrochloric acid is more effective than sulfuric acid in the complete hydrolysis of corn starch.

c. The effect of rapid cooling of acid-thinned mashes. It was considered possible that rapid cooling of acid-thinned mashes would result in improved saccharification, and hence higher yields. Since quick cooling must be accomplished by the addition of cold water to the hot mash, it was necessary to cook the corn in half the usual volume of acid, as previously described. Thus the mash during cooking consisted of 50 grams of corn in 100 ml. of acid, instead of 50 grams of corn in 225 ml. of acid, as in the previous experiments. It was therefore necessary to use stronger acid to thin this thicker mash. A concentration of 0.08 normal acid was used, and was found to thin the mashes satisfactorily.

Both hydrochloric and sulfuric acids were used as thinning agents

Table 11

Ethanol Yields from Mashes Thinned with 0.04 Normal Acids and Allowed to Cool Slowly

Mold-bran, g. per 100 g. corn*	Ethanol yields, % of theory	
	Thinned with 0.04 N HCl	Thinned with 0.04 N H_2SO_4
1.5	73.9	74.3
2.0	77.6	78.9
2.5	84.5	82.3
3.0	88.3	86.8
4.0	92.8	90.8
6.0	92.7	90.0

*Corn from Lot No. 2.

for testing the effect of quick cooling. Sodium carbonate solution was used to adjust the pH of the mashes to 5.0. Mold-bran was used as the saccharifying agent, and was mixed into the mashes at 30° C.

The data in Table 12 show that ethanol yields were definitely increased by cooking the mashes with 0.08 normal acid and cooling them rapidly, as outlined above. The maximum yield, 93.8 per cent of theoretical, was obtained from mashes thinned with hydrochloric acid and saccharified by 3.5 per cent mold-bran. Although this yield was only one per cent higher than the maximum obtained from the mashes cooked with 0.04 normal acid and cooled slowly, the increased yields are particularly evident from those mashes containing lower proportions of mold-bran. Mashes containing only 2 per cent mold-bran gave a yield of 90.3 per cent of theoretical, when thinned with 0.08 normal hydrochloric acid and cooled rapidly. The corresponding yield from mashes thinned with 0.04 normal acid and cooled slowly was only 77.6 per cent. The improvement was also quite marked in mashes thinned with sulfuric acid. This acid was as good as hydrochloric acid for the "quick-cooling process," whereas it was shown to be inferior for the "slow-cooling method."

It was necessary to ascertain whether the advantageous effects of the "quick-cooling procedure" were due to the quick cooling itself or to the different cooking method. A series of mashes was therefore prepared using 0.08 normal hydrochloric acid and allowing the mashes to cool slowly to 30° C. They were then mixed with water at 30° C., and with sufficient sodium carbonate solution to raise the pH to 5.0. Mold-bran was added, and after one hour the mashes were inoculated. The results of this series are shown in Table 13.

Table 12

Ethanol Yields from Mashes Thinned with 0.08 Normal Acids and Cooled Rapidly

Mold-bran, g. per 100 g. corn*	Ethanol yield, % of theory	
	Thinned with 0.08 N HCl	Thinned with 0.08 N H_2SO_4
2.0	90.3	88.7
2.5	92.5	91.8
3.0	92.7	93.2
3.5	93.8	93.5
4.0	93.8	93.1
6.0	93.6	93.4

*Corn from Lot No. 2.

Table 13

Ethanol Yields from Mashes Cooked with 0.08 Normal Hydrochloric Acid and Allowed to Cool Slowly

Mold-bran, g. per 100 g. corn*	Ethanol yields, % of theory
2.0	86.9
2.5	91.2
3.0	91.3
3.5	93.3
4.0	92.9
6.0	92.4

*Corn from Lot No. 2.

The data presented in Table 13 indicate that ethanol yields were not significantly lowered by allowing the mashes cooked with 0.08 normal acid to cool slowly. A comparison of the data in Tables 11, 12, and 13 shows that, although the "quick-cooling procedure" using 0.08 normal acid for thinning resulted in much better yields than did the "slow-cooling procedure" employing 0.04 normal acid for thinning, the better yields were due to the method of cooking used, rather than to the rate of cooling of the mashes.

d. The use of calcium carbonate for neutralizing acid-thinned mashes. Calcium carbonate is a cheaper and more convenient neutralizing agent than is sodium carbonate. It has a particular advantage in that it is insoluble in neutral solution, and therefore a slight excess can be added without ill effects. In order to test the effects of calcium carbonate upon ethanol yields, two series of mashes were prepared, using the "quick-cooling procedure" and employing 0.08 normal hydrochloric and sulfuric acids respectively for thinning. Exactly 0.45 gram of calcium carbonate was added to the water used for the rapid cooling of each mash. This amount of calcium carbonate raised the pH to only 4.5 instead of the 5.0 desired, but it was found by experimenting with test flasks that the addition of larger amounts would not increase the pH further. The corn apparently acted as a buffer, since a mixture of 100 ml. of 0.08 normal hydrochloric acid and 0.45 g. of calcium carbonate had a pH of 5.7. The fermentation results for this series are presented in Table 14.

In no case were yields as good when calcium carbonate was used as when sodium carbonate was employed. This is apparent from a comparison

Table 14

Ethanol Yields from Mashes Thinned by Acids and Neutralized by Calcium Carbonate

Mold-bran, g per 100 g. corn*	Ethanol yields, % of theory	
	Thinned by 0.08 N HCl	Thinned by 0.08 N H_2SO_4
2.0	89.2	88.5
2.5	91.3	89.9
3.0	92.3	90.6
3.5	92.7	90.1
4.0	92.5	90.8
6.0	92.5	92.3

*Corn from Lot No. 2.

of the data in Tables 14 and 12. The differences were not appreciable in the case of mashes thinned by hydrochloric acid. However, in the case of those thinned by sulfuric acid, the use of calcium carbonate decreased the yields by 1 to 3 per cent. Although such a decrease may be considered significant, the advantages of calcium carbonate as a neutralizing agent might well outweigh this disadvantage.

e. The use of malt for saccharifying acid-thinned mashes. For this series of experiments the mashes were prepared exactly as for saccharification by mold-bran, as previously described. However, the malt was added at 55° C., and the mashes were kept at that temperature for one hour. They were then cooled to 30° C. and inoculated. Both hydrochloric and sulfuric acids were used, and both the "slow-cooling method" using 0.04 normal acids for thinning and the "quick-cooling procedure" using 0.08 normal acids for thinning were investigated. All mashes were adjusted to pH 5.0 with sodium carbonate solution. The ethanol yields are listed in Table 15.

None of the yields were satisfactory, the highest being only 83.3 per cent of theoretical. Hydrochloric acid was much better than sulfuric acid as a thinning agent. Thinning the mashes with 0.08 normal acids and quick cooling was quite advantageous. All the mashes were quite fluid before inoculation, but apparently they were not completely fermentable. Thinning with the aid of mineral acids proved to be undesirable when malt was used as the saccharifying agent.

f. The effect of the mixing temperature upon ethanol yields from acid-thinned mashes. The temperature at which the saccharifying agent

Table 15

Ethanol Yields from Acid-Thinned Mashes Saccharified by Malt

Malt, g. per 100 g. corn*	Ethanol yields, % of theory			
	Slow cooling		Quick cooling	
	Thinned by 0.04 N HCl	Thinned by 0.04 N H_2SO_4	Thinned by 0.08 N HCl	Thinned by 0.08 N H_2SO_4
2	69.8	63.3	----	----
4	73.0	65.6	80.6	74.7
6	76.8	67.2	83.3	78.8
8	76.0	69.2	82.0	78.1
10	75.9	68.2	81.7	77.8

*Corn from Lot No. 2.

is mixed into the mash was shown to be of importance in the experiments on two-stage mashing. Similar experiments were therefore carried out to test this effect in the case of acid-thinned mashes. To test the effect upon saccharification by mold-bran, mashes were prepared by thinning with 0.08 normal hydrochloric acid and cooling the mashes rapidly. The pH was adjusted with sodium carbonate solution. The mold bran was mixed with the mashes at 55° C. for 2 minutes, which were then cooled immediately to 30° C. The ethanol yields from mashes prepared in exactly the same manner but mixed at 30° C. have already been listed in Table 12. The yields from both series are presented in Table 16.

The mashes mixed at 55° C. all gave lower yields than did the corresponding mashes mixed at 30° C. The differences were about one to three per cent. This indicates that in the case of acid-thinned mashes, as well as those thinned by malt at 80° C., mold-bran should be added at 30° C. rather than at a higher temperature.

In testing the effect of the mixing temperature upon the action of malt, mashes thinned by 0.04 normal hydrochloric acid were employed. These mashes were not cooled rapidly. The pH was adjusted to 5.0 with sodium carbonate solution. The mashes were allowed to cool to 30° C. before the malt was added. The ethanol yields are shown in Table 17, together with those of a series prepared in exactly the same manner but mixed at 55° C., and maintained at that temperature for one hour. The latter data are repeated from Table 15.

A saccharification period at 55° C. was definitely beneficial to the action of malt upon acid-thinned mashes. Omission of this malting period resulted in a decrease in yields of from 4 to 10 per cent.

Table 16

Influence of the Mixing Temperature upon Ethanol Yields from Acid-Thinned Mashes Saccharified by Mold-Bran

Mold-bran, g. per 100 g. corn*	Ethanol yields, % of theory	
	Mixed at 30° C.	Mixed at 55° C.
2.0	90.3	89.4
2.5	92.5	91.3
3.0	92.7	90.8
3.5	93.8	90.4
4.0	93.8	91.3
6.0	93.6	91.8

*Corn from Lot No. 2.

Table 17

Influence of the Mixing Temperature upon Ethanol Yields from Acid-Thinned Mashes Saccharified by Malt

Malt, g. per 100 g. corn*	Ethanol yields, % of theory	
	Mixed at 30° C.	Saccharified at 55° C. for 1 hour
2	63.3	69.8
4	67.2	73.0
6	66.7	76.8
8	72.3	76.0
10	70.6	75.9

*Corn from Lot No. 2.

4. Viscosities of mashes thinned by various methods

A good thinning method must not only attack the starch so that it is readily saccharified, but must also produce a fluid mash that is easily handled and pumped in the distillery plant equipment. In order to obtain an indication of the liquefying power of the different thinning methods, viscosity measurements were made on the mashes of certain representative series before inoculation. Only enough measurements were made to give a general idea of the efficiency of the liquefaction process.

Viscosity measurements were made by the process previously described. Although the apparatus was somewhat crude, it was found that the viscosity of a given mash could be checked quite closely, whereas there was often considerable difference between the viscosities of duplicate flasks. Therefore the method was more than sufficiently accurate for this type of work. Values which appear to be out of line are due to variability of the mashes rather than to lack of precision of the measurements. The method had the limitation that viscosities of very thick mashes could not be measured.

The viscosities of mashes thinned by quick cooling are presented in Table 18. It can be seen that viscosities decreased with an increase in saccharification time. This was to be expected, as diastasis is a relatively slow process. It should be noted that mold-bran produced thinner mashes after one hour at 30° C. than after one hour at 55° C., a fact which once more demonstrated that mold-bran should be used at 30° C. Malt produced much thinner mashes than did mold-bran. The

Table 18

Specific Viscosities of Mashes Thinned by Quick Cooling

Per cent saccharifying agent	Saccharified by mold-bran				Saccharified by malt		
	2 minutes at 55°, 1 hour at 30°	1 hour at 55°	2 hours at 55°	3 hours at 55°	1 hour at 55°	2 hours at 55°	3 hours at 55°
1.0	----	10.6	9.4	7.8	----	----	----
1.5	----	12.5	12.9	7.8	----	----	----
2.0	----	15.2	11.9	7.4	12.8	10.0	5.3
2.5	----	14.6	16.2	11.0	----	----	----
3.0	----	12.9	14.8	8.0	----	----	----
3.5	10.0	13.8	11.1	13.6	----	----	----
4.0	8.7	12.6	9.6	4.9	11.8	7.0	4.1
6.0	9.1	15.5	15.5	12.2	8.8	6.2	4.8
8.0	----	----	----	----	8.0	6.0	4.5
10.0	----	----	----	----	6.8	5.2	3.8
12.0	----	----	----	----	7.1	5.4	4.0

thinnest mashes were those containing the most malt. In the case of mold-bran, the thinnest mashes were frequently those containing the least mold-bran. Viscosities increased, then decreased, and finally increased again as the amount of mold-bran was increased. This effect was probably due in part to the absorption of water by the bran. Quick cooling alone, without the addition of a saccharifying agent, did not thin the mashes sufficiently so that the viscosities could be measured.

A series of mashes prepared exactly as the preceding ones, but allowed to cool slowly, had the viscosities shown in Table 19.

Table 19

Specific Viscosities of Mashes Allowed to Cool Slowly*

Percent malt	Viscosity
4	8.6
6	7.8
8	8.6
10	8.6
12	9.9

*Saccharified by malt for one hour at 55° C.

The mashes were saccharified by malt for one hour at 55° C. When these viscosities are compared with those of the rapidly cooled mashes which were malted for one hour (data in Table 18), it is demonstrated that

the slowly cooled mashes were not as a whole more viscous than the quickly cooled ones. At 4 and 6 per cent malt, the former were considerably the thinner, while at 8, 10, and 12 per cent malt the reverse was true. Obviously, the rapid cooling of a corn mash thins it little, if at all.

Mashes prepared by two-stage mashing were generally quite fluid. The only mashes of this type which were too thick to be handled in plant equipment in a distillery were those thinned by mold-bran at 75° C., and those thinned by malt at 85° C. Data on three series of mashes are listed in Table 20. The mashes saccharified by mold-bran were mixed at 30° C., and viscosity measurements were made one hour later. The malt series was saccharified for one hour at 55° C., then cooled to 30° C. before the viscosities were measured. All three were thinned with 2 per cent malt in the first stage.

The data in Table 20 show that the mashes in the malt series were unusually thick, while those saccharified by mold-bran were quite thin. Apparently the thinning treatment with malt at 70-80° C. affects the starch in such a way that it is readily attacked by mold-bran. In the series thinned at 70° C., one mash had a specific viscosity of 13.8 after it was cooled to 30° C. and before any mold-bran was added; such a mash could be readily handled in distillery plant equipment. When 3.5 per cent mold-bran was added, the viscosity immediately increased to 19.8. One hour later, it was 5.8.

The acid-thinned mashes were by far the thinnest of all those prepared. They were quite fluid in all cases. Table 21 shows the viscosities of some mashes saccharified by mold-bran. All three of these

Table 20

Specific Viscosities of Mashes Prepared by Two-Stage Mashing*

Per cent saccharifying agent, second stage	Specific viscosities when saccharified by		
	Malt Thinned at 80° C.	Mold-bran Thinned at 70° C.	Mold-bran Thinned at 75° C.
0.0	----	13.8	----
3.5	----	5.8	6.8
4.0	15.3	12.8	9.2
4.5	----	7.2	21.5
5.0	----	7.1	----
6.0	17.3	----	----
8.0	18.1	----	----
10.0	10.5	----	----
12.0	9.5	----	----

*2% malt used in the first stage.

Table 21

Specific Viscosities of Acid-Thinned Mashes Saccharified by Mold-Bran

Per cent mold-bran	Thinned by: Cooled:	0.08 N H_2SO_4 rapidly	0.08 N HCl rapidly	0.08 N HCl slowly
0.0		2.6	2.1	2.1
2.0		2.3	----	----
2.5		2.3	----	2.0
3.0		2.4	2.1	1.9
3.5		2.4	2.2	1.9
4.0		2.2	2.1	2.2
6.0		2.1	2.2	2.0

series were thinned with 0.08 normal acid, neutralized, cooled to 30° C., and mixed with mold-bran. Viscosities were determined after one hour. The mashes cooked with hydrochloric acid were a little thinner than those cooked with sulfuric acid. The mashes which were allowed to cool slowly were slightly thinner than those which were cooled rapidly, indicating that quick cooling did not have any thinning action. In most cases the mashes were almost as thin before any mold-bran was added as they were afterwards.

In Table 22 are listed the viscosities of the acid-thinned mashes saccharified by malt. Data are given on both hydrochloric and sulfuric acids, and slow and rapid cooling. All mashes were saccharified at 55° C. for one hour before the viscosities were measured, except those to which no malt was added at all. The mashes which cooled slowly are shown to be less viscous than those cooled rapidly. The differences cannot be ascribed to the method of cooling, however, as the quick-cooling method made it necessary to cook the mash at twice the desired concentration of corn, and in twice the normal concentration of acid, so that cold water could be added for cooling. Hydrochloric acid gave slightly thinner mashes than did sulfuric acid in the slow-cooling process, but slightly thicker ones in the quick-cooling process. Unlike mold-bran, the malt caused the acid-cooked mashes to become thinner in most cases.

It has not been possible to find any correlation between the viscosities, as measured, and the fermentability of the mashes, either within a series or in the comparison of different series. In some instances the least viscous mashes gave the highest ethanol yields, but frequently

Table 22

Specific Viscosities of Acid-Thinned Mashes
Saccharified by Malt

Per cent malt	Thinned by: Cooled:	0.04 N HCl slowly	0.04 N H_2SO_4 slowly	0.08 N HCl rapidly	0.08 N H_2SO_4 rapidly
0		2.2	1.9	2.1	2.6
2		1.3	1.4	---	---
4		1.3	1.4	---	---
6		1.3	1.4	1.9	1.7
8		1.3	1.3	2.0	1.8
10		1.3	1.4	2.0	1.8

the most viscous ones gave the best yields. The thinnest mashes observed were those thinned by 0.04 normal hydrochloric acid, and saccharified by malt. The highest yield of ethanol from this series was 76.8 per cent. The thickest mashes dealt with were those referred to in the discussion of two-stage saccharification, in which mold-bran was used for both stages. Although these mashes were heavy pastes and entirely too thick to be handled in distillery plant equipment, they fermented readily and gave ethanol yields of from 85.3 to 90.4 per cent of theoretical.

From the standpoint of the production of a fluid mash, thinning by means of acid was the best method investigated. Mashes thinned by 2 per cent malt at 70-80° C. were satisfactorily thin. Quick cooling of the mashes did not thin them perceiptibly; however, mashes prepared by this method were sufficiently thin after a period of saccharification.

IV. DISCUSSION

In the present investigation all ethanol yields have been expressed as per cent of the theoretical yield, the latter figure being based on the starch content of the corn as determined by the Official Diastase Method followed by Acid Hydrolysis previously referred to. It is believed that this method of expressing yields furnishes the best basis for a comparison of experimental results, especially if there is to be a comparison of yields from mashes prepared from different grains, or grains from different sources. It is common practice for commercial producers of alcohol to express their yields in the form of gallons (or proof gallons) of ethanol per bushel of grain, or as gallons per 100 pounds of dry grain. The latter figure is preferred, and is a convenient unit for commercial usage, when the analysis of the grain is not known. However, this figure will obviously vary with the starch content of the grain. Another method of expressing yields that is sometimes used is as per cent of theory, based on the starch content as determined by acid hydrolysis of the grain. Analysis by acid hydrolysis is much more rapid and convenient than by diastase hydrolysis. However, acid treatment of whole grain hydrolyzes pentosans, and the apparent starch content is too high. Moreover, grains from different sources vary in their content of pentosan, so that the error is by no means a constant one.

Two different lots of corn were used in the present investigation, the analyses of which varied appreciably. In Tables 7 and 8 are recorded the results of two experiments performed in exactly the same

manner, but using different corn. Each series of mashes was prepared by the two-stage mashing procedure, using 2 per cent malt for thinning and 3 to 6 per cent mold-bran for saccharification. The data for yields from these two experiments are repeated in Table 23, expressed in the three ways discussed above.

The data in Table 23 illustrate clearly the fact that the method of expressing experimental results may affect considerably the conclusions reached. When yields were recorded as per cent of theory on the basis of diastase analysis, results were practically the same from the two lots of corn, except at mold-bran concentrations of 5 and 6 per cent. However, yields as per cent of theory on the basis of acid hydrolysis of the corn were widely different for the two series. This was probably due to the fact that the corn of Lot Number 1 contained more pentosan than that of Lot Number 2, thus introducing a larger error into the calculation of the theoretical yield. Yields calculated as proof gallons of ethanol per 100 pounds of dry corn agree somewhat more closely, but are consistently higher for corn from Lot Number 2, except at mold-bran concentrations of 5 and 6 per cent. One would expect this to be the case, as this corn contained the higher percentage of starch. On the basis of the above comparisons, it is believed that the ethanol yields as calculated throughout this thesis provide a true measure of the effectiveness of the mashing procedure used, regardless of whether the corn was from Lot 1 or Lot 2.

The experiments on the rapid cooling of mashes have shown that the process was somewhat beneficial when used with malt. However, it was

Table 23

Ethanol Yields from Mashes Prepared from Different Lots of Corn, and Calculated by Different Methods

Mold-bran, g. per 100 g. corn	Ethanol yields					
	% of theory, diastase analysis		% of theory, acid analysis		Proof gallons per 100 pounds corn*	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
3.0	87.0	87.0	77.9	83.5	9.75	10.07
3.5	88.3	88.4	79.0	84.9	9.88	10.22
4.0	89.1	88.3	79.8	84.8	9.98	10.21
5.0	91.5	88.6	81.8	85.0	10.23	10.23
6.0	91.4	88.4	81.7	84.9	10.22	10.22

*Dry weight basis.

entirely ineffective when used with mold-bran, as evidenced by thick mashes and low ethanol yields. It may be that a portion of the amylase of the mold-bran was destroyed at 55° C., the temperature at which the mold-bran was mixed into the mash. Only a portion of the amylase could have been destroyed, as continued heating at 55° C. for 1 to 3 hours did not result in appreciably lower yields, indicating that saccharification had continued to take place. The reason for poor ethanol yields appeared to be incomplete liquefaction; the ultimate cause may only be surmised.

It did not appear to be necessary to hold the quickly-cooled mashes at 55° C. for long periods of time in order to obtain the maximum action of the malt enzymes. Saccharification for only 2 minutes at 55° C. appeared to be sufficient in most cases though not always optimum. The optimum saccharification period varied with the yeast culture used for fermentation.

Two-stage mashing was found to be quite satisfactory, using malt for thinning and mold-bran for saccharification. Mold-bran was entirely without effect as a thinning agent at 75° C. Apparently the heat inhibited all enzyme action. Mold-bran was much better than malt for saccharification, once the mashes had been thinned. The best temperature for thinning with malt appeared to be between 75° and 80° C. Below this range the action was less complete, while at 85° C. there was very little thinning action at all. At 85° C. the rate of enzyme destruction by heat was so much greater than the rate of enzyme action that the starch paste did not appear to be attacked at all. The temperature at which the mold-

bran for saccharification was mixed into the mashes was found to have an influence upon ethanol yields. A temperature of 30° C. was better than 55° C. This demonstrates once again that mold-bran should not be used at high temperatures.

In the case of mashes thinned by malt at 80° C. and saccharified by malt, a saccharification period of 2 minutes at 55° C. was found to give somewhat higher ethanol yields than did a period of one hour at 55° C. However, when the longer saccharification period was employed, much less malt was necessary to obtain maximum yields. Eight per cent malt gave the highest yields from mashes saccharified for 2 minutes, while 4 per cent proved to be optimum in the case of mashes saccharified for one hour. Whereas malting for only two minutes would save considerable time and heat in a distillery, and would result in higher yields, malting for a full hour would effect a very considerable saving in malt. Which process would be preferable in distillery plant practice would depend upon economic considerations.

Mashes thinned with dilute mineral acids were quite fluid, and produced high ethanol yields when saccharified by mold-bran and fermented by yeast. However, mashes thinned in this manner gave poor yields when malt was used as the saccharifying agent. The acid apparently affects starch in such a manner that the malt enzymes cannot convert it completely to fermentable sugars, whereas the enzymes of mold-bran are able to do so. The same situation is encountered when starch is completely hydrolyzed by acid and the hydrolyzate is fermented, as has been shown by the work of Schoene, Fulmer, and Underkofler (1940) and of Goering (1941).

The above-mentioned investigators found that acid-hydrolyzed mashes fermented poorly, and that the addition of malt did not increase the ethanol yields; however, the addition of mold-bran resulted in good yields of ethanol. This phenomenon may be explained by the fact that acid acts upon starch to produce dextrose, and upon dextrose to form disaccharides and polysaccharides. Berlin (1926) and Coleman, Buchanan, and Paul (1935) have shown that the unfermentable disaccharide, gentiobiose, is found in considerable quantities in hydrol, the mother liquor from the commercial manufacture of crystalline dextrose by acid hydrolysis of starch. Other unfermentable carbohydrates, largely unidentified, are also present. Apparently mold-bran contains enzymes which are capable of breaking down these reversion products, while malt does not.

It was found that cooking a 50 per cent corn mash with 0.08 normal acid, then diluting it to 22 per cent, was preferable to cooking a 22 per cent mash with 0.04 normal acid. The stronger acid gave better liquefaction, and resulted in higher ethanol yields. Cooking the mash at a concentration of 50 per cent corn would have an additional advantage from a commercial standpoint, as it would reduce the volume of the mash cooked and thus reduce considerably the cost of cooking and cooling the mash. Rapid cooling of acid-thinned mashes was found to have little effect upon ethanol yields. This is not surprising, as "irreversible changes" in the starch would not be so likely to occur during the cooling of partially hydrolyzed mashes.

Acid-thinned mashes were more completely saccharified by mold-bran when the latter was added at 30° C., rather than at 55° C. Malt was more

effective when added to the mashes at 55° C. and allowed to stand for one hour at that temperature, than when added at 30° C. This illustrates the difference in the manner in which heat affects the action of the two materials, mold-bran and malt. Malt amylase is more active at 55° C., while mold amylase is sufficiently active at 30° C.

Since ethanol yields are not ordinarily calculated as per cent of theory in commercial practice, some of the best yields obtained in this investigation have been calculated on other bases. In Tables 24, 25, and 26 are listed the best yields obtained from mashes thinned by acid, two-stage mashing, and quick cooling, respectively, calculated as per cent of theory, as proof gallons of ethanol per 100 pounds of dry corn, and as per cent of the total dry matter in the original mash. The latter two methods of computing yields take into account the moisture content of the substrate, but not the starch or sugar content. Yields based on the total weight of dry matter present include the alcohol derived from the malt, mold-bran, and inoculum, while these quantities are deducted in the other two methods of calculation.

The ethanol yields shown in Table 24 were the highest obtained from any series in this entire investigation. The mashes were prepared by cooking the corn, at a 50 per cent mash concentration, with 0.08 normal hydrochloric acid, and then diluting to a 22 per cent mash concentration and adjusting the pH to 5.0 with sodium carbonate solution. Mold-bran was added at 30° C. Not only did this process result in the highest ethanol yields obtained, but on a commercial scale it would also have the advantage previously mentioned, that it would require only half the

Table 24

Ethanol Yields from Mashes Thinned with 0.08 Normal Hydrochloric Acid and Saccharified by Mold-Bran, Calculated by Different Methods

Mold-bran, g. per 100 g. corn*	Ethanol yields		
	% of theory	Proof gallons per 100 pounds dry corn	% of total dry matter
2.0	90.3	10.5	34.6
2.5	92.5	10.7	35.4
3.0	92.7	10.7	35.3
3.5	93.8	10.9	35.6
4.0	93.8	10.9	35.5
6.0	93.6	10.8	35.2

*Corn from Lot No. 2.

Table 25

Ethanol Yields from Mashes Thinned with 2% Malt at 75° C. and Saccharified by Mold-Bran, Calculated by Different Methods

Mold-bran, g. per 100 g. corn*	Ethanol yields		
	% of theory	Proof gallons per 100 pounds dry corn	% of total dry matter
3.0	88.4	10.3	33.4
3.5	90.0	10.5	34.4
4.0	90.5	10.5	34.6
4.5	91.1	10.6	34.6
5.0	91.5	10.6	34.6
6.0	91.1	10.6	34.3

*Corn from Lot No. 2.

Table 26

Ethanol Yields from Quickly-Cooled Mashes Saccharified by Malt, Calculated by Different Methods

Malt, g. per 100 g. corn*	Ethanol yields		
	% of theory	Proof gallons per 100 pounds dry corn	% of total dry matter
2	81.8	9.16	31.0
4	87.1	9.77	32.8
6	87.7	9.83	33.2
8	86.8	9.72	32.9
10	86.6	9.70	32.9
12	87.6	9.81	33.2

*Corn from Lot No. 1.

cooker volume and would bring about a large saving in cooking and cooling costs. However, it would have the disadvantage that acid-resistant equipment would be required.

Table 25 shows the best yields obtained from mashes prepared by two-stage mashing. Two per cent malt was used for thinning, at 75° C. The mold-bran was added at 30° C. These yields were quite satisfactory, though a little lower than those in Table 24. All yields in this series were well over 10 proof gallons per 100 pounds of dry corn. The two-stage mashing procedure should offer no particular difficulties on a commercial scale. It would make possible a very large saving in malt, if mold-bran were made available in large enough quantities for commercial use.

Where malt alone was used for saccharification, quick cooling was found to be superior to acid thinning, but not to two-stage mashing. The best yields obtained by this process are given in Table 26. This series of mashes was saccharified at 55° C. for one hour. The yields were considerably lower than those shown in Tables 24 and 25. Yields expressed as proof gallons per 100 pounds of dry corn and as per cent of total dry matter are particularly lower, as the corn from Lot Number 1 contained more unfermentable matter than did that from Lot Number 2. The quick-cooling process, though the poorest of the processes investigated, might possibly be of value so long as mold-bran is unavailable to the distilling industry.

V. SUMMARY AND CONCLUSIONS

1. An investigation has been made of three methods of preparing corn mashes for fermentation by yeast: (1) rapid cooling of the mashes, (2) two-stage mashing, and (3) thinning by means of mineral acids.

2. Rapid cooling of corn mashes from 100° C. to 55° C. resulted in an increase in ethanol yields of from 0.6 to 4.5 per cent when the mashes were saccharified by malt, and reduced the quantity of malt required by about one third.

3. The optimum length of time for the saccharification of rapidly-cooled mashes by malt at 55° C. varied with the yeast culture used for fermentation. Saccharomyces cerevisiae number 43 produced most ethanol from those mashes saccharified for either 2 minutes or 3 hours, while Saccharomyces cerevisiae number 51 produced highest yields from mashes saccharified for 1 hour. Differences were small, and a two-minute saccharification period was satisfactory for either yeast culture.

4. Malt was superior to mold-bran for the saccharification of quickly-cooled mashes.

5. The quick-cooling process was the poorest of the three mashing procedures investigated. The mashes were not well thinned by the cooling, and fermented to give comparatively low ethanol yields. The highest yield was 87.7 per cent of theory, from mashes saccharified for one hour at 55° C. by a malt concentration of 6 per cent of the weight of the corn.

6. In the two-stage mashing procedure, the corn mashes were adequately thinned by adding a small amount of malt to the mashes at 70° to 80° C. Two grams of malt per 100 grams of corn were found to be better than one or 3 grams of malt. Mold-bran had no thinning effect when used in this manner.

7. Best results using the two-stage mashing procedure were obtained when thinning took place at 75° or 80° C. Mashes thinned at 70° C. gave slightly lower ethanol yields upon fermentation, while those treated at 85° C. were quite viscous and fermented poorly.

8. Mold-bran was better than malt for the saccharification stage of two-stage mashing, and resulted in about 3 per cent higher yields of ethanol. A quantity of mold-bran equal to 5.0 per cent of the weight of corn resulted in an ethanol yield of 91.5 per cent of theoretical. The best yield using malt was 88.2 per cent of theoretical, at a malt concentration of 8.0 per cent.

9. The two-stage mashing process would probably be satisfactory in commercial practice, as the mashes are quite fluid and ethanol yields are high. This process was found to be the best of the three investigated for use with malt, but not for mold-bran.

10. Both hydrochloric acid and sulfuric acid, at a concentration of 0.04 normal, thinned 22 per cent corn mashes readily upon cooking for one hour at 15 pounds per square inch steam pressure. The resulting mashes were adjusted to pH of 5.0 with sodium carbonate solution before saccharification. Mold-bran was far superior to malt for the saccharification of these mashes.

11. Mashes thinned with 0.04 normal hydrochloric acid fermented to give about 2 per cent higher yields of ethanol than did those thinned with 0.04 normal sulfuric acid.

12. Fifty per cent corn mashes were cooked with 0.08 normal hydrochloric acid or sulfuric acid for one hour at a steam pressure of 15 pounds per square inch, and were then diluted to concentrations of 22 per cent corn. These mashes were quite fluid. They were adjusted to a pH of 5.0 before saccharification. Rapid cooling of these mashes did not prove beneficial.

13. Mashes thinned with 0.08 normal acids gave rise to higher ethanol yields than did those thinned with 0.04 normal acids, and required a smaller amount of saccharifying agent for saccharification.

14. Mold-bran was superior to malt for the saccharification of mashes thinned with 0.08 normal acids. The highest ethanol yield obtained was 93.8 per cent of theory, from mashes thinned with hydrochloric acid and saccharified by 3.5 per cent mold-bran.

15. Sulfuric acid proved to be as effective as hydrochloric acid at a concentration of 0.08 normal, in the case of mashes saccharified by mold-bran. Hydrochloric acid was much the better for mashes saccharified by malt, and resulted in about 5 per cent higher ethanol yields.

16. Calcium carbonate was tested as a neutralizing agent for mashes thinned with 0.08 normal acids, and was found to raise the pH to only 4.5. Fermentation results with calcium carbonate as neutralizing agent were only slightly poorer than with sodium carbonate in the case of mashes thinned with hydrochloric acid, but ethanol yields were about 3

per cent lower with calcium carbonate as neutralizing agent than with sodium carbonate when sulfuric acid was used for thinning.

17. Mashes thinned with 0.08 normal hydrochloric acid, adjusted to pH 5.0 with sodium carbonate, and saccharified by mold-bran were quite fluid and fermented to give higher yields of ethanol than mashes treated by any other mashing procedure investigated. Yields were about 2 per cent higher than any produced by two-stage mashing, and about 6 per cent higher than the highest yield obtained from quickly-cooled mashes.

18. Mold-bran saccharified the corn mashes more effectively at 30° C. than at 55° C., in both the two-stage and acid-thinning procedures. Malt was more effective at 55° C. than at 30° C., as shown by its action upon acid-thinned mashes.

19. No correlation was found between the viscosity of a saccharified mash and its fermentability by yeast.

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