Effect of salt concentrations upon the fermentation of grain mashes

Frank Major Shipman
Iowa State College

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UMI
EFFECT OF SALT CONCENTRATIONS UPON THE
FERMENTATION OF GRAIN MASHES

By

Frank Major Shipman

A Thesis Submitted to the Graduate Faculty
for the Degree of
DOCTOR OF PHILOSOPHY
Major Subject Chemical Engineering

Approved:

Signature was redacted for privacy.

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Iowa State College

1938
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INTRODUCTION
The object of the work presented in this thesis is to determine the effects of various chemical compounds upon the fermentation of grain mash. The compounds of most interest are those that are normally found in the natural limestone waters, although some other chemicals are also given consideration.

Any study of the fermentation of grain mash must take into consideration all factors involved, which include the grains, and their chemical and physical characteristics; the enzymes present in the grains and the effect of chemical compounds upon them; the chemical compounds in the waters used; yeast, its growth and various functions, with the effect of chemicals upon these functions; and the possible resolution of these findings to a practical application.

The actual research work could not cover all of the many factors which would naturally be encountered in so complex a mixture as grain mash. Therefore, it was limited to the over-all effect, which was considered from the standpoint of the yield of alcohol, as well as the concentration of certain groups of compounds that are normally distilled over with the alcohol-water mixture.

The history and general discussion are presented with the hope that they may be of value to those interested in this field of work.
HISTORICAL
The early history of fermentation, as a means of producing spirituous beverages, is lost in antiquity. Fermentation is as old as vegetation, and its use by man goes back to the beginnings of recorded time.

The early Egyptians had their wines and something resembling a beer. Practically all native tribes, when they have been discovered, have had some type of alcoholic beverage made from a local grain or fruit. The Chinese are supposed to have had a knowledge of distillation and its utilization long before the birth of Christ.

Although wines and beers were known in western Europe during its early history, the art of distillation was not introduced until the alchemical period. It is these pseudo-scientists who developed the first efficient pieces of chemical equipment, among them the still. In the hands of the early craftsmen, this equipment was used to make a product known as "spirit of the fruit". This was really a brandy or rum.

Although fermentation had intrigued the scientists since the Sixteenth Century, the scientific knowledge of fermentation was very meager up to the last half of the Nineteenth Century. During this period, yeast had been taken from the field of chemical reagents and many different yeasts had been isolated. Fermentation of sugars had been shown to be due to a catalytic agent, an enzyme, which was produced
It was called "The American Druggist," a famous
pharmaceutical publication, and one of the first American
books ever published. "In 1804, while fermentation was being taken from the
palm trees to be made into bread, some palm trees were encountered, and on some of the
palm trees, the starchy parts of the plants were destroyed. By the latter part of the
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Or, The Theory and Practice of Distilling. The importance which he placed upon this publication is shown in that he dedicated it to Thomas Jefferson, then President of the United States, in the following words:

"To His Excellency Thomas Jefferson, Esquire, President:

In dedicating this work to you I had two important objects in view — I was anxious to avail myself of the protection which the name Jefferson, so eminently distinguished as the parent of his country's welfare, and the munificent patron of the sciences, could afford to a work of this kind, as one whose fostering care this work looks up to as a safeguard against its falling into the general wreck of oblivion, to which the frowns of envy, malice or neglect, may wish to consign it.

I was also desirous under your excellency's patronage, to recommend the investigation and pursuit of the objects contained in this treatise to the immediate attention of the patriotic and scientific, as opening a vast field for the agriculturist to transport his superfluous grain in a different form to a market, to which the raw materials could never get.

I have the honor to be your Excellency's most humble servant,

Michael Krafft,

May 25, 1804."
Thus, it can be seen that two groups were simultaneously working towards the development of the fermenting industries: the scientists and those men actually engaged in the production of spirits. These two groups were far apart and had little use for each other. It was not until the early part of the Twentieth Century that the scientist was called into the practical field. He first entered the field of brewing, and in 1914 - 1917 he entered the distilled spirits field. Today, the whole field of the fermenting industries is being controlled by the technically trained man who is rapidly applying to practice many of the theories and facts that have been developed in the research laboratories.

The history of the effects of chemical substances upon fermentation covers such a wide variety of subjects that it must be divided into groups based upon the raw materials or upon the effects of chemicals on certain reactions which are common to all yeast fermentations.
WATER
The quality and composition of the water used in the fermenting industries have long been considered of prime importance. In the early days there was no explanation of why a certain water was selected, and many stories are told of plants that were moved to localities which had better water supplies. It was known that the water used influenced the yield of fermented products as well as the flavor which was secured. Consequently, the industries segregated themselves in those localities having a plentiful supply of water which had proven satisfactory.

Only in later years has there been a concerted effort on the part of the scientists to give a full explanation of the effects of the chemical and biological composition of waters on fermentations. Much of this work has been done in studying water in its relation to the brewing industry. The importance of water composition and control in the distillery has been summarized by R. Deplanque (126).

Not only has the original composition of the water been studied but also methods of purification and sterilization have been perfected and classified. W. Kluger (303), in his studies on brewing waters, recommended precipitation of organic colloids with aluminium sulfate followed by filtration through sand filters. He also found that chlorination was best for biological purification while
the "Katadyn" method could be used only with limitation, as it had an unfavorable effect upon the yeast and enzyme actions.

There is a considerable difference of opinion regarding the effects of various chemical compounds, normally present in water, upon the production of malt and in brewing.

E. Jalowetz (232) found little difference in the action of lime and gypsum steep waters on the extract, acidity, and proteins of the finished malt.

W. Duncan (134) found in brewing that calcium salts lower the extract considerably when correction is made for the salts. This is caused by the precipitation of mineral matter, chiefly the phosphates.

A. Ball (31) found that excessive concentrations of magnesium sulfate, chlorides, carbonates, nitrites, nitrates, and ammonia cause trouble in brewing, but small amounts are beneficial.

W. Windisch (637) stated that good drinking water may be a poor brewing water. He found that ammonia, nitrogen pentoxide, sodium chloride, and sodium sulfate have little effect in low concentrations but that calcium carbonate and magnesium carbonates play an important role. He assumed that the necessary calcium and magnesium salts
could be secured from the grains. W. Windisch (640) further found that in the evaluation of waters it is necessary to know in which combinations the magnesium and calcium salts are present as well as their ratio to each other. Certain concentrations are permissible while others are harmful.

It is common practice in all the fermenting industries to first decarbonize the water by heating. W. Windisch (651) found this operation to be retarded by the presence of carbon dioxide and the decarbonization is best when the ratio of calcium sulfate to magnesium carbonate is four to one. W. Windisch (645) showed the action of salts in water to be two-fold: first, they cause a change in the acidity and pH value, and second, they react with the salts of the grains. A high magnesium content precipitates the phosphates causing bad results in fermentation.

P. Kolbach (309) found the permissible concentration of calcium sulfate in brewing liquor to be from 30 to 175 grams per hecto-liter. Calcium sulfate lowers the pH value and phosphate content of the wort. K. Maurer (379), using synthetic waters, found the most favorable action of calcium sulfate at a concentration of 0.6 gram per liter. E. Herman (260) stated that waters containing calcium carbonate and magnesium carbonate have little effect on the extract of beer and that addition of calcium sulfate increases the
yield only at a temperature of 70° C.

F. Pott (486) found that solid calcium carbonate reduces the acidity of the wort about 50 per cent. He also found that calcium carbonate has no effect upon the aroma or saccharification. With bicarbonate, the acidity of the wort is decreased, saccharification retarded, and aroma affected.

The alkalinity of the water used and of the resulting mash appears to play an important part. G. Stricht (581) found that the carbonates and bicarbonates decrease the extract in the wort, decrease the maltose, increase the dextrin content, increase the time for saccharification and reduce the soluble and amino nitrogen in the wort. W. Windisch (642) showed that calcium sulfate, calcium carbonate, and magnesium salts in high concentrations have a bad effect upon the resulting beer, causing darkening and a decrease of yield. Using brew water with equivalent alkalinity, E. Noufang (404) found that the degree of acidity of the wort depends upon the mashing methods employed.

Today it is an accepted fact that small concentrations of various minerals are necessary for enzyme action and fermentation. E. Bekaert (40) showed that a high iron content in the brewing water is removed during the brewing process. This may be partially due to its utilization but is probably due largely to its removal by the grains. A small
concentration of iron was found to be beneficial.
H. Lüers (355) investigated the effect of silicic acid and found 1-2 parts per 100,000 to be harmless, while 4-10 parts per 100,000 deteriorate the yeast and retard fermentation.

F. Emslander (141) compared distilled water and untreated water in their effect on the wort. He found that the untreated water decreases the maltose and proteins while it increases the dextrin, the color of the wort, the time for saccharification, the viscosity, and the fermentation. H. Heron (262) summarized the action of inorganic salts from water as having a pronounced effect on the flavor, influencing enzymic conversion by disturbing the acid-base equilibrium in mashing and having an effect upon the fermentation.

Although there seems to be much difference of opinion regarding the exact effects of mineral waters on the products of the fermenting industries, it is agreed that the minerals present in the water play an important role. The varied results secured by different investigators are probably due to the fact that the research work has been done under different conditions, using different raw materials and yeasts.

The practices used in handling the water in the distilling
industry today are based upon economics and conservation of heat energy. The water that is to be used for mashing is first used in the condensers. This heats the water to about 180°F., and the water is then run to storage tanks and saved for the mashing operation. This preheating in open top condensers carries out the decarbonization process.

Waters are selected for the fermenting and distilling industries not only for their mineral content, but also for their biological analysis. In most cases the water used is secured from deep wells but, where these are not available, the water is secured from artificial lakes and reservoirs. Where this surface water is used, the yield of alcohol appears to be a function of the rainfall. The yield of alcohol decreases during the rainy seasons and increases again during the dry seasons.

The waters from the limestone regions of Kentucky have long been considered as the best available for the fermenting industries. This is probably due to the fact that these waters as a rule contain a high percentage of calcium and phosphates with a relatively low concentration of magnesium, silicates, and iron.
RAW MATERIALS
There is a number of raw materials that is used in the fermenting industries. These may be divided into two general classifications: Group I, Those materials containing soluble sugars; Group II, Those materials containing starch.

Group I. Materials containing soluble sugars
Sugar cane molasses
Sugar beet molasses
Other sugar wastes
Fruits and fruit juices

Group II. Materials containing starch
Corn, rye, barley, wheat,
barley malt, etc.
Potatoes, etc.

Very little need be said about those materials containing soluble sugars, as their handling is principally fermentation preceded by an adjustment of mineral content, acidity, and pH value.

The raw materials of most interest are the cereal grains common to the United States, namely, corn, rye, and barley. When these grains are mentioned, it is generally believed that each grain has certain unalterable characteristics regardless of strain used, climatic conditions during the growing season, or the soil on which the grain was grown. This
is a mistaken idea, as grains of the same general classification will vary widely in both chemical and physical characteristics.

J. Maschaupt (374) investigated the nitrogen and ash content of various grains grown in different soils and found the structure as well as the constituents to vary. O. Lemmermann (335), in studying the effect of various compounds upon the utilization of ammonia and nitrates by grains, found that calcium carbonate and barium carbonate increase the formation of protein from ammonium sulfate, while they decrease the protein formation when sodium nitrate is used. Magnesium carbonate and ferric hydroxide lessen the formation of protein with both sources of nitrogen. Much work has been done on the individual grains and will be considered under their respective headings.

While the greater part of the work has been done upon the content of starch, protein, fat, enzymes, and ash in the grain upon fermentation and its products, little has been done upon the effect of various other compounds that are present in small percentages. These compounds may play a rather important part in the yields and products that are secured. E. Nelson (421) found the following acids to be present in maize, oats, and rye - aconitic, malic, citric, and oxalic. Tricarballylic acid was found in maize and malonic in oats.
CORN

This important raw material belongs to the family known as Zea mays. The species used in the fermenting industries are the giants grown in the United States. Originally, there were three main varieties; namely, dent, flint, and horsetooth. "Dent" was grown in the midwestern sections, including Ohio, Indiana, Illinois, and Iowa. This is a moderately hard corn which gives good yields. "Flint" was grown in the eastern states and in Kentucky. "Horsetooth" was grown in the south, and blended indiscriminately into the other two classes.

Today, the principal source of supply is the "dent" variety. This classification means little, however, for the Bureau of Agriculture, in cooperation with the agriculture departments of the various corn growing states, has developed numerous hybrids. These hybrids have been developed for their drought resistant and insect resistant properties. This work has been done for the benefit of the farmer, to increase the yield and to insure a suitable yield even under the most severe growing conditions. Although the general appearances of these hybrids are the same, they give varying results when used in the fermenting industries. Their chemical composition varies as well as the structure of the grains. In order to insure uniform results both in yield of alcohol and in the by-products secured, it is necessary to change continuously the operating
conditions of a plant. Much work is being done to develop quick methods of determining the characteristics of the grains, but to date the best that can be done is a rough estimation.

The chemical composition of corn varies with the climate during the growing season and also with the ground on which it is grown. One of the variables is the ratio of soluble to insoluble nitrogen. Other variables are the percentage of dextrins present and the ratio of maltose to dextrin that can be secured under standard methods of conversion with malt diastase.

Not only do the growing conditions affect the corn but there is also a difference in the action of kiln dried and naturally dried corn. Naturally dried corn has a more uniform texture throughout, while kiln dried corn tends toward a hard exterior with a tough film and a softer center. Operating conditions must be varied to meet these differences. With the kiln dried corn, a longer steeping and heating period is necessary.

In the handling of corn, much care must be given to the storage and grinding of the meal. J. McHargue (383) found that cornmeal absorbs moisture. In air of high humidity, it will reach an equilibrium at about 20 per cent moisture. He also found that mold growth starts when the corn contains 15 per cent moisture. It is a well known fact
that there is a gradual change in corn meal when it is exposed to the air with the formation of free fatty acids.

Some analyses of typical American corns show the following percentages:

<table>
<thead>
<tr>
<th></th>
<th>High Protein</th>
<th>Low Protein</th>
<th>Hybrid</th>
<th>Hybrid</th>
<th>Hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>11.5</td>
<td>13.6</td>
<td>12.8</td>
<td>13.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Ash</td>
<td>1.2</td>
<td>1.41</td>
<td>1.4</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Protein (N X 6.25)</td>
<td>11.3</td>
<td>2.23</td>
<td>2.2</td>
<td>3.8</td>
<td>10.5</td>
</tr>
<tr>
<td>Ether extract</td>
<td>5.1</td>
<td>4.2</td>
<td>4.6</td>
<td>4.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.1</td>
<td>1.62</td>
<td>1.9</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Starch</td>
<td>63.2</td>
<td>62.8</td>
<td>62.2</td>
<td>63.9</td>
<td>63.5</td>
</tr>
<tr>
<td>(Diastase convertible)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>71.1</td>
<td>69.3</td>
<td>72.8</td>
<td>70.6</td>
<td>73.2</td>
</tr>
<tr>
<td>(Acid convertible)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Corn of good quality will contain at least 62.5 per cent of diastatic convertible starch and sugars. Under the correct conditions and control, it should give 0.067 gallons of alcohol for each pound of starch.

Corn is generally purchased on the basis of the government grading. This grading is based upon the percentages of moisture and damaged grains present. It has no bearing upon the essential physical and chemical properties of the grain.
This classification is based upon the following percentages:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Moisture</th>
<th>Damaged Grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14.0</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>15.5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>17.5</td>
<td>7</td>
</tr>
<tr>
<td>D</td>
<td>20.0</td>
<td>10</td>
</tr>
</tbody>
</table>

Only grain of the better grades can be used in the production of the best potable spirits. Low grade grains may be used in the production of alcohol or products that are to be prepared by high temperature sterilization of the mashes, followed by a high proof distillation of the resulting beers.

RYE

Rye, agriculturally speaking, is a minor crop but it is of prime importance to the potable spirits industry. It is used to give a certain aroma and flavor to the finished spirit.

Rye is grown principally in Michigan, Wisconsin, Minnesota, North and South Dakota and the northern part of Indiana. There are several strains of rye, but the bulk of that reaching the market is generally a cross bred. Rye, like wheat, is grown as both a winter and spring crop. The commercial gradings follow the same
general methods as those for corn. Only Number 1, or a
good grade, rye is used in the spirits industry.

The general classifications are Rosen and Rosen type,
with further specifications as to the particular state in
which it is grown. Michigan Rosen rye commands the
highest premium on the market, but it is not always
superior to some other ryes as far as the fermentation
and yield of spirits are concerned.

Ryes are also classified as "country run" and
"elevator delivery". "Country run" means that the rye
is as it has been received from the farm and has not been
mixed in the elevators with low grade ryes. "Elevator
delivery" rye is generally a mixture of some very good rye
with some of poorer quality. The point of origin is not
always determinable. It is the lowest grade sold to the
distilling industries.

A typical analysis of a good grade American rye
shows the following percentages:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>12.5</td>
</tr>
<tr>
<td>Ash</td>
<td>1.0</td>
</tr>
<tr>
<td>Protein (N x 6.25)</td>
<td>11.2</td>
</tr>
<tr>
<td>Ether Extract</td>
<td>1.5</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>1.0</td>
</tr>
<tr>
<td>Starch (diastase convertible)</td>
<td>59.6</td>
</tr>
<tr>
<td>Carbo hydrates (acid convertible)</td>
<td>71.1</td>
</tr>
</tbody>
</table>
BARLEY AND BARLEY MALT

Barley, although used in smaller quantities in the distilling industries than rye or corn, is the most important due to its ability to produce a high concentration of the enzyme, diastase, when it is allowed to sprout. In the brewing industry it is the principal grain used and is, therefore, of great importance.

There are several kinds of barley, but the one that is of most importance is "Hordeum vulgare" or the ordinary six-row barley grown in Illinois, Iowa, Wisconsin, and Minnesota. It has a high protein content and yields a malt of high diastatic power.

A typical analysis of a good barley gives the following percentages:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8.6</td>
</tr>
<tr>
<td>Ash</td>
<td>2.8</td>
</tr>
<tr>
<td>Protein (N x 6.25)</td>
<td>11.8</td>
</tr>
<tr>
<td>Ether extract</td>
<td>2.1</td>
</tr>
<tr>
<td>Fiber</td>
<td>5.9</td>
</tr>
<tr>
<td>Starch (diastase convertible)</td>
<td>59.2</td>
</tr>
<tr>
<td>Carbohydrates (acid convertible)</td>
<td>62.1</td>
</tr>
<tr>
<td>Weight per bushel</td>
<td>47.5 lbs.</td>
</tr>
</tbody>
</table>

Barley, to be suitable for malting, must be clean, of uniform grains, good germinating power and good odor. Cleanliness is necessary because dirt is a source of
bacteria and molds. These, when present, will not only affect the production of the malt but also may seriously affect the fermentations in which this infected malt is used.

Because barley is the principal source of commercial malts, it has been given greater consideration in its application to malting and fermenting than any of the other grains. Much work has been done on its growth, chemical composition, and changes in chemical composition during the malting process. The phosphate content of barley and malt has received much consideration, as phosphates play such an important role in fermentation.

W. Windisch (652) found that barley contains no inorganic phosphates but that organic phosphates are broken down during the germination and that light accelerates this action. E. Crowther (110) could find no general relationship between the phosphate content of barley and the fertilizer used. M. Van Lear (615) found 1.047 grams of phosphorous pentoxide in 1000 grams of barley with 10 per cent being lost in malting.

The extract value of barley malt is of prime importance to the brewer. L. Bishop (53) stated that the nitrogen content of barley is in a reverse relation to the extract value of the malt produced. He gave the following formula
for predicting the extract value:

Extract value (in lbs. per quarter of malt) =

\[ 110.1 - 11.2 \text{ (per cent Nitrogen) } 0.18 \text{ (weight of 1000 kernels)} \]

G. Bode (60) in investigating barley found the sucrose content to be hereditary. A high sucrose content indicates high germinative power. H. Rultan (275) doubted if the total protein content of barley was any measure of its malting value, although it might be if the individual proteins could be accurately determined. T. Chrsasszos (99) stated that there is no relation between the total protein content of a barley and the amount of amylase which it produces. He found the amylase content of barley to vary from 36.8 to 434.

G. Mezzadroli (390) subjected barley to the action of short electro-magnetic waves for 30 minutes before soaking. He found that this treatment causes a more rapid development of the saccharification power as well as a 19 per cent higher production of maltose. K. Giri (222) found a 100 per cent increase in the diastatic power of the malt when the barley has been previously soaked in water for 24 hours, treated with 0.1 per cent solution of sodium nitrate and then subjected for 2 hours to an alternating current of 25 cycles, 210 volts and \( 4.3 \times 10^{-3} \) amperes. He stated that the wort from the treated barley malt is
deeper in color, has a better odor, and contains 60 per cent more sugars.

R. DeFazi (119) found that exposure of the barley to ultra-violet rays accelerates germination, prevents mold growth, increases the vitamin content, and facilitates the formation of the enzymes.

A. Niethammer (443) found that various nickel compounds have an injurious effect on germination with the order of injury being nickel cyanide, nickel nitrate, nickel carbonate, nickel chloride, and nickel sulfate. He further found that a concentration of 0.1 per cent nickel nitrate and nickel sulfate accelerates germination.

Barley contains numerous bacteria and molds, and it is often necessary to use disinfectants. C. Becker (38) found hydrogen peroxide to be satisfactory as a disinfectant and also to have a more beneficial effect upon the germinating power than the lime water treatment. M. Popp (495) investigated barley which poisoned live stock. He found that the grains had been attacked by microorganisms, particularly Gibberella saubinetti. Chemical analysis showed the proteins to have been altered with the formation of toxic albumins and other nitrogenous compounds. This indicates that too much care cannot be exercised in the selection of barley, whether it is to be used as such or as barley malt.
MALT

The general term "malt" refers to any grain that has been allowed to germinate. Barley, rye, and wheat are used, but unless specified, any future statement will refer to malt made from barley. Malting is done for one purpose, namely, to produce the enzyme, diastase, which has the power to convert the insoluble starch over to soluble sugars.

The procedures followed in the malting of barley, after the selection of the raw grain, can be divided into three methods based upon the germination. These methods are known as floor, drum and compartment. The handling of the barley up to the germinating operation and the drying of the malt are the same regardless of the method used for germination. The general operations in malting are: 1, cleaning of the barley; 2, steeping; 3, germination; 4, drying.

The cleaning is done by screening and air cleaning. This eliminates foreign seeds, dirt, and chaff.

Steeping is for the purpose of thoroughly wetting the grain. Steeping tanks are filled with water of the correct composition and the barley is added. The chaff and undeveloped grains float on the surface of the water and are skimmed off. The steeping period is from 48 to 72 hours. During the first 24 hours, the water is changed every
12 hours and every 24 hours thereafter until the steeping is finished and the grain is thoroughly wetted. The water is then drained off and the grain is ready for germination.

The floor method of germination is carried on by spreading the steeped grain in layers of 8 to 12 inches thick on large floors over which filtered air is blown. The grain is turned periodically by hand to prevent the temperature rising above 75° F. Germination proceeds, and in from 120-190 hours the acrospire or young plant has grown to about the length of the kernel. Growth must then be stopped by drying the green malt in a kiln.

The compartment method and drum method carry out the same operations as does the floor method except the grain is handled mechanically instead of manually. In the compartment method the grain is placed in oblong tanks and is turned by revolving screws traveling along the top of the tank. In the drum method the grain is turned by the action of the revolving drum. There is little difference in the malts secured from the three methods.

After the germination of the grain has reached the desired stage, the green malt is dried. This drying may be done on the floor of buildings or in continuous kilns. In floor drying, the green malt is first spread on the top floor of a three story building. Heated air is forced up through the building from the first floor. After the
green malt has been partially dried, it is dropped to the second floor where it encounters air of a higher temperature. It is eventually dropped to the first floor where it is finished by the incoming hot air. The starting temperature should be approximately 90°F, with a finishing temperature of 125°F. There are no set conditions of steeping or drying the malt as the processes must vary according to the particular characteristics of the individual barley.

Many factors are involved in the malting of a grain and many conditions and substances affect the quality of the finished product. The grains themselves vary as was shown by P. Petit (486) who found that barley grown in dry seasons has a more compact and refractive starch. This "dry season" barley requires longer mashing time, and the hordein nitrogen in the resulting malt is increased while the soluble nitrogen remains constant. J. Reichers (622) found that ether, ethyl alcohol, methyl alcohol, ethylurethan, acetaldehyde, and various organo-mercury compounds stimulate the formation of the enzyme, diastase. He further found that diastase formation is inhibited by chloroform, chloralhydrate, the higher alcohols, aniline, methylene blue, and tannins.

E. Mousfang (406) used the method of hot steeping at 47°C for 1½ hours followed by alternately aerating and
steeping at 10° C. for 24 hours. After 7 days the malt was of good quality with a total loss of 6 per cent instead of the normal loss of 10 per cent.

E. Jalowetz (282) compared the malts secured from floor temperatures of 10-13° C. and 20-22° C. He found that the lower temperatures give a malt richer in extract, dextrin, soluble albumins, formal and ammoniacal nitrogen, and acidity.

Many tests have been used to determine the quality of barley malt. The Lintner method of determining diastase activity is the base for a number of these tests. Before the advent of the technical man into the distilling industries, the "Sinker test" was commonly used. This test was based upon the fact that well modified malts would float while poorly modified grains would sink when placed in water. This test has been proven to be very unreliable and of little value.

MALT ENZYMES

Malt contains certain substances known as enzymes. These are diastase or amylase, peptase, maltase, and catalase. By far the most important are the diastases or amylases. The exact nature of these enzymes has long been a point of controversy. They have been defined as "definite material catalysts of organic nature with specific powers of reaction, formed indeed by living cells, but
independent of the presence of the latter in their operation".

Although extensive research has been carried on in trying to isolate these substances as chemical compounds, this has not as yet been accomplished. The Willstätter School believes enzymes to be chemical individuals, while others believe that these substances are mixtures of chemically familiar substances. The colloidal concept of enzymes has adherents who claim that the activity of the enzymes is dependent upon the degree of division, and the loss of this division causes the inactivation of the enzyme processes.

The activity of diastase in its conversion of starch to sugar was long considered a single action. It was then found that there was a distinction between the enzyme property causing liquefaction and the one causing saccharification. Workers in recent years have been able to suppress one individual action, while retaining the other. Even in the light of these later discoveries, the existence of two enzymes is still controversial.

Another division of the amylases is a-amylase and b-amylase. This division was brought about by the optical analysis of sugars formed during the degradation of starch. This leads to the assumption of the presence in starch of alternating a- and b- glucosidic linkages and non-furoid
grape sugar residues. Malt amylase is principally b-amylase and yields b-maltose from starch. G. van Klinkenberg (302) gave complete information as to the methods used in separating the a- and b-amylases.

Many reports have been published as to the kinetics of the diastatic degradation of starch. O. Bailly (28) showed that the law of mass action may govern diastatic activity. However, there is still a controversy as to whether starch degradation by diastase follows an equation of the first order.

Since there is still a doubt regarding the exact nature of the enzymic material and its mode of action the greater part of research has been considered from the over-all effect. O. Bergheim (44) showed that lime-softened water inhibits amylase activity due to the adsorption of the enzymes by magnesium hydroxide which is always present in this treatment.

H. Nakamura (417) showed that yeast juice has little protective effect on taka-diastase when heated to 50°C while malt infusions prepared cold, then boiled and filtered, have good protective properties. He further stated that the protective action is due to the calcium content. From this he concluded that amylase is an organo-calcium compound and its loss in activity when heated is due to the loss of combined calcium.
M. Holderer (268) found that if the diastase solution is alkaline and is filtered through a Chamberlain filter, it remains active. Solutions which are acid or neutral become totally inactive. He concluded that the colloidal substance of the enzyme is adsorbed by the porcelain in acid or neutral solutions.

The pH value seems to play a rather important role in diastatic activity. A. Oparin (455) found that in a pH range of 3.5 to 6.2 any changes in activity depend upon physical alterations in the substrate, while in pH values outside this range there is a loss of enzyme activity. L. Adler (10) found that at 20° C, diastase is most active at a pH of 4.9 and the optimum pH range is from 4.6 to 5.2. Any change from these conditions quickly retards the enzyme. A pH value of 2.1 causes complete inactivation as does a pH of 8.1. At a pH of 7.07 there is only a slight activity. H. Sherman (552) found malt diastase activity in pH range of 2.0 to 9.0 with the maximum at a pH of 4.4 to 4.5.

W. Biedermann (49) believed the effect of pH value on diastatic activity was over-estimated and that other ions had a pronounced effect. He stated that if the enzyme concentration is high enough the pH value will have no effect on the reaction velocity.

The effects of short wave radiations on diastase were
studied by K. Kosieradski (314). Irradiation of diastase solutions with waves of 2, 4, 10 and 15 u. has no effect on the activity. S. Loewenthal (351) reported that radium emanations accelerate the action of diastase. H. Collier (107) found that ultra-violet radiation (200-313 m.μ.) destroys malt diastase, but visible radiations stimulate the starch conversion slightly. S. Banerjee (33) found that malt diastase is completely inhibited by ultra-violet radiations but it is protected from this action by asparagine, formic acid, ammonium citrate, gelatine, etc. The protective action depends upon the capacity of the substance to absorb the destructive wave lengths of the ultra-violet light.

T. Panzer (473) found ammonia reacts with diastase, but the exact nature of the reaction can not be determined. He found that aldehydes are not essential for diastatic activity. T. Panzer (478) further reported that the action of ammonia is the same with active diastase as with that which has been inactivated by heat. T. Panzer (474) found that hydrogen chloride stops diastatic action and the original activity is only partially restored by ammonia gas. T. Panzer (472) found that large amounts of hydrogen chloride are absorbed by diastase with a loss in conversion power, but the hydrogen chloride can be removed from the atomic complexes by a vacuum, while the hydrogen chloride is not readily liberated from other groups.
According to A. Thomas (525) bromides have an inhibitory action even in small amounts and an activating action when their concentration is increased. This is contrary to the actions of chlorides, nitrates, sulfates, and phosphates of sodium and potassium. U. Olsson (453) found iodide and fluoride ions to have no inactivating effect upon diastase. L. Hawkins (252) found all chlorides to accelerate diastatic activity at various concentrations. Ferric chloride was the most effective with an increased activity of 291 per cent at a concentration of 0.000125 molar. There is no influence of one salt upon the other in its action on diastase.

L. Berczeller (43) found the diastatic activity of malt to be inhibited by halogens in the order of iodine, bromine, chlorine. The protective action of starch is greatest with chlorine and least with iodine. G. Doby (129) found that amylases are strongly activated by chloride ions and weakly activated by potassium, nitrate, and fluoride ions.

The effect of neutral salts on diastase varies considerably depending upon the concentration of the salt and of the enzyme. A. Hahn (239) found neutral salts to accelerate activity when present in diluted buffer solutions that are more acid than the optimum for the enzyme. In concentrated buffer solutions, neutral salts retard activity. W. Haarmann (236) found similar results with the salts of the Hofmeister series.
R. Heyl (263) found that primary potassium phosphate stimulates the action of diastase while the secondary potassium phosphate inhibits freshly prepared diastase solutions and stimulates old solutions. From this work he concluded that the rate of hydrolysis of starch solutions follows the simple (logarithmic) formula indicating that only part of the starch is acted upon.

The effect of many heavy metals on the diastatic activity has been studied. U. Olsson (452) found the amylase activity to be halved by a concentration of $2.1 \times 10^{-7}$ normal silver nitrate. Other heavy metals inhibit the activity but not to such an extent. A. Baumgarten (36) found dilute solutions of salts of metals generally have an inhibitory action. G. Mori (396) found that the inhibitory action of silver chloride, silver bromide, silver nitrate, copper sulfate, and lead acetate is a function of their concentration. Inhibitory action of silver, gold, copper, and lead stands in ration of $1 : 1/18 : 1/30 : 1/1400$.

Organic as well as inorganic compounds affect changes in the rate as well as the extent of diastatic activity. R. Clark (104) found that chlorohydrin, thiourea, and potassium thiocyanate increase the activity at low concentrations (0.1 per cent solutions) and that higher concentration (5 per cent solutions) decreases the activity. E. Ohlsson (450) found that citrates have no influence on
diastase at the optimum pH but retard the activity at high or low pH values. H. Pringsheim (502) found that barley amylase cannot be activated by either glutathione or yeast compounds. H. Pringsheim (501), however, found that a 0.0001 molar solution of glutathione activates malt amylase. E. Wystkowski (414) found that glycine stimulates amylase activity but that this activation can be suppressed with calcium chloride. T. Chrzaszcz (96) claimed that the action of malt amylase is a function of two ferments - 1, liquefies starch; 2, changes it to sugar. The production of dextrin may be due to one of these or to a special enzyme. He found that the dextrinizing power is inhibited parallel to the liquefying power. He also found that bases in suitable concentration activate the liquefying and dextrinizing power but inhibit the saccharifying power. Ethyl alcohol inhibits all functions of the enzyme.

Other enzymes are always found along with the amylases and may affect their activity. W. Biedermann (50) found that pepsin attacks and retards diastatic activity while trypsin has little effect. W. Schneidewind (535) investigated the effect of numerous substances and found that peptone, asparagine and albumin exert a favorable influence while alcohol and ether are injurious. He also found that citric and acetic acids in concentration of 0.001 per cent have a favorable action while they retard
action in concentrations of 0.01 per cent. F. Caujolle (63) found that tetramethylethanolium hydroxide and choline have no effect on the amylolytic activity.

Maltase, another enzyme which always accompanies the amylases, is also affected by foreign substances, as was shown by W. Kopaczewski (311), who found that there is little difference between the action of organic and inorganic acids in respect to their effect on the maltase activity. He also found that boric acid favors the activity and that, in organic acids, an increase of - C H₂ decreases the activity. Chlorine and phenol in low concentration intensify the maltase activity.

Malt catalase, another enzyme associated with the amylases, is determinable by its ability to decompose hydrogen peroxide. N. Matsuyama (375) found that at 20° C. in a 0.05 normal solution of hydrogen peroxide, the optimum pH value for the reaction is between 7 and 8. He also found that the inactivation coefficient of catalase is doubled when the concentration is reduced one-fourth. M. Charmandaryan (93) found the inhibition of catalase by metallic ions to follow the order of their molecular weight. He concluded that the effect of the salt is through its action on the state of dispersion of the enzyme. A. Tyutyunnikova (607) found that both organic and inorganic poisons may stimulate catalase activity. He found barium chloride has the
strongest stimulating action of the inorganic salts while magnesium chloride is next.

THE ACTION OF AMYLASES ON STARCH

The action of amylases on starch is two-fold; 1, liquefaction; 2, saccharification. There is a possibility of there being a third action, namely, that of forming dextrin. Since starch is in a grain formation, it must first be treated in some manner to break the grains. Even after this is done the starch is in a colloidal state which varies with the grains used and the treatment followed.

E. Sym (586) found that the colloidal character of the starch has no influence on the amylase activity but that changes in the colloidal state of the enzyme solution affect it slightly. In acid range, the anions effect hydrolysis of the starch while in alkaline range the cations effect it.

E. Szego (507) found that the small grains of starch although having a high viscosity give a better yield of conversion.

P. Esmander (147) found that, if the surface of corn is covered with a protective substance such as toluene, the dextrin content increases during conversion.

In the amylase degradation of starch, both maltose and dextrin are formed. In practice the degradation desired favors the formation of the highest possible percentage of maltose. S. Pronin (503) succeeded in securing 95.9 per cent
conversion by using a large excess of malt extract. D. de Hoop (269) found that if a sufficient amount of coenzyme is present a yield of 96 per cent of maltose can be secured. With small amounts of the coenzyme the reaction stops at 80 per cent maltose. J. Baker (29) found that the addition of excess malt increases the activity and suggested it is due to the proteolytic enzyme of malt which liberates more amylase. W. Windisch (643) believed the physical nature of the grist and kind of starch affect the saccharifying power. By a 4 hour mashing at 60° C. he secured a 93 per cent solubility of the starches of barley and wheat, 53 per cent solubility of potato starch, and 18 to 20 per cent solubility of corn and rice starch. At 65° C. for 4 hours, 50 per cent of rice starch and 55 per cent of corn starch are dissolved. H. Eckhard (136) stated the optimum conversion of starch to maltose is at 50-55° C. and under these conditions the best yields will show 80 per cent maltose and 20 per cent dextrins.

It has been suggested that light affects the degradation of starch by amylases. A. Navez (419) found that a photo-sensitization effect produced during hydrolysis by internal screens of fluorescein dyes increases the rate of production of sugars. He believed a loose compound is formed between the dye and amylase which, on the addition of light, forms
a peroxide capable of removing the diastase inhibition.

Many salts, both organic and inorganic, influence the action of amylase on starch. W. James (283) found that potassium chloride activates the enzyme responsible for the formation of dextrin, while it has little effect upon the enzyme producing sugar from dextrin. C. Gerber (210) showed that salts of the monobasic acids, when present in low concentrations, accelerate the hydrolysis of starch by the amyloytic enzymes. C. Gerber (214) also found that low concentrations of neutral salts of ammonia from mineral acids accelerate conversion of starch, while they retard it in high concentration (1-5 per cent). The bicarbonates, if neutral to methyl orange, act similarly to the ammonium salts. The chlorides of rubidium and caesium accelerate the action only slightly, while lithium chloride retards it in proportion to the salt concentration.

As shown by H. Sherwood (557) the order of stimulation by neutral salts under unfavorable conditions of pH is sodium chloride, potassium chloride, sodium sulfate, and sodium nitrate, while under favorable pH conditions, the neutral salts are not essential to the activity of amylase. H. Sherman (555), using hydrochloric and phosphoric acids and primary potassium phosphate, determined the optimum pH for starch degradation to be 4.4 in each case. The maltose formation was directly proportional to the enzyme concentration,
and he could not find a cessation of hydrolysis at 30 per cent equilibrium as claimed by some investigators.

G. Kita (300) found that calcium sulfate and calcium chloride retard both the liquefying and saccharifying of starch at 25° C., but at 50° C. liquefaction is accelerated. According to G. Gerber (217), cadmium chloride, potassium oxalate, sodium citrate, zinc chloride, and calcium chloride in low concentrations have no effect upon the conversion of soluble starch, while mercury and silver salts retard conversion, but this latter action is due to a direct action on the amylase. C. Gerber (213) further determined that chromium, magnesium, and manganese salts accelerate amylolytic conversion while ferrous salts retard it in all concentrations. The retardation of hydrolysis by cadmium and zinc salts was found by G. Gerber (210) to be due to action on both the enzymes and the starch.

G. Gerber (212) found that copper chloride retards conversion in low concentrations, accelerates in larger concentrations, and retards again in still larger concentrations. Gold chloride retards action in proportion to its concentration, while platinic chloride when added to starch accelerates the conversion up to a concentration of 1. milligram mole and retards it above that. When platinic chloride is added to the diastase solution a retarding action is present in all concentrations.
U. Olsson (454) in his studies on the effects of poisons on the conversion of starch showed that copper sulfate and iodine decrease both liquefaction and saccharification. H. Sherman (554) found that glycine, alanine, phenylalanine and tyrosine increase the rate of hydrolysis of starch by protecting the enzyme from deterioration in the aqueous dispersion in which it acts. H. Sherman (553) also found that aspartic acid and asparagine accelerate the action of malt amylases.

E. Ohlsson (451) studied the action of alkaloids on the hydrolysis of starch and determined that quinine, cinchonine, and optochine, retard the action. C. Gerber (216) found that the basic salts of quinine accelerate the conversion of starch by amylases. Some of the salts accelerate the action in all proportions. He also reported that caffine and codeine accelerate in small amounts and have no retarding action until much larger amounts are added. According to F. Caujolle (36), ethylenediamine, putrescine, and cadaverine retard the amylolytic activity, while their hydrochloric acid salts have no effect.
MASHING
"Mashing" is the term applied to the processes involved in the conversion of the starch of grains over to sugars. There are two general methods used in the distilling industries: 1, conversion with acids; 2, conversion with malt. Only the second method will be considered, and it also can be divided into two methods: 1, atmospheric mash tub mashing; 2, high pressure cooker mashing.

In mashing three changes in the starch must take place: 1, pastification; 2, liquefaction; 3, saccharification. The operation of the atmospheric pressure mash tub is generally as follows:

The mash tub is charged with water until a volume is secured which represents 20 gallons for each bushel (56 pounds) of grain to be mashed. The acidity of this water is adjusted until it has a pH of 5.5. This adjustment may be done with sulfuric acid or with spent beer from a previous fermentation. The temperature of the water is then raised to 150° F, and a uniformly ground corn meal is run in. This temperature is maintained for fifteen minutes so as to thoroughly wet the meal. The charge is then slowly heated to boiling with live steam. Care must be exercised in this heating, for if heated too rapidly, a film of gelatinized starch will form on the surface of each grain of meal and the interior will be unaffected. The charge is boiled for 15 minutes and cooling is started. This
cooling is done by water coils placed in the mash tub. The tub is also equipped with revolving rakes which travel at a speed of about 12 R.P.M. High speed rakes not only require excessive power but also cause the thick, viscous mass to move with the rakes thus preventing good stirring.

If rye is to be used in the mash, the pastified corn mash is cooled to 160° F. and the ground rye is added. This temperature is maintained for 10 minutes, and the charge is then cooled to 148° F. The crushed barley malt is added, and a temperature of 145° F. maintained until liquefaction and saccharification are complete. This generally requires about 45 minutes. The finished mash is then cooled to the temperature desired for fermenting. This cooling may be done in the mash tub or in heat interchangers while the mash is being pumped to the fermenting room.

The above conditions must be varied to meet the physical and chemical characteristics of the grains. Certain other practices may be used, such as adding 1 per cent of the malt with the corn. This helps prevent the formation of the viscous coating on the meal grain and allows a better pastifying action. It also thins the mash, thereby reducing the power necessary for the rakes.

Another practice, which may be desirable, is the addition
of disinfecting compounds at the same time that the rye is added. This is done to reduce the bacteria and molds on the rye and malt, as these grains are not heated to a temperature high enough to sterilize them.

High pressure mashing is done in order to get complete conversion of all the starch in the grains. The process is essentially as follows:

The equipment is a long cylindrical container capable of holding pressures up to 90 pounds per sq. in. It is equipped with an off center shaft to which are fastened the stirring arms. This "cooker", as it is called, is charged with water until it contains 20-22 gallons for each bushel (56 lbs.) of corn that is to be mashed. The acidity of the water is adjusted with sulfuric acid or spent beer until a pH of 5.5 is reached. The water is heated to 150° F. and the cracked corn is added. The charge is then heated with live steam until a pressure of 70 to 80 pounds per sq. in. is secured. The pressure is then slowly released and a vacuum is drawn in the cooker. This reduction of the pressure cools the charge to the temperature for the addition of the malt or the rye, if rye is to be used. After cooling, the charge is run to a "drop tub" which is essentially a mash tub and the malting is carried on in the same manner that is used in the mash tub method.
In the high pressure cooking of the corn, care must be taken in heating the charge with the high pressure steam. If heated too rapidly excessive caramelization takes place which causes a loss in yield as well as developing a burnt odor and flavor to the finished distillate.

Although the high pressure cooking of the corn allows a much better conversion of the starch in the grain, resulting in better yields, it also causes a degradation of proteins and fats. This latter action gives compounds that are acted upon by the yeasts during fermentation. The resulting beer must be distilled at a high proof in order to give a satisfactory finished product.

The processes involved in mashing have received considerable attention, although the greater part of the work has been done on brewery marshes.

E. Moufang (399) found that a thorough digestion of the grain with water, prior to mashing, increases the yield from 0.3 to 5 per cent. W. Windisch (680), from his studies on barley mashes determined that the higher the temperature, up to 75° C., the greater the conversion and that prolonged mashing increases the percentage of maltose. E. Lampe (329) reported the yield of alcohol to be smaller when malt is added to the mash in two portions instead of all at one time.
W. Windisch (653) showed that mashing under pressure gives about the same yields as the method involving predigestion and protein rest. The amount of protein in solution is about the same for both methods. W. Windisch (649) also found that the optimum temperature for the formation of permanently soluble and formal nitrogen varies with the time of mashing. The formal nitrogen decreases with increased temperature. According to R. Hopkins (270) low temperature mashing of corn leaves the nitrogen in a form not readily assimilable by the yeasts. H. Kelley (285) showed that salts affect the solubility of the proteins during mashing and that the soluble nitrogen content is a parabolic function of the pH value for calcium sulfate, sodium chloride, calcium chloride, and potassium sulfate, while the sulfates of magnesium, copper, ferrous iron, and aluminium all show an inhibition at a pH value of 4.8. According to E. Staker (574) the 0.5 molar aqueous solutions of neutral inorganic salts increase the solubility of the proteins in grains during mashing.

The pH values used in the mash tubs should coincide with those given as the optimum for the amylase activity. In actual practice, slightly higher values are used due to the inability to make corrections later on in the process. K. Benedek (42) reported that the optimum pH is 4.5-5. at a malting temperature of 50° C; is 4.8-5.4 at 60° C.; and
5.6 at 70° C. E. Mounfang (403) found that a reduction in acidity during mashing causes a decrease in soluble nitrogen. E. Mounfang (402) also found that by the addition of phosphoric and lactic acid different effects upon mashing are secured. Phosphoric acid gives best results at a mashing temperature of 60° C. while lactic gives best results at 45° C. Lactic acid causes a better saccharification, an increased yield, and an increase in the amount of soluble albumins.

The mashing operation leaves considerable quantities of the starch in a form which is not attacked by the yeast enzymes. The change of this material over to a form which can be acted upon is known as after-conversion. Therefore, sufficient malt enzymes must be present to carry out these reactions. R. Deplanque (125) stated that any method used to determine this after-conversion power must be based upon the consideration of the acidity of the mash; otherwise, incorrect results would be secured. H. Eck (135) found that fermentation of acid converted mashes gives much better results when 2 to 5 per cent of malt is added.
YEASTS
Yeast is the term generally applied to all those microorganisms which decompose sugar solutions into alcohol and carbon dioxide. Botanically speaking, yeasts are unicellular fungi, which are spherical or oval in shape and multiply by budding. They belong to the second broad group of vegetable growths, i.e., those which do not contain chlorophyll and are, therefore, incapable of manufacturing their own food. There are a large number of specific strains of yeasts, but the two most important are: 1, saccharomyces cerevisiae or beer and distillery yeasts; 2, saccharomyces ellipsoideus or wine yeasts. The first group, the saccharomyces cerevisiae, contain a large number of individual yeasts which are differentiated from each other by their growing conditions, giant colonies, the carbohydrates which each will ferment and the amount of alcohol and other products they will produce.

Yeast occur in nature in mixtures not only with other yeasts but also with bacteria. To secure a suitable strain for fermenting purposes, it is necessary to isolate these yeasts from each other. There are two general methods for accomplishing this: 1, the physiological method; 2, the single cell culture.

The physiological method is based upon the fact that one race of yeast may grow readily in a certain nutrient medium,
while other races and bacteria will either be killed or will remain practically dormant. By growing successive generations in a medium of the same composition, it is possible to gradually develop a pure race. This method is not very reliable and in most cases has been supplanted by the single cell method of isolation.

The single cell culture is based upon the actual isolation of one cell. The method originally used was to dilute the yeast with distilled water until there was one cell in the liquid for each one or two cubic centimeters of liquid. By inoculating flasks, containing a nutrient medium, with one cubic centimeter of this diluted yeast solution, part of the flasks would contain one cell of yeast, while others would remain sterile.

Many variations of this method have been developed, one of which is to use a sterile agar-agar nutrient solution for the growing medium. After the diluted yeast solution is added, the mixture is thoroughly shaken and then set aside to gel. If more than one cell is present, they will be separated by the gel and the colony desired can be cut out and placed in a nutrient solution for growing.

Still another method is the drop culture method. This consists of diluting the yeast until each drop contains a single cell. Drops of the diluted liquid are then placed on
ruled cover slides and these placed on moist chambers. The cells are examined under the microscope and their growth followed. The cells desired can be removed to nutrient solutions by means of a sterile platinum wire.

Yeast, being unable to manufacture its own food, requires that nutrient materials be supplied. Not only must the actual food, as sugars, be supplied but also other elements which are essential to the growth of yeast must be present. The first attack on this problem was by the analysis of the dry residues secured from yeast. A typical analysis of a dry yeast shows the following percentages:

<table>
<thead>
<tr>
<th>Material</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogenous matter</td>
<td>40.98</td>
</tr>
<tr>
<td>Fatty Matter</td>
<td>2.8</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.47</td>
</tr>
<tr>
<td>Starchy Material</td>
<td>44.10</td>
</tr>
<tr>
<td>Other Organic matter</td>
<td>1.06</td>
</tr>
<tr>
<td>Mineral matter</td>
<td>5.54</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0.05</td>
</tr>
</tbody>
</table>

J. Neisenheimer (387) showed the analysis of the nitrogen in yeast to have the following percentage distribution:

<table>
<thead>
<tr>
<th>Nitrogen Form</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia nitrogen</td>
<td>11</td>
</tr>
<tr>
<td>Nuclein base nitrogen</td>
<td>7</td>
</tr>
<tr>
<td>Arginine-histidine</td>
<td></td>
</tr>
<tr>
<td>nitrogen</td>
<td>22</td>
</tr>
<tr>
<td>Lysine-choline nitrogen</td>
<td>4</td>
</tr>
<tr>
<td>Monoamino acid nitrogen</td>
<td>56</td>
</tr>
</tbody>
</table>
Many mineral analyses have been reported on the ash of yeast. A typical bottom yeast shows the following percentages:

<table>
<thead>
<tr>
<th>Mineral Oxide</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium oxide</td>
<td>26.07</td>
</tr>
<tr>
<td>Sodium oxide</td>
<td>2.26</td>
</tr>
<tr>
<td>Calcium oxide</td>
<td>7.58</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>6.34</td>
</tr>
<tr>
<td>Ferric oxide</td>
<td>0.70</td>
</tr>
<tr>
<td>Phosphorous pentoxide</td>
<td>54.31</td>
</tr>
<tr>
<td>Sulfur trioxide</td>
<td>0.51</td>
</tr>
<tr>
<td>Silicon dioxide</td>
<td>0.92</td>
</tr>
</tbody>
</table>

GROWING OF YEAST

The commercial growing of the yeasts begins where the laboratory stops, namely, with a small flask of a pure culture. There are two general methods for utilizing the pure cultures in commercial plants: 1, the pure culture apparatus of the Schwarz laboratories or the Hansen-Kühle apparatus; 2, the jug yeast method.

The pure culture yeast apparatus is designed so that it can be completely sterilized and charged with sterile wort, and yeast, without air coming in contact with them. After the apparatus is charged, sterile air is bubbled through the mass and the yeast is grown rapidly. The yeast charge is forced from the apparatus by air pressure and is
sent to the day yeast tubs or fermenters. It is customary to have at least two of these apparatus set up as a battery so that yeast can be charged into one from the other. In this way one stocking of a pure yeast culture may last for a long time as part of the yeast can be stocked back, while the rest is sent to the day yeast tubs. This method has the advantage that once a pure culture is delivered to the plant, it can be kept pure.

The jug yeast method has long been used in the spirits industry. In this method a pure culture is used as the starter yeast. A wort is prepared by boiling for 15 minutes 1 pound of hops in 5 gallons of water. The hops may be kept in a sack in the water and in this case, the boiling can continue for 45 minutes or the temperature may be kept just below boiling (135°F.) for 3 hours. The hops are then strained from the solution and 9 pounds of good grade barley malt are added. This is allowed to stand at a temperature of 140°F. for 1 hour and the liquid is again strained from the malt. The liquid wort is then sterilized by boiling. It is then cooled to 80°F., and is stocked with a pure culture yeast. The temperature is allowed to rise to 85°F., and the yeast is grown until the liquid has a Balling sugar content of 6 to 8°. The yeast is then placed in tin lined copper jugs of a suitable capacity. One charge of this yeast as outlined above should
give 2 to 2.5 gallons of jug yeast. The equipment used in making the above jug yeast must be a steam jacketed, tin lined container with a close fitting top. If the operations have been carried on correctly, the jug yeast secured is still a pure culture and can be kept in cold storage until needed in the distillery.

The growing of the jug yeast is only part of the operation as this yeast is only a stock from which the daily needs are drawn. The yeast to be used for stocking the fermenters must be grown. There are two general methods for growing the day yeast, namely, the "sweet yeast method" and the "sour yeast method".

The "sweet yeast method" is as follows:

A "dona" is first prepared by soaking hops (1 pound for each 5 gallons of water) in water at 180°F for 3 hours. The hops are strained off and the liquid boiled and then cooled to 140°F. To this liquid are added 8 pounds of barley malt for each 5 gallons of liquid and the temperature of 140°F maintained for 2 hours. The mash is then boiled for 15 minutes, cooled to 80°F, and stocked with jug yeast. 1 gallon of jug yeast will stock 50 gallons of dona mash. The dona is allowed to rise in temperature to 88°F and in 10 to 12 hours is ready to be added to the sweet yeast mash.
The "sweet yeast mash" is prepared by mashing equal proportions of rye and barley malt in water. The water is heated to $150^\circ F.$ and 5 pounds of rye, for each 5 gallons of water, are added. This temperature is maintained for 15 minutes, and the mash is then cooled to $140^\circ F.$ and an equal amount of barley malt is added. This temperature is maintained for 1 hour, then the mash is boiled for 10 minutes, cooled to $80^\circ F.$ and stocked with the dona yeast. The yeast grows rapidly and is ready for the fermenters in about 5 hours.

The "sour yeast method" varies somewhat from the previous method and is as follows:

The jug yeast and the dona are prepared in the same way as for the "sweet yeast method", but the handling of the main yeast mash is considerably different. This mash may be prepared in the same manner and with the same grain proportions as the main mash for the sweet yeast up to the final boiling of the mash. Instead of boiling the mash for 10 minutes, it is boiled for 30 minutes. It is then cooled to 120-122$^\circ F.$ and is stocked with 1 pint of a pure culture of *Delbruckii* lactic acid bacteria. The temperature is held at 122$^\circ F.$ for 36 to 40 hours, at which time the pH value will have reached 3.8 to 4.0, and the acidity expressed as lactic acid is about 0.15 molar. The mash is again boiled for 30 minutes to kill the
bacteria, cooled to 80°F, and stocked with the dona yeast or with a cut back yeast from another yeast tub that is ready to go to the fermenters. The stocked mash is allowed to stand at 85-88°F for 16 to 20 hours, at which time it is ready for "pitching" to the fermenters.

The use of cut back yeast is recommended, as it gives a higher fermenting power since it is already acclimated to the high acid condition in the yeast mash and is not affected by the high acidity encountered in the fermentations. It also gives better yields of alcohol and allows for a better development of desired congeners.

The subject of yeast growth is one which has received much attention both as to the optimum conditions and the effects of various chemical compounds upon it. C. Funk (203) discovered that yeast did not grow in a nutrient medium, which was made up of compounds that had been purified by adsorption with fullers earth which rendered them free of vitamin D. E. Fulmer (199) found that sterilization of certain media under pressure produces a growth stimulant for saccharomyces cerevisiae. E. Fulmer (201) also found that caramelization produced by sterilization under pressure may lead to the formation of growth stimulants for yeasts. E. Fulmer (202) in studying the effect of magnesium salts, reported that magnesium sulfate in the presence of bios
preparations increases the rate of growth of yeasts, while magnesium chloride and nitrate do not show the same results.

Raw grains have been considered as acceptable sources of nitrogen for yeasts, but F. Wyatt (661) found that all proteins in the raw cereals are precipitated by boiling with hops and that the nitrogenous yeast food in worts and beers is in direct proportion to the kind and quality of malt used.

Yeast, like all organisms, requires some oxygen for satisfactory growth. According to W. Richards (512), the rate of oxygen consumption, for saccharomyces cerevisiae grown at 28.2° C., reaches a maximum at 65 hours, at which time there is an equilibrium between the first and second cycles of growth. This rate decreases gradually and reaches an equilibrium at 130 hours. T. Stier (578) showed that the constancy of metabolic activity, measured as a function of temperature, could be utilized for precise investigations of respiratory systems. The constant (u) in the Arrhenius equation for the relation of oxygen consumption and temperature with yeasts was reproducible over a wide range of temperature.

An unidentified compound was secured by H. Lutndin (561) when yeasts utilized alcohol in the presence of oxygen. An increased fermentative action was observed by F. Windisch (635)
when yeast is cultivated in the absence of oxygen. He also found that the respiratory activity increases parallel to the fermentative activity.

Nitrogen in some form is an essential to yeast growth and yeast is capable of utilizing most forms of nitrogen. E. Fulmer (194) found that saccharomyces cerevisiae, Race F., could utilize atmospheric nitrogen for growth. E. Fulmer (199) also found that the fixation of atmospheric nitrogen by yeasts is a function of the pH value and there are two optima, one at a pH of 6 and the other at a pH of 7. F. Swoboda (585) showed that proline, lysine, and arginine activate yeast growth, while cystine, tryptophan, and glucosamine retard growth.

P. Thomas (596) found that acetamide, propionamide, and butyramid retard yeast growth, while formamide, containing free ammonia, is more readily utilized. From this, he concluded that organic nitrogen must be converted to ammonia before it is utilized by the yeast. H. Fringsheim (498) showed that all nitrogenous foods, except ammonium salts, producing active fermentating yeast cultures contain either the grouping \(-NH-CH-CO\) or \(-NH-CO\). Other nitrogen groupings give yeast growth but not active fermentations. He also showed that there is a difference in the nitrogen requirements of different yeasts as well as a variation in
nitrogen requirements of the same yeast depending upon the nitrogen compounds used. He found that fusel oil decreases with decreased nitrogen content and increases with temperature. He also secured a decrease in fusel oil formation when ammonium sulfate was added to a natural mash. Although ammonium chloride is changed by yeast to a yeast protein, C. Hoffman (268) found that it is not an enzyme stimulant.

The rate of yeast growth has been studied by a number of investigators. W. Clark (102) found that the rate of reproduction of saccharomyces cerevisiae at 25° follows the formula \( \log \frac{C}{C_0} = 0.160 \) t. until the count reaches 100 million cells per c.c. According to A. Slator (561) the logarithmic law of increase holds good on yeast growth provided the growth is unrestricted. A. Slator (560) also found that if the number of yeast cells are plotted against the time, the curve rises at an increasing rate to a point of inflection and then decreases. In this case, there was a brief induction phase followed by a logarithmic rate of growth, then retarded growth. The rate of growth was independent of the sugar concentrations except in very dilute solutions.

An organism such as yeast, offers the opportunity of studying its respiration and growth independently of its
function as a producer of enzymes. Some foreign substances have been found to affect one function without affecting the others. Of all its functions, the growth is the easiest to follow.

A. Hardt (247) found that in the presence of mercuric chloride yeast follows the Arndt-Schulz law which states that "very weak stimuli incite, weak stimuli enliven, moderate stimuli hinder, and very strong stimuli inhibit cell activity". The respiration of yeast was shown by M. Geiger-Huber (206) to increase proportionally with the concentration of glucose from 0.0001 to 0.002 molar while it is independent of the glucose solutions with concentrations from 0.01 to 0.16 molar. M. Church (100) showed that yeasts have a high sugar tolerance and can live in 70 per cent sugar solutions having an osmotic pressure of 260 atmospheres. H. Schopmeyer (544-545) found some molds to be symbiotic to yeasts, producing a substance which activates yeast growth by an extra cellular action.

The exact action of some foreign substances on yeast is little understood. W. Bancroft (32) discovered that yeast cells coagulated by a 2 per cent solution of amyl alcohol are brought back to normal transparency on removal of the alcohol. This favors Claude Bernard's theory of narcosis which states that "anesthesia is due to a reversible coagulation of proteins of the sensory nerves". C. Winslow (655)
reported that certain cations coagulate cell colloids and that their order of increasing activity is sodium, potassium, lithium, barium, magnesium, calcium, manganese, zinc, and cadmium. G. Knaysi (506) found no difference in the manner of death in small and large cells and that cells take up much iodine in the colloidal parts and give it up on dilution. In this respect the mechanism of disinfection closely resembles dyeing. T. Bokorny (66) found that poisons are taken up by the protoplasm of the cells and that the solutions are decreased in concentration by this amount. O. Rahn (506) in studying the order of death of organisms found that one or more inactivated molecules make the cells appear dead but that more than one molecule must be destroyed before yeast is killed.

Grains contain substances that are harmful to yeast growth as does yeast itself. P. Hayduck (254) found that crushed rye, wheat, and barley have a poisonous effect upon yeasts, killing 99 per cent in 5 to 7 minutes. Witte's peptone and white of egg have the same effect; therefore, the poison must be an albuminoid. This poison is not extracted from the grain by water but is extracted by yeast juice of 0.1 per cent hydrochloric acid. These poisons are destroyed by proteolytic enzymes and are counteracted by calcium carbonate, sulfate and chloride as well as by salts of barium and zinc. The poisonous property differs with grains as different
temperatures are required to destroy it. He also found that yeast produces similar compounds that act as poisons on yeast. H. Lange (330) reported that the yeast poisons in grains vary according to temperature, aeration, nutrition, and stimulative influences. Wheat, rye, and barley show a marked poisonous effect while corn and oats do not show the presence of poisons. He found that distillery yeast is less sensitive to these poisons than are beer yeasts. T. Bokorny (64) studied the harmful action of enzymes and found that diastase, pepsin, trypsin, papystin, and rennet are poisonous to yeasts while bacteria is less affected.

The action of disinfectants on yeasts has had considerable attention. From his studies, E. Fulmer (191) stated that yeasts grown in dilute alcohol solutions (up to 4 per cent) are more resistant to the action of phenol than those from water worts. E. Fulmer (195) also found that the resistance of individual cell varies although yeast, subjected to the action of phenol or benzol and alcohol, does not give a quantity of cells that fall on the logarithmic curve. P. Lindner (349) found that the temperature of cultivation affects the resistance of yeast to the action of antiseptics, and the optimum cultivation temperature for this resistance varies with the yeast.

E. Navassart (418) stated that boric acid (2-5 per cent)
benzoic acid (sat.), salicylic acid (sat. and 1/2 sat.),
alcohol (5-10 per cent), and toluene (sat. and 1.2 sat.) do
not influence yeast autolysis or nuclease, while a 1 per cent
solution of formaldehyde inhibits autolysis completely.
H. Euler (152) found that, in adopting itself to sodium
fluoride, yeast forms protective substances and these substances
are capable of retarding fermentation. The degree of retardation
depends upon the length of time the yeast is in contact with
the salt solution. W. Newton (441) determined the lethal
power of certain substances as phenol coefficients. With
phenol 1:110 as 1, hexylresorcinol 1:15000 is 135, mercuric
chloride 1:9000 is 81.4, picric acid 1:250 is 2.3, and silver
nitrate 1:9000 is 81.4. Cadmium sulfate, potassium di-
chromate, urea, potassium iodide, and sodium benzoate in the
concentrations used were not lethal to the yeast. According
to W. Cruess (112), the concentration of sodium benzoate,
sulfite, and salicylate required to prevent the growth of
yeasts, molds, and bacteria is greater at a pH of 5-9 than at
a pH of 2-4.5. The growth preventing concentration of sodium
chloride and formaldehyde is affected only moderately from
pH values of 2 to 9.

The acidity and pH value play an important part not only
in the effect of foreign substances on the growth of yeast
but also in its growth in normal mediums. W. Van Lear (612)
found that with Pasteur's solution the optimum growth is
secured at a pH of 6.8, while with a wort of equal proportions of malt and raw grains, it is 416. H. v. Buler (166) showed that the maximum alkalinity tolerated by Frohberg yeast is at a pH of 7.7-8.0 and by saccharomyces ellipsoides at 7.9. According to G. Svanberg (584) the optimum conditions for top yeast is pH 3.6 and bottom yeast 4-6. Torula have two optima, pH 2.5 and 6. He found no relation between acid production and acid tolerance of the yeasts. The addition of mineral acids transforms yeast from the flocculent to the powder form, which has a high peptic property, as was shown by F. Schöenfeld (543). W. Henneberg (257) found that treatment of yeast with 5 per cent sulfuric acid for 30 minutes kills the young, unripe cells. E. Moufang (399) found that phosphoric acid treatment of yeasts has a mechanical disinfecting and physiological action. From studies on degenerated yeasts, E. Moufang (400) was able to reactivate them by washing with phosphoric acid. This treatment increases the germinative property and fermenting power of the yeast. J. Brown (77) found that hydrogen peroxide added to aerated cultures of yeast causes a gradual reduction in pH value from 7 to 2; however, this treatment inhibits growth and in some cases stops it entirely.

Ozone was used by G. Nowark (448) as a disinfectant, and it freed yeast of objectionable organisms which were more susceptible to destruction. It also eliminated the weakened
cells and stimulated the activity of the surviving ones.

The halogens and their compounds affect yeast in various ways depending upon their concentration as well as what they are combined with. K. Scharrer (533) found that inorganic salts of iodine stimulate the rate of reproduction of yeasts and that iodine plays an insignificant role in yeast metabolism. K. Scharrer (534) also found that both the iodide and iodate ions stimulate growth and that although molecular iodine in alcoholic or aqueous solution is more toxic than the iodide ion, it also stimulates growth when present in very small concentrations. He also found that solutions of alival and iodoethyl-thiosinamine stimulate growth in concentrations of 0.000001 per cent of iodine, while they are toxic in concentrations of greater than 0.00005 per cent of iodine. A. Kossowicz (316) secured only very small amounts of free iodine when yeast was grown in a potassium iodide-mineral-sugar solution.

H. Speak (569) found that sodium chloride is stimulating to yeast in concentrations up to 1.5 per cent and that the logarithmic phase is lengthened by increasing concentrations. The action of sodium chloride is on the cell division and not on its metabolism. I. Moni (445) showed that the growth of Saccharomyces cerevisiae is retarded in concentrations of sodium chloride of greater than 0.5 per cent, while it is
accelerated by potassium chloride solutions up to 2 per cent. According to E. Bachrach (25) yeast cultivated in potassium chloride solutions of 100 parts per 1000 has an optimum growing temperature of 33° C., whereas the normal yeast has an optimum of 30° C.

S. Mizia (394) found that the optimum stimulating concentrations of some salts are potassium chloride 0.2 M.; magnesium chloride 0.1 M.; calcium chloride 0.01 M.; sodium chloride 0.001 M., while the inhibiting concentrations are potassium chloride 2.2 M.; and sodium chloride 0.2 M. F. Boas (59) reported that the addition of bile salts to the nutrient media reduces the tolerance of saccharomyces cerevisiae to sodium chloride in concentrations of 0.6 to 0.8 M. E. Caserio (84) showed that bromates act similarly to other halogen compounds, 0.04 per cent stimulate, while 0.1 per cent causes the growth of saccharomyces cerevisiae to cease.

The phosphates have long been considered as one of the mineral substances necessary to the correct functioning of yeasts, and analysts have found them to be one of the principal minerals of yeast ash. According to E. Elion (143) distillery yeast absorbs phosphorous in direct proportion to the quantity of yeast formed, and the absorption is greater and more rapid in neutral than in acid media. H. v. Euler (150) found that yeast dried at less than 40° C. yields with water
a solution of an enzyme which causes phosphates to enter completely into organic combination with the products of partial fermentation of glucose and sucrose. This same action does not take place with unfermented sugars nor does the action on partially fermented sugars proceed to the evolution of carbon dioxide. E. Caserio (84) determined that phosphates stimulate growth of Saccharomyces cerevisiae at an optimum concentration of 0.2 per cent, while growth ceases at 0.5 per cent. The presence of phosphates causes an increase in the rate of fat formation and storage by yeast cells as was shown by T. Maclean (364).

The effects of various cations have been investigated although in some cases the results secured may be a composite of the action of all the ions present instead of that of an individual ion. F. Genaud (207) discovered that there is a rapid interchange of cations between the cellular membrane and the surrounding fluid when yeasts are placed in solutions of dissociable salts. Complete equilibrium is secured in 60 minutes except with silver which is aided by reduction. With potassium, ammonium, and lead ions the vacuoles contain one-half as much of the ion as does the membrane.

A. Lasnitzki (332) cultivated yeasts in synthetic media to which was added the cation being studied. He found that lithium ions increase growth slightly, sodium and caesium ions intermediately, and potassium and rubidium ions
strongly. He also found that magnesium can not replace calcium for yeast growth.

T. Bokorny (62) studied the effects of a large number of compounds on yeast. He found that potassium oxalate (0.5 to 10 per cent), secondary potassium phosphate (1 per cent), potassium sulfate (4 per cent), potassium chlorate (0.1 per cent), potassium perchlorate (0.5 per cent), sodium fluoride (0.05 per cent), sodium chloride (24 per cent), sodium sulfate (1 per cent), and dilute arsenic acid are practically harmless to yeast while potassium bromide (0.5 per cent), cerium sulfate (0.05 per cent), potassium iodide (over 0.5 per cent), rubidium sulfate (0.05 per cent), titanium chloride (0.05 per cent), sodium carbonate (0.05 per cent), zinc sulfate (1 per cent), nickel sulfate (0.5 per cent), potassium permanganate (0.01 per cent), zinc chloride (0.2-1 per cent), silver nitrate (0.001 per cent), gold chloride (0.1 per cent), osmium oxide (0.001 per cent), and caesium sulfate inhibit growth to a more or less degree. The salts, cadmium sulfate (0.025 per cent), ferrous sulfate (0.5 per cent), lead acetate (0.1 per cent), copper sulfate (0.1 per cent), and mercuric chloride (0.01 per cent), are all sufficiently toxic to kill the yeast cells.

The ammonium salts can be utilized by yeasts as a source of nitrogen and the effect of various concentrations of these salts, as well as of ammonia itself have been investigated.
T. Bokorny (61) found that free ammonia in aqueous solution of more than 0.05 per cent retards yeast growth. In solutions of lower concentrations yeast fixes chemically a considerable quantity of ammonia. The ammonium salts are less toxic than ammonia while phenyl-hydrazine is one of the strongest yeast poisons encountered. He stated that some ammonia is absorbed by the yeast protoplasm.

E. Fulmer (197) stated that the concentration of ammonium chloride, sulfate, nitrate, and tartrate, which produces the greatest yeast growth is identical with the concentration causing the least swelling in wheat gluten. This concentration is 0.0353 N., and the action is due to the ammonium ions and is not a function of the pH value. E. Fulmer (190) reported that a 4.8 per cent solution of ammonium fluoride kills yeast cells in 38 minutes, while the addition of 2.5 per cent alcohol causes completion of kill in 13 minutes. Yeasts can be acclimated to the action of ammonium fluoride either by stepping up the concentration on successive seedlings or by planting direct in a concentrated solution. The maximum concentration for yeast to grow was found to be 7 grams of ammonium fluoride per liter. He stated that a portion of the cell becomes paralyzed and then reproduces resistant cells.

F. Lieben (341) found that chloride, nitrate, and ammonium ions have no effect upon sugar assimilation by oxygenated
yeast, while the sulfate, thiocyanate, iodide, potassium, and magnesium ions increase and the fluoride ions decrease the assimilation. The Hofmeister series does not hold true in this case. According to A. Kossowicz (315), the nitrates are not suitable sources of nitrogen for yeasts. However, they are changed to nitrites and ammonia by molds. S. Nicolau (442) showed that the action of nitrates depends upon the seedings of yeast. For a fixed concentration, the action of small seedings is retarded. There is no action on medium seedings but large seedings are stimulated. He found that nitrates have no effect upon the normal products but nitrites have a general unfavorable action and eventually destroy the cells.

The action of the heavy metals upon yeasts is of importance due to the fact that these materials are used for construction of yeast tanks and also are found in waters that are used in the fermentation industries. K. Trautwein (602) showed that 0.0001 M. aluminium sulfate reduces fermentation by 50 per cent and inhibits growth, while 0.01 M. kills the yeast cells.

From his studies with different yeasts, H. Green (224) decided that microorganisms vary widely in their tolerance for arsenates and arsénites. J. Effront (138-139) showed that yeast is killed by 75 milligrams of arsenous oxide per liter but doses of three times this concentration can be
used after a gradual acclimatization of the yeast. Yeast produces a substance to neutralize some poisons; for arsenic, it is hydrogen sulfide; for fluorides, it is calcium; and for formaldehyde, it is an oxidase.

H. Bortels (75) stated that iron and zinc are essential to yeast life, while copper is of no importance. According to C. Elvelyen (147), small amounts of iron accelerate the yeast growth and increase the cytochrome content, while the addition of both copper and iron further increase the yeast growth. Copper has the property of stimulating the formation of certain hematin compounds with 0.02 milligrams per 200 c.c. of medium being the optimum concentration. He also found that iron is not assimilated by yeast at a pH of 7 but is readily taken up at a pH of 4. J. McHargue (334) found that copper stimulates cell division with the optimum concentration at 7.5 p.p.m., while manganese and zinc have a less stimulative action at their optimum of 10 p.p.m.

High concentrations of these ions eventually cause the death of the cells. E. Kayser (294) showed that different yeasts act differently to manganese salts and that sugar concentration and acidity of the media affect the allowable concentrations. G. Nadson (415) found that metal plates, suspended from the top of Petri dishes and 1-2 m.m. away from the culture retard the development in the order of lead, copper, and aluminum.

The salts of mercury have been used as disinfectants for
microorganisms, but F. Nottin (447) found that as a metal, mercury increases the growth of yeast, although in acid worts it produces salts which delay or retard growth. He showed that if the concentration of the mercury salts is not too great, they destroy the supersaturation of carbon dioxide and allow a better respiration by the yeast.

E. Hanorlow (372) stated that the salts of nickel are less destructive and are less inhibitory on the growth of microorganisms than copper and most other heavy metals. E. Zerner (669) reported that the toxicity of silver salts depends upon the amount of yeast present and the difficultly soluble salts owe part of their toxicity to the ratio of salt to the amount of yeast settling out of suspension. Silver nitrate and silver carbonate are quite toxic, but metallic silver is without effect.

The necessity of vitamins for yeast growth is a controversial subject, although the increase in yeast activity is one of the methods of estimating vitamin B. According to F. Bachmann (24), yeasts grow better and ferment more readily in mediums containing vitamins. Some require very little vitamins, while others need large quantities. R. Baeste (27) showed that solutions rich in vitamins increase yeast crops at least one-third. However, amino acids from malt give a similar crop with a higher nitrogen assimilation. According to E. Fulmer (192), vitamin B is
not necessary for yeast growth. He found that extracts of wheat embryo and alfalfa contain materials which maintain yeast growth. Although no vitamins were added, he reported the most satisfactory medium for growing yeast at 30°C is 0.189 grams ammonium chloride; 0.1 grams calcium chloride; 0.1 gram secondary potassium phosphate; 0.04 gram precipitated calcium carbonate; 0.6 gram dextrin; and 10 grams sugar, per 100 c.c. of water. V. Lepeshkin (338) found that yeast does not grow normally in synthetic media when seeded with 1-2 cells but that large seedings do develop normally. The addition of vitamin B shows a decided increase in reproduction in comparison to the control.

T. Philipson (489) separated vitamin B, Activator Z and bios from each other. He found that the separated Activator Z does not show the growth promoting action similar to bios. He concluded that Activator Z is not an individual compound but did not determine whether its components have additive effects or neutral influence. T. Philipson (488) further found that autolysates from bottom yeast give twice as much Activator Z as from top yeast and that normally it is stable to both acids and alkalies. Bios stimulates the respiration of yeast, and R. Norris (446) found that this stimulation is similar to that produced by exposure of yeast cells to ultraviolet light. R. Fulmer (193) determined that bios is composed of at least two yeast growth promoting materials
and that a mixture of the two give greater growth than either one separately. O. Richards (511) found that certain samples of asparagin contain thallium which may be one of the yeast stimulants referred to as bios. He found that 0.001 milligram of thallium per c.c. increases the yield of yeast by 80 per cent. T. Philipson (490) prepared extracts from barley, tea powder, green peas, wine, and yeast and found that only the green pea extract strongly stimulated yeast growth.

Organic compounds vary in their effect on the growth of yeast and although there is some relationship in groups, each compound presents an individual problem requiring study. N. Clark (103) found that phenol (0.1 per cent), methyl green (0.01 per cent), acetone (1.0 per cent), and acetic acid (0.07 per cent) retard the growth of yeast but that in most cases organisms can acclimate themselves to these chemicals if the concentration is gradually increased. H. v. Euler (154) showed that 0.05 per cent of sodium salicylate, guaiacal, and acetaldehyde activate yeast, while greater concentrations are toxic. He found resorcinol and hydroquinone have little effect, while acutanilide and quinine sulfate are toxic. According to H. Rufferath (323), 1.1 per cent oxalic acid inhibits, while malic acid is utilized and did not inhibit growth of yeast up to a concentration of 7.4 per cent. Lactic acid is utilized by
some yeasts up to a concentration of 10 per cent.

Many dyes affect yeasts and some are used to distinguish the percentage of dead cells as well as to differentiate the different parts of the yeast cell. H. Fink (182) recommended the following solution for determining dead cells. 1 part methylene blue solution (1:10,000) and 1 part buffer solution (1 part 0.2 M secondary sodium phosphate and 329 parts 0.2 M primary sodium phosphate). H. v. Euler (165) concluded that dyesuffs penetrate the cells and are retained by chemical combination or precipitation. They become united to the surface by chemical combination with the proteins or by adsorption, with the degree of adsorption being affected by previous treatment of the yeast, concentration of the dye, pH value, and activity of the yeast. M. Geiger (205) found that added neutral red (1:10,000) raises respiration as much as 30 per cent.

The action of some miscellaneous compounds on yeasts is as follows:

P. Rona (519) found that quinine hydrogen chloride (0.002 M) inhibits respiration and that this effect increases with pH value and time, due to the permeability of the cell walls. According to G. Lejhanec (334), quinine in a concentration of 1:500,000 increases oxygen consumption,
lowers multiplication, and increases mortality, while weaker solutions have no effect and stronger solutions increases mortality. E. Sechi (551) showed that caffeine in dilutions of 1:1000 to 1:10,000 stimulate growth. O. Schwarz (548) found that adrenaline causes yeast cells to assimilate substances otherwise not available because they are not diffusible. Some of these are glycogen, casein and alanine which are changed to fermentable compounds.

T. Bokorny (67) determined that the following substances can be utilized by yeasts: citric, acetic, and tartaric acids, glycerol, asparagin, peptone, mannitol, pentoses, and alcohol. Hexylresorcinol was shown by J. Brown (78) to completely inhibit yeast growth and fermentation in concentrations of 0.1-0.3 grams per liter. According to C. Bertrand (48) chloropicrin (1 miligram per liter) retards fermentation, while 5 to 6 miligrams per liter completely arrest it without killing the yeast. L. Plantefol (494) found that 2, 4-dinitrophenol retards growth but increases respiration of yeast. It is reduced by some yeasts to 4-amino - 2-nitrophenol and by others to 2-amino - 4-nitrophenol. I. Asheshov (20) found yeast is not killed by toluene in 72 hours and that oxygen makes yeast more resistant to toluene. A. Ling (350) reported that cultures of certain common yeasts have been kept alive for 34 years which proves the longevity of the average yeast.
The effects of heat, light, electric currents, and ultra-violet light have been investigated with varying results. P. Sherwood (556) found the optimum temperature to be 42° C. for growing yeasts in beer wort.

R. Tracy (601) stated the lethal effect of a 60 cycle alternating current varies with the current density and quantity of current and may be due to the formation of temporary toxic substances. C. Winslow (656) determined that yeast cells carry a negative electric charge and migrate when exposed, in solution, to a direct current field.

A. Remhard (509) found that yeast develops more rapidly when exposed to normal light. Of the other light rays, yellow light is the most favorable, blue light least favorable, while ultra-violet light has an inhibitory action. J. Woodrow (657) showed that yeast culture media, when exposed to ultra-violet light radiations, develops a toxicity which is non-volatile and results from action of short waves (300-425 u.u) on sucrose. According to H. Valentine (609), ultra-violet rays inhibit the growth of yeast.

G. Palmieri (469) found that ultra-violet and infrared rays exert a mitogenetic effect which seems to follow the Arndt-Schultz law. B. Feuer (177) stated that yeast is destroyed in a few minutes when exposed to ultra-violet
light. H. Cernovodeane (31) showed that exposure to ultra-violet light causes the protoplasm of yeast cells to become granular. J. Beauverie (37) found that certain yeasts resist the lethal action of ultra-violet light and that this resistance increases with the glycogen content. R. Oster (46), using monochromatic ultra-violet light, found the older cells more resistant than those in the reproductive stage but that there is no toxic substance given off by the killed cells. He believed that the effect of ultra-violet light may be due to the absorption of energy by the nucleoproteins of yeast.

R. Oster (46) could not secure any stimulative action on saccharomyces cerevisiae with ultra-violet light.

P. Wels (625) found that exposure of yeast cells to Röntgen rays causes a decrease in their rate of reproduction, while the oxygen consumption and carbon dioxide production is not changed. K. Sugiura (583) reported that the growth promoting factors in yeast are partially inactivated by exposure to radium emanations.
THE ENZYMES OF YEAST
For a long time the action of yeast was considered a simple cellular function and this action was merely the changing of sugars to alcohol and carbon dioxide. The mechanism of the action on sugars was not understood, and the requirement for other substances was considered as being purely one of growth necessity without regard to the products that may be formed. Today, yeast appears as it is, a very complex organism producing and secreting a number of substances that either react with the chemicals present in the substrate, or, due to their catalytic property, cause chemical changes to take place. These secreted substances are the enzymes, and it is on their actions that all of the fermenting industries are based.

These enzymes can be grouped according to their action upon different types of compounds, but a further classification of the individual groups has not been possible due to the lack of knowledge of the mechanism of some of their actions. The general groupings with their members and each individual enzyme's specific action are as follows:

1. Enzymes which act upon the carbohydrates.
   (a) Sucrase or invertase - changes saccharose to glucose and levulose.
   (b) Maltase - changes maltose to glucose.
   (c) Trehalase - changes trehalose to glucose and levulose.
(d) Raffinase - changes raffinose to levulose and melibiose.

(e) Melibiose - changes melibiose to dextrose and galactose.

(f) Melizitase - changes melizitose to glucose and turanose.

(g) Glycogenase - changes glycogen to dextrose.

(h) Amylase - changes amylose to maltose.

(i) Amylopectinase - changes amylopectin to dextrin.

(j) Dextrinase - changes dextrin to maltose.

2. Enzymes responsible for the cell functions and fermentation.

(a) Zymase - a group of enzymes which accomplish the change of sugars to alcohol and carbon dioxide.

(b) Carboxylase - decomposes pyruvic acid to carbon dioxide and acetaldehyde.

(c) Hexases and phosphatases - change hexoses in presence of phosphates to hexose diphosphate.

(d) Aldehydase - breaks down phosphoglyceraldehyde - a Cannizaro reaction.

(e) Co-zymsae - an activator of zymase.

3. Enzymes which act upon the proteins - proteases and creptases.

(a) Endotryptase - dissolves albuminous materials.

(b) Guanase - changes guanine to xanthine.
(c) Amidase - changes amino acids to ammonia and volatile acids.
(d) Anti-protease - protects albuminous material from endotryptase.
(e) Peptidases - changes peptides into amino acids.

4. Enzymes which act on fats. Esterases.
(a) Lipase - changes fats to glycerine and fatty acids.

5. Oxidizing and reducing enzymes.
(a) Catalases - reduces hydrogen peroxide to water and oxygen and probably acts on organic peroxides which are formed as intermediates.
(b) Hydrogenase - decolorizes methylene blue and forms hydrogen sulfide with sulfur.
(c) Oxidases - changes aldehydes to acetic acid and can oxidize alcohol in presence of air.

Of the above enzymes, the first of importance from the standpoint of fermentation are the enzymes which act upon the carbohydrates. Under the Enzymes of Malt there may be found a brief general discussion of the theory of enzymes. The same general theories will also govern the reaction and mechanism of these actions of the enzymes of yeasts. However, B. Woolf (659) concluded that enzymes are chemical compounds able to form addition compounds with all their substrates. The process of catalysis consists
of a series of tautomeric changes in the enzyme - substrate complex as a result of which it can dissociate into free energy and reaction products. This theory accounts for the observed effects of substrate concentration, pH values, and salts, on the velocity of the catalyzed reaction. Although this theory answers some questions pertaining to enzyme actions, it is not confirmed by the isolation of the enzymes as chemical compounds, nor does it explain the difference in ultimate analyses of the same enzyme showing the same concentration.

The specific actions and conditions which affect the principal enzymes are as follows:

Sucrase, saccharase or invertase.

This enzyme causes the hydrolysis of sucrose according to the equation:

\[ \text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + \text{C}_6\text{H}_{12}\text{O}_6 \]

Sucrose \hspace{1cm} glucose \hspace{1cm} levulose

This enzyme is an intra cellular one; however, small amounts of it diffuse into the surrounding liquid. Sucrose is converted by the enzymes in the cells as well as the part that diffuses out. In the former case, the sucrose diffuses into the cell and the hydrolyzed products diffuse out. This enzyme is destroyed by pasteurization temperatures and its absence in fermented liquids shows pasteurization.
The enzymes were divided by E. Pantanelli (471) into ecto-enzymes or a secreted enzyme and endo-enzymes or a contained enzyme. He classed invertase as an ecto-enzyme and found that the permeability of yeast cells for invertase decreases similarly to their permeability to sodium and ammonium chlorides and ammonium sulfate. The highest permeability of the salts was found to be at the time of the most vigorous fermentation and is decreased by gum arabic and peptone. He concluded that the secretion of invertase is a true secretion caused by an increase in the permeability of the cell membranes during fermentation.

H. v. Euler (157) found that invertase is increased as much by the fermentation of pyruvic acid as of sucrose and mannose.

Various methods of expressing the purity of invertase preparations are all based upon the "time-value" test. In this test the "time-value" of an invertase preparation is expressed as the time, in minutes, required for 0.05 gram of the preparate to invert 4.0 grams of sucrose (in 25 c.c. of a 1 per cent solution of primary sodium phosphate at 15.5 c.c.) to zero rotation with sodium light.

The hydrogen-ion concentration has been shown to be of greater importance to the action of invertase than the acidity. The optimum range of pH seems to be from 3.5 to 5.5 at 55° C. H. v. Euler (161) showed that at 16° C. the pH optimum for invertase action is 4.67-5.07. Increasing
the pH to 7.0 causes a decrease in invertase activity to 10 per cent of its original value. By using a pH value of 2, R. Willstätter (632) found the fermentation is retarded 20-30 per cent and the invertase reduced to 7 per cent of its original value. He also showed that dilute acids and alkalies diminish the invertase content of yeast without killing the organism or affecting fermentation. 95 per cent of the invertase is destroyed in one hour by 0.15-0.3 N sulfuric acid or sodium hydroxide.

F. Stoward (580) showed that sulfuric, hydrochloric, nitric, acetic, and phosphoric acid in certain concentrations exert a favorable influence on invertase. However, a 0.2 per cent solution of sulfuric, hydrochloric, and nitric acids almost completely check the action of the invertase.

Since the action of invertase of yeast is dependent not only upon itself but also on its ability to diffuse through the cell membrane, any substance which will affect either of the above will cause an over-all effect upon activity. T. Panzer (475) found that treatment of invertase with hydrogen chloride inhibits its action due to the formation of an internal anhydride and not an addition compound. An inhibitory action on invertase by the chlorides of the alkalies, alkaline earths, and magnesium was shown by S. Neuschlossz (440) to be related to the valency of ions used.
He believed that the inhibition is due to the diminution of the active enzyme surface, since it parallels the disperseness of the solution of enzymes.

The invertase concentration of yeast may be increased or accelerated by the presence of certain substances. E. Miller (333) found that an alcoholic extract of yeast, when added to growing yeast, increases the amount of invertase but does not accelerate its action. F. Pavy (482) found that the inverting power of yeast invertase is increased 10-15 times by adding a boiled extract of yeast. He stated that this action is due to the acidity, as analogous results were secured by adding acetic acid. S. Lögren (353) after studying the effects of solutions of various neutral salts, yeast water, alcohol, acetone, etc., as a pretreatment of yeast, concluded that the neutral salts have little effect on the inverting power of top yeasts. He also concluded that the inverting power can be increased by pretreatment and that the nitrogen nutrient should be regarded as essential if the increase in inverting power is due to an augmented vitality due to the pretreatment.

H. Colin (105) concluded that there is a relation between the concentration of sucrose, which gives the maximum initial velocity of inversion with a fixed amount of enzyme, and the point at which this velocity begins to diminish. The products of hydrolysis do not affect this relationship.
Substances such as methyl and ethyl alcohol, glycerol and silver nitrate displace the point of maximum velocity.

T. Bokorny (70) found that invertase is not destroyed by 0.1 per cent solutions of formaldehyde, mercuric chloride, or silver nitrate, although these solutions affect the other functions of yeast. According to T. Panzer (477) a part of nitrous oxide is bound chemically to invertase and is further oxidized by atomic oxygen. W. Gies (219) showed that invertase is very active in the presence of a 2 per cent solution of beryllium sulfate.

From extensive work with the alkaloids of the atropine, cocaine, and morphine groups, P. Rona (520) concluded that their inhibitory effect on invertase is proportional to the logarithm of the alkaloid concentration. When the alkaloid concentration is constant, the inhibitory effect increases with the pH value.

Invertase, like most enzymes, is affected by light of different wave lengths. C. Gorbach (223) found that brief irradiation with ultra-violet rays increases the invertase activity of yeast and that repeated irradiations of short intervals are better than one of a long interval.

MALTASE

This enzyme causes the hydrolysis of maltose according
to the equation:

\[ C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6 \]

Maltose \hspace{1cm} Glucose

According to A. Taylor (593), this hydrolysis follows the course of a mono-molecular reaction. Other investigators believe that the reaction proceeds more slowly than one of the first order.

The "apparent maltase unit (A.E.)" has been set up as a standard of enzyme evaluation. This unit represents "The quantity of enzyme which splits half of 2.5 grams of maltose hydrate in 50 c.c. of solution at a pH of 6.8 and 30\(^\circ\) C. in 1 minute".

B. Inonye (276), in his studies on the heat produced by enzymes, found the enzyme hydrolysis of 1 gram of maltose to produce 4.66 calories.

This enzyme generally occurs in conjunction with the amylolytic enzymes, the amylases. It is an endo-enzyme, and it is believed that maltase is accessible to the diffused sugars only after the cell is dead.

F. Schönfeld (541), after killing yeast by adding toluene and filtering, found the cell walls of bottom yeast are impermeable to maltase, while those of top yeast are permeable.
F. Schönfeld (540) found that the maltase activity of brewers yeasts is a function of the fermenting activity.

The optimum pH for the action of maltase varies with the temperature, condition of the yeast, and substrate. According to P. Rona (518) the optimum action is between a pH of 5.8 and 6.6, while K. Isaiw (278) reported the optimum to be from 6.1 to 6.7. R. Willstätter (631) found the optimum to be at a pH of 6.75 to 7.25 with a pH of 6.1 being less favorable than a pH of 7.

R. Willstätter (630) also reported that for yeast rich in maltase its action is completely suppressed at a pH of 4.5, while for yeasts poor in maltase it requires a pH of 3.5 for the same action.

Many substances affect the action of maltase, and L. Michaelis (392) concluded the retardation effect is due to either the added substances causing a reduction in the reaction constant of the enzyme action or these substances uniting with the enzyme because of chemical differences.

F. Schönfeld (542) found the production of dextrose to be much slower when toluene was added to a yeast - beer mixture (containing 2 per cent maltose) than from a pure maltose - yeast suspension. Since pastuerization also reduces the rate of maltase activity, he concluded that this reduction in activity is due to the elimination of oxygen. F. Schönfeld (539)
found that a 4 per cent solution of potassium phosphate or a 0.6 per cent solution of lactic, phosphoric or tartaric acids stimulate the activity of maltase.

H. v. Euler (151) showed that toluene and thymol diminish the maltase activity of yeast while chloroform inhibits it entirely. According to H. Roger (517) uranium acetate in small amounts inhibits maltase activity, but the addition of secondary sodium phosphate, to neutralize the uranium, removes the inhibitor.

The reducing power of yeast enzymes was found by R. Murakami (411) to be inversely proportional to the intensity of the lights.

The other enzymes of yeast which act upon the carbohydrates changing them to fermentable products are raffinase, trehalase, melibiase, melizitase, glycogenase, amylase, amylopectinase, and dextrinase. These enzymes, with the exception of amylase, have not had the attention of the more important ones. However, the actions of some of the above enzymes are the controlling factors in the production of good yields from grain mashes. The specific actions of these enzymes except amylase, which was discussed under malt, are as follows:

Raffinase

\[ \text{C}_{18}\text{H}_{22}\text{O}_{16} + \text{H}_2\text{O} = \text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{C}_6\text{H}_{12}\text{O}_6 \]

raffinose melibiase levulose
Trehalase
\[ \text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O} = \text{C}_6\text{H}_{12}\text{O}_6 + \text{C}_6\text{H}_{12}\text{O}_6 \]
trehalose glucose levulose

Malibiose
\[ \text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O} = \text{C}_6\text{H}_{12}\text{O}_6 + \text{C}_6\text{H}_{12}\text{O}_6 \]
melibiose dextrose galactose

Melizitase
\[ \text{C}_{18}\text{H}_{32}\text{O}_{16} + \text{H}_2\text{O} = \text{C}_6\text{H}_{12}\text{O}_6 + \text{C}_{12}\text{H}_{22}\text{O}_{11} \]
melizitose glucose turanose

Glycogenase
\[ \text{C}_6\text{H}_{10}\text{O}_5 + \text{H}_2\text{O} = \text{C}_6\text{H}_{12}\text{O}_6 \]
glycogen glucose

Dextrinase
\[ 2\text{C}_6\text{H}_{10}\text{O}_5 + \text{H}_2\text{O} = \text{C}_{12}\text{H}_{22}\text{O}_{11} \]
dextrin maltose

The enzymes that catalyze the cell functions and fermentation are responsible for the degradation of the carbohydrates to the finished products, principally alcohol and carbon dioxide, although many other substances may be formed in small amounts. Of these enzymes the most important is the complex enzyme zymase.
ZYMASE

Zymase is considered today as a complex system and may be made up of a number of similar substances acting in unison or consecutively one on the products of the other. This enzyme is responsible for the changing of the fermentable monosaccharides into alcohol and carbon dioxide, although the reaction may take place in stages with the formation of intermediate products. The fermentation reaction was originally associated with the cell metabolism of yeast until the zymase activity was isolated from the life process by mechanically destroying the yeast cells and filtering the liquid free of the cellular structure. This filtrate has the power to ferment sugars and is termed yeast "press-juice".

Originally the action of zymase was considered as catalyzing the reaction:

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

Today the above equation has been shown to express only an over-all effect and not the actual processes involved. There are many theories for the reactions involved in the enzyme degradation of sugars. The basis for these theories is the fact that this degradation has been stopped at intermediate stages and the intermediate compounds determined. This division of the action of the zymase complex into stages
has made it possible to answer many questions pertaining to
the effects of the absence or presence of chemical substances.
One of the newer theories of the formation of intermediate
compounds was advanced by C. Meyerhof (390) and is as
follows:

\[ \text{Hexose} + \text{Hexose-diphosphate} \]

1.

\[ \text{glucose} + \text{hexose-diphosphate} \rightarrow \text{phosphoglyceraldehyde} \]

2.

\[ \text{Phosphoglyceraldehyde} \rightarrow \text{Phosphoglycerol} \]

3.

\[ \text{Phosphoglyceric acid} \rightarrow \text{pyruvic acid} \]

4.

\[ \text{pyruvic acid} \rightarrow \text{acetaldehyde carbon dioxide} \]
5.

\[ 2\text{CH}_2(\text{PO}_4\text{H}_2)\cdot\text{CHOH.CHO} + 2 \overset{\text{(Oxidation)}}{\rightarrow} 2\text{CH}_2(\text{PO}_4\text{H}_2)\cdot\text{CHOH.COOH} \]

Phosphoglyceraldehyde  \hspace{1cm} \text{Phosphoglyceric acid}

\[ 2\text{CH}_3\text{CHO} + 2 \rightarrow 2\text{CH}_3\cdot\text{CH}_2\text{OH} \]

\text{(Reduction)} \hspace{2cm} \text{acetaldehyde} \hspace{2cm} \text{alcohol}

This theory brings into play the various enzymes belonging to the zymase complex. By this theory the hexoses are changed to hexose-diphosphate by phosphatase and its co-enzymes. In the presence of phosphoric acid and hexose-diphosphate the sugar is changed to phosphoglyceraldehyde. This in turn is catalyzed by aldehydase in a Cannizaro reaction into phosphoglycerol and phosphoglyceric acid. The pyruvic acid formed from phosphoglyceric acid is changed by carboxylase to acetaldehyde and carbon dioxide. The acetaldehyde and phosphoglyceraldehyde are changed by an aldehydase into ethyl alcohol and phosphoglyceric acid which reenters the reactions. These reactions will proceed as long as sugars and phosphates are present.

Many factors affect the enzymes which cause the change of sugars into alcohol and most of these will be considered under the heading of fermentation. Some of these actions can be directly attributed to the individual enzymes such as was shown by H. v. Euler (151), who found that vacuum dried yeast
retains one-tenth to one-thirtieth of its zymase activity. J. Glocja (221) found that toluenized yeast loses up to 96 per cent of its fermenting activity, but that this loss is not necessarily due to the destruction of the zymase. He further decided that only about 5 per cent of the fermenting activity of yeast is attributable to zymase. According to A. Fernbach (176) nitrates are accelerators to zymase but have no effect on the other enzymes present.

H. Agulhon (15) found that zymase is sensitive to boric acid in very small amounts as a N/620 solution completely inhibits its action. H. v. Euler (171) showed that magnesium was necessary for zymase activity, and that the optimum concentration is between 0.1 and 0.001 molar. By substituting glycerol, ethyl alcohol, or pyridine for water, W. Falladin (468) found that the action of zymase is retarded or stopped. According to W. Van Lear (611) the action of zymase is destroyed by the addition of papain.

One of the principal discoveries relating to the action of enzyme systems was made by A. Harden and W. Young (240) in 1906 when they found in yeast a substance which is heat resistant, and is a specific activator of alcoholic fermentation which is termed "Co-zymase". According to this work fermentation is not observed in the absence of this activator, and the activating action cannot be replaced by other
substances such as vitamins. Although this activator has been concentrated, it has not been isolated as a chemical compound. H. v. Euler (172) defined cozymase as "that substance which brings about the typical carbohydrate cleavage in an otherwise inactive mixture of sugar, phosphate, zymophosphate, apozymase, and magnesium salts and is necessary for fermentation". He concluded that cozymase is a separate entity and is necessary for fermentation. According to K. Myrböck (413), cozymase retains its full activity after digestion with trypsin, diastase, urease, catalase, phosphatase, and erepsin, but is partially destroyed by esterase while bacterial infection removes the cozymase by adsorption. A. Lennerstrand (336) found that a fermenting system of apozymase, cozymase, glucose, hexose-diphosphate, and phosphate buffer is inactivated by sodium fluoride due to its action on the cozymase. A. Lennerstrand (337) also found the addition of more cozymase will reactivate a fermenting system which has been inactivated by fluorides. Although cozymase has been considered essential, C. Neuberg (437) showed that the potassium or calcium salts of pyruvic, a-ketobutyric, a-ketocaproic, hydroxypyrvic, and other acids are capable of activating "coenzyme free" yeast juice and dry yeasts so that they ferment sugars.

In any modern theory on the action of the fermentation enzymes, phosphates always play a major role and at least
one of the reactions is based upon the phosphate combination with the hexoses. J. Weichberz (624) stated that the phosphates form phosphatides which act as buffers and that there is no demonstrable evidence of a zymophosphate as an intermediate step in fermentation. K. Lohmann (352) using neutral mediums found no support for the Waldschmidt-Leitz theory that "oxidation reduction processes govern the intracellular reaction of phosphoric acid and carbohydrates". I. Yamasaki (663) decided that sodium moniodoacetate in low concentrations inhibits phosphatase, but has little effect upon the glycolase and carboxylase. M. Macfarlane (363) determined that hexose-phosphatase is inhibited by sodium moniodoacetate and that arsenates have no accelerating effect upon the liberation of phosphates during the induction period of zymin fermentation. M. Macfarlane (362) also found that the rate of production of phosphate from hexose-phosphate is accelerated by arsenates only in the presence of yeast extracts. Magnesium was shown by H. Albers (16) to have an inhibitory action on phosphatase independent of the pH value.

PROTEASES, AND EREPTASES

It has been known that yeast juice could hydrolyze proteins with the formation of large quantities of amino acids and they named the active enzyme endo-tryptase. R. Willstätter (630) and various co-workers have reported
that there are three proteolytic enzymes in yeast, one acting on proteins at an optimum pH of 5.0, one on polypeptides, and one on the dipeptides with the optimum pH of the latter two being 7.0 to 7.8. Although the presence of proteins in fermentations causes the formation of small quantities of products other than alcohol, it is not within the scope of this work to consider the theoretical actions of the various proteases on the respective protein substances. It is sufficient to say that the proteases are specific in their action towards the proteins.

ESTERASE

The esterases which act upon fats are present in yeast in the form of lipase. This enzyme changes fats into fatty acids and glycerol both of which are found in the products of fermentation. Generally, the lipase of yeast is an intercellular enzyme which acts upon the fats in the cell itself. In some yeasts the lipase seems capable of diffusing through the cell membranes and decomposing the fats in the substrate. The action of foreign substances upon the function of lipase of yeast has had little attention, but the work done on the esterases from other sources should approximate the results that would be obtained.

N. Deleano (123) found that 1 per cent solutions of methyl and ethyl alcohol has a favorable effect on the general
activity of yeasts but little effect upon lipase.

S. Anberg (17), working on food products, has shown that the greater the molecular weight of the acids formed, the less is the effect of fluorides on the action of lipase.

**OXIDASE, CATALASE AND HYDROGENASE**

The oxidases and peroxidases present in yeast have been assumed to have definite functions and have a part in most of the theories of fermentation. The oxidase enzymes have their function in the cell metabolism, while the peroxidases cause the peroxides that are formed to be changed over to non-toxic compounds. In this respect the peroxidases act similarly to the catalases but the existence of the two has been established. Some of the theories of oxidation in cells have been based upon the presence of iron and its ease of changing from the ferrous to the ferric condition and vice versa. However, A. Bach (22) found the presence of manganese and iron is not necessary for oxidase activity. He decided that oxygen is first changed to the peroxide and this acts on the substrate forming primary oxidative products which are changed into end products by peroxidases. According to C. Bertrand (46) the acids form a continuous series of decreasing activity, following the order of electrical conductivities and catalytic effect on the hydrolysis of saccharose.
Catalase is the term applied to an enzyme which decomposes hydrogen peroxide. This enzyme is known to be present in yeast, but its action other than as a hydrogen peroxide is not known as it cannot decompose the organic derivatives of hydrogen peroxide. Therefore, it has not been given a definite function in cell metabolism or in fermentation.

Hydrogenase is an enzyme of yeast that has the ability to change sulfur into hydrogen sulfide. Its action appears in some of the theories of fermentation whereby intermediate aldehydes are reduced to alcohols. The specific functions of hydrogenase as well as other reductases are not well understood. Therefore, their reactions on other enzymes and the reactions of substances upon them have generally been considered in the over-all effect of fermentation.

There has been a great amount of work done on the over-all actions of the yeast enzymes and the effects of certain substances on these actions. K. Myrbäck (412) decided there were three types of enzyme inactivation: 1, a reversible inactivation caused by an equilibrium between the enzyme and the substance; 2, an irreversible inactivation by certain substances such as iodine; 3, an irreversible destruction of the enzyme due to the limited stability of the substance.
According to E. Abderhalden (3), an extract prepared from yeast by boiling with 10 per cent H₂SO₄ accelerates the enzymic hydrolysis of sucrose and maltose and also accelerates the fermentation of dextrose, levulose, and galactose. T. Bokorny (72) found yeast cells could be killed with sulfuric acid, formaldehyde or mercuric chloride without inhibiting the enzyme activity. It has been shown by R. Labes (325) that elementary tellurium and selenium inactivate enzymes. A. Herbert (259) noticed no effect on the enzymes by the addition of the sulfates of thorium, cerium, lanthanum, and zirconium.
YEAST FERMENTATION
Many theories have been advanced as to the mechanism of fermentation by yeasts. One theory is that fermentation is a form of respiration by the yeasts when they find themselves out of contact with oxygen. The yeasts will decompose the available oxygen compounds, thereby securing the oxygen necessary for life. Fermentation then is the equivalent of respiration and the yeasts will decompose the carbohydrates in resisting suffocation and securing the energy for life.

This theory placing fermentation as a type of respiration has been expanded by many workers, some of whom believe that yeasts in their utilization of sugar carry on the first stage of respiration which is anaerobic and is

\[ C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \]

The second phase of respiration takes place in contact with oxygen and is

\[ C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O \]

Some have advanced the theory that fermentation is comparable to the secretion of a toxin. In this theory, the alcohol is the poison by which yeast is able to compete with other organisms, as yeasts can survive an alcoholic concentration of 18 to 22 per cent while most other organisms are killed at a concentration of 5 to 12 per cent.
Other theories of fermentation deal with the mechanism of the reactions. In 1904, after his work on oxidases, J. Grüss (229) advanced the theory that fermentation is composed of the decomposition of glucose by zymase according to the equation:

\[ C_6H_{12}O_6 = 2CH_2OH.CHOH.COH \]

Under the action of the oxidases this intermediate compound is oxidized:

\[ CH_2OH.CHOH.COH + 6O = 3CO_2 + 3H_2O \]

These reactions represent ordinary respiration.

In the absence of air, the enzyme hydrogenase acts on the decomposition product of glucose in two stages as follows:

\[ CH_2OH.CHOH.COH + 3H_2O = 3CO_2 + 12H \]
\[ 2CH_2OH.CHOH.COH + 12H = 3C_2H_5OH + 3H_2O \]

This theory gives full importance to the action of the oxidases and hydrogenases.

A. Lebedeff (333) advanced the theory of the utilization of phosphates in fermentation and presented the following equations as the steps required:

1. \[ C_6H_{12}O_6 = 2(C_3H_6O_3) \]
2. \[ 2(C_3H_6O_3) + 2RHPO_4 = 2(C_3H_5O_2RPO_4) + 2H_2O \]
3. \[ 2(C_3H_5O_2RPO_4) = C_6H_{10}O_4(RPO_4)_2 \]
4. \[ C_6H_{10}O_4(RPO_4)_2 + H_2O = C_2H_5OH + CO_2 + C_3H_5O_2 + 2RHPO_4 \]
   or \[ 3C_6H_{10}O_4(RPO_4)_2 + 2H_2O = 2(C_2H_5OH) + 2CO_2 + 2RHPO_4 \]
C. Neuberg (422-439) and his co-workers have been able to secure acetaldehyde and glycerol from fermentations by the addition of sodium sulfite. This has led to his theory of fermentation which can be expressed as follows:

1. \( \text{C}_6\text{H}_12\text{O}_6 - 2\text{H}_2\text{O} = \text{C}_6\text{H}_8\text{O}_4 \)

   Methylglyoxal-aldol

2. \( \text{C}_6\text{H}_8\text{O}_4 = 2\text{CH}_2 : \text{C(OH)}\text{.COH or 2CH}_3\text{COCCHO} \)

   Methylglyoxal

3. \( \text{CH}_2: \text{C(OH)}\text{.COH} + \text{H}_2\text{O} \quad \text{H}_2 = \text{CH}_2\text{OH.CHOH.CH}_2\text{OH} \quad \text{Glycerol} \)

   \(+\text{(Reduction)}\)

   \( \text{CH}_2: \text{C(OH)}\text{.COH} \quad \text{O} = \text{CH}_3\text{CH(OH)}\text{.COOH} \quad \text{Lactic acid} \)

   \( \text{(Oxidation)}\)

4. \( \text{CH}_3\text{CO.COOH} = \text{CO}_2 + \text{CH}_3\text{CHO} \)

   Acetaldehyde

5. \( \text{CH}_3\text{CO.CH}_2 \quad \text{O} = \text{CH}_3\text{CO.COOH} \quad \text{(Oxidation)} \quad \text{Pyruvic acid} \)

   \(+\text{(Reduction)}\)

   \( \text{CH}_3\text{CHO} \quad \text{H}_2 = \text{CH}_3\text{CH}_2\text{OH} \quad \text{Ethyl alcohol} \)

C. Neuberg (439) has also divided fermentation into three forms. These expressed in equations without consideration are:

1. Normal yeast fermentation

   \( \text{C}_6\text{H}_12\text{O}_6 = 2\text{CH}_2\text{H}_5\text{OH} + 2\text{CO}_2 \)

   Dextrose   Alcohol   Carbon dioxide
2. Fermentation in solutions containing alkaline sulfite

\[ C_6H_{12}O_6 = CH_3CHO + C_3H_6O_3 + CO_2 \]

Dextrose  Acetaldehyde  Glycerol  Carbon dioxide

3. Fermentation in alkaline salt solutions in absence of sulfites

\[ 2C_6H_{12}O_6 + H_2O = CH_3COOH + C_2H_5OH + 2C_3H_6O_3 + 2CO_2 \]

Dextrose  Acetic acid  Alcohol  Glycerol  Carbon Dioxide

The study of the intermediates formed during fermentation, and the role of phosphates has attracted many workers. W. Young (665) found the addition of soluble phosphates increases the rate of carbon dioxide evolution while the phosphates are no longer precipitated by magnesium mixture. The addition of lead nitrate to the solution, after the free phosphates have been removed, precipitates a compound of empirical formula \( C_3H_5O_6 \cdot P\cdot Pb \). A. Harden (241), working with yeast juice, showed the addition of phosphates to glucose and yeast juice causes an increase in the total fermentation, as well as accelerates fermentation. He believed phosphates acted according to the equations:

1. \[ 2C_6H_{12}O_6 + 2R_2HPO_4 \rightarrow 2CO_2 + 2C_2H_5OH + C_6H_{10}O_4 \cdot (PO_4R_2)_2 + 2H_2O \]

2. Then \[ C_6H_{10}O_4 \cdot (PO_4R_2)_2 + 2H_2O \rightarrow C_6H_{12}O_6 + 2R_2HPO_4 \]
The hydrolysis of equation (2) determined the rate of fermentation, but the total action takes place only in the presence of the ferment and co-ferment. A. Harden (242) found the addition of small amounts of phosphorus increases fermentation up to 700 per cent over phosphorus free mixtures. This shows that phosphorus is necessary for alcoholic fermentation. E. Büchner (79) found no relation between the fermenting power of yeast juice and its phosphorous content. H. v. Euler (153) showed that fermentation begins with an enzyme change which causes a union of the carbohydrate and the phosphates. This change is shown by a loss in inorganic phosphates during the first stages of fermentation and by the formation of a triose monophosphate, as well as a hexosediphosphate.

O. Meyerhof (390) described three reactions which were involved in the action of the phosphorylated intermediate compounds. These reactions were

1. \[ C_6H_{12}O_6 + 6H_3PO_4 \cdot (H^+P_0_4)^2 + 2H_2PO_4 = 4C_3H_5O_2 \cdot H_2PO_4 + H_2O \]
   Dextrose \ Hexose \ phosphoric acid \ Triosephosphoric acid

2. \[ C_3H_5O_2 \cdot H_2PO_4 = 2 \cdot C_3H_5(0H)_2 \cdot PO_3 + 2CH_2 \cdot (PO_4H_2) \cdot CHOH \cdot COOH \]
   Triosephosphoric \ Glycerophosphoric \ Phosphoglyceric acid
   acid \ acid \ acid
2.  
\[2\text{CH}_2\text{(PO}_4\text{H}_2\text{)}\cdot\text{CHOH}\cdot\text{COOH} = 2\text{CH}_3\text{PO}_4 + 2\text{CH}_3\cdot\text{CO}\cdot\text{COOH}\]
Phosphoglyceric acid  
\[2\text{CH}_3\cdot\text{CO}\cdot\text{COOH} = 2\text{CH}_3\cdot\text{CHO} + 2\text{CO}_2\]
Acetaldehyde  carbon dioxide

3.  
\[\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_3\text{PO}_4 + 2\text{CH}_3\cdot\text{CHO} = 2\text{C}_3\text{H}_5\text{O}_2\cdot\text{H}_2\text{PO}_4 + 2\text{CH}_3\cdot\text{CHO}\]
Dextrose  acetaldehyde  Tricosephosphoric acid  
\[2\text{C}_3\text{H}_5\cdot\text{O}_2\cdot\text{PO}_4 + 2\text{CH}_3\cdot\text{CHO} = 2\text{CH}_2\cdot\text{(PO}_4\text{H}_2\text{)}\cdot\text{CHOH}\cdot\text{COOH} + 2\text{CH}_3\cdot\text{CH}_2\cdot\text{OH}\]
Phosphoglyceric acid  Alcohol

During this investigation, Meyerhof found that reactions 1 and 3 are inhibited by monochloacetic acid, but not by fluorides, while reaction 2 is inhibited by fluorides but not by the iodoacetic acid.

According to L. Adler (11) about 80 per cent of the phosphates in beer are in the inorganic form. However, the organic phosphorous remains constant during fermentation except with increased temperatures when more is developed. The power of esterification of phosphates by yeast was shown by H. v. Buler (18) to depend upon the water content of the yeast.

According to A. Malkov (367) phosphates inhibit oxidations and accelerate fermentation through the formation of compounds between iron and phosphate ions. A. Crooke (109) found a high
phosphate content accelerates fermentation but less fermentable material is removed by the yeast.

Zymase was considered by S. Veibel (616) as a number of enzymes each with a specific action. He found methyl-glyoxal, aldot, glycerol, acetaldehyde, pyroracemic acid as intermediates in alcoholic fermentation. According to C. Schwerzei (550) the transformation of substances of aldehyde group follow the Cannizaro reaction

\[ 2 \text{RCHO} + \text{H}_2\text{O} \rightarrow \text{RCH}_2\text{OH} + \text{RCOOK}. \]

A. Fernbach (176) decided that acetaldehyde acts in fermentations as follows:

\[ \text{C}_6\text{H}_12\text{O}_6 \rightarrow \text{CH}_3\text{CHO} + \text{C}_3\text{H}_5(\text{OH})_3 + \text{CO}_2 \]

In presence of sulfites

\[ \text{C}_6\text{H}_12\text{O}_6 + \text{Na}_2\text{SO}_3 + \text{H}_2\text{O} \rightarrow \text{C}_3\text{H}_5(\text{OH})_3 + \text{CH}_3\text{CH(\text{OH})}_2\text{SO}_2\text{Na} + \text{NaHCO}_3 \]

and

\[ 2\text{CH}_3\text{CHO} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{CH}_2\text{OH} + \text{CH}_3\text{COOH} \]

C. Neuberg (422) found that fermentation is generally accelerated by most aldehydes except vanillin, while the keto-acids with secondary potassium phosphate act as coenzymes.

The general methods of studying fermentation are to determine the carbon dioxide liberated either volumetrically or by weight lost. In the former case, the carbon dioxide liberated is collected and measured, while in the latter methods
the gas is allowed to escape and the loss in weight of the apparatus is calculated as carbon dioxide. One method in use is to determine the "half-fermentation" period or the time required to liberate one-half of the theoretical carbon dioxide that should be liberated. E. Köhler (308), by measuring the carbon dioxide, found that the course of fermentation is irregular and dependent upon the concentration of the sugar. Increasing alcohol concentration causes fermentation to be rhythmical which is true of the growth. L. Ornstein (459) found the rate of alcoholic fermentation can be expressed by the equation

\[
\frac{dx}{dt} = Ky (1 - \frac{c}{x} + \frac{x}{2L})
\]

where
- \( y \) = yeast concentration
- \( x \) = sugar concentration at time \( t \)
- \( c \) = sugar concentration at time 0.
- \( L \) = alcohol concentration at time \( t \).
- \( K \) = a constant

O. Rahn (505) showed that the decreasing rate of fermentation is proportional to the increase in concentration of fermentation products and that this can be expressed by the equation

\[
-Kt = \ln \left( \frac{2L}{(2L-x)} \right)
\]

Where
- \( K \) = a constant
- \( t \) = time in hours
- \( L \) = limiting concentration of alcohol
- \( x \) = amount of sugar decomposed
The number of cells must also be taken into consideration although it does not appear in the formula.

N. Bestestone (39) found that the rate of diffusion into and out of the yeast cells is of logarithmic character, and the osmotic effects of a 10 per cent sugar solution and a 5 per cent alcohol solution are approximately the same. From this he decided that the change of carbohydrates into alcohol has little effect upon the osmotic conditions of the cells.

Much work has been done on the determination of the theoretical amount of alcohol that can be secured from yeast fermentation. Originally, it was considered to be that amount which would follow the equation

\[ C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 \]

It has been shown that some of the sugar must be used by the yeast for energy, while a small per cent is changed to compounds other than alcohol and carbon dioxide. This theoretical recoverable amount has been placed from 88 per cent to 95 per cent. D. Sidersky (559) was able to obtain 60.8 liters of alcohol or 96.3 per cent from 1 kilogram of sucrose, while the total theoretical is 64.33 liters. G. Staiger (572) found that fermentation stops at a concentration of about 16 per cent alcohol. The alcohol production is not increased by seeding with increased yeast.
concentrations, but fermentation is completed sooner with the larger seedings. The type of yeast as well as its previous culture plays an important role in the yield of alcohol which can be secured. G. Tumang (606), using pure culture yeast, was able to secure a 73 per cent yield, while with adventitious yeast the best yield obtained was 65.14 per cent.

The yeasts do not ferment all sugars at the same rate, and they also carry on selective fermentations as was found by H. Sobotka (563), when he determined that all types of yeast prefer glucose to fructose at a rate of 2 to 1. H. Sobotka (562) also found that the pentoses retard the rate of fermentation of the hexoses, although sometimes the volume of carbon dioxide evolved is increased.

Yeasts can be acclimated to fermenting sugars which they ordinarily would not attack. H. v. Euler (169) showed that yeast would ferment galactose if it had been previously treated with this carbohydrate. Research carried on in recent years shows that the yeasts will utilize many organic compounds as a source of the necessary carbon and nitrogen when the normal nutritional compounds are absent.

The effects of the natural waters upon fermentation have been noticed almost as long as fermented beverages have been produced. Certain mineral waters have been found detrimental
to fermentation. K. Mayer (381) showed that the Karlsbad water causes the fermentations to favor the splitting of the carbohydrates in the third type of fermentation, with a decrease in the formation of alcohol and an increase of glycerol. Sodium carbonate and magnesium sulfate are held responsible since, in the presence of the sodium chloride and sodium sulfate, normal fermentation occurs. Sea water has been found by L. Sanzo (530) to stimulate fermentation and this activation is increased if the water is exposed to the radiations of ultra-violet light. If the irradiated water is boiled for 20 minutes the effect of irradiation is lost.

E. Fasce (480) tried fermenting glucose in deuterium and found this water decreases the rate 90 per cent, while a 60 per cent deuterium, 40 per cent ordinary water mixture, decreases the rate only 16 per cent. A. Hughes (274) found that deuterium inhibits both respiration and fermentation.

One of the methods for treating infected yeasts is the use of organic and inorganic acids. It has been shown that some of these acid treatments have detrimental effects upon the resulting yeasts. G. Staiger (571) found that most yeasts cannot resist the effect of sulfuric acid, and yeast treated with this acid loses some of its fermenting power.

T. Bokorny (69) found that all acids hinder fermentation with oxalic and formic being the most harmful. According to
E. Hagglund (237) the toxic action of sulfurous acid is due to the undissociated part, but there is an optimum concentration of 0.005 N where the enzymes are activated.

The action of the acids on fermentation is partly due to the ions themselves and partly due to the pH value of the solutions. E. Hagglund (238) found that decomposition of sugars is constant from pH of 4 to 8.5, while there is no fermentation at a pH of 10. H. v. Euler (164) found little difference between the action of acids upon the enzymes and the cells of bottom yeasts. From this he concluded that the enzymes must exist in the living cells. H. v. Euler (160) showed that lowering the pH below 5 causes a rapid decrease in fermentation and the amount of carbon dioxide secured. K. Trautwein (603) showed that pH curves drop rapidly on the alkaline side with fermentation being suppressed at a pH of 8 and replaced by respiration up to a pH of 11.8. The respiration and fermentation curves are parallel up to a pH of 7.

L. Casale (83) found the maximum velocity of fermentation occurs between 3.3 and 4.0 pH and the maximum yield from 2.8 to 5 pH. pH values of 2.6 and 2.8 arrest the zymase activity. According to J. Dehnicke (122) the highest yields of alcohol are secured from grain mashers with a pH from 4.6 to 5.3 and values outside this range cause longer periods for complete fermentation. H. v. Euler (155) showed that
yeasts can ferment dextrose in 0.05 N ammonium hydroxide solutions, although the rate is decreased. G. Wilenko (627) found that high pH values cause fermentation of glucose, by yeast, to take place without formation of carbon dioxide.

The activation and retardation of the fermentation by some substances is well understood while others have effects that cannot be easily determined. G. Neuberg (434) concluded that the effects of catalysts for alcoholic fermentation could classify into (1) substances of sugar-like structure as glycolaldehyde, (2) aldehydic and ketonic alkaloids, as pelletierine, (3) natural and synthetic diketones and quinones, as isatin and quercetin, (4) nitrogen and oxygen containing organic compounds, as m-dinitrobenzene, (5) sulfur and selenium compounds as cystine and sodium thiocyanate, (6) metallic compounds, as iron, copper, etc. Classes 1, 2, and 3 activate fermentation while 4, 5, and 6 stimulate carbon dioxide production; however, both effects are dependent upon the concentration of the reacting substances.

Many substances can stop or inhibit fermentation, and L. Genevois (209) divided these into three classes; (1) narcotics, which arrest fermentation by and respiration and growth of yeast, with this action being reversible if not allowed to act for too long; (2) antisepsics, which kill the cells; (3) specific inhibitors, which under certain
conditions stop fermentation without affecting growth and respiration.

Of the less understood substances which have accelerating effects on fermentation the Activator Z and the vitamins are the more important. Activator Z is prepared from boiled yeast extract. O. Meyerhof (389), using Saccharomyces ludwigii, found the presence of Activator Z increases the fermentation by this yeast to 200 per cent of that of a sugar-phosphate solution, while it is 100 per cent greater than for pressed yeast. The Z factor is continuously produced by the yeast, and its effect on the fermentation is independent of the sugar concentration. Malt germ extracts were found by H. v. Euler (173) to contain about the same Activator Z concentration as yeast extract. He also showed that caffeine, xanthine, tryptophan, adenosine, and hemin have no influence on the rate of fermentation even in the presence of Activator Z.

According to P. Gayrol (88) cysteine completely inhibits the accelerating effect of factor Z, although it has no effect on the rate of fermentation in the presence of phosphates. S. Frankel (185) found that choline and choline act as inhibitors, while the alcoholic extracts of vegetables, probably due to their vitamin content, activate fermentation. At a pH of 5, E. Lindberg (343) showed that the water extracts of yeasts and milk accelerate fermentation, but
actic acid and cholesterol have no effect. S. Hermann (261) found cholesterol inhibits fermentation by living yeasts both in the normal solutions and also those activated with insulin. E. Vahlen (608) prepared metabolin and antibolin from yeast and found the former accelerates while the latter inhibits fermentation.

Under the discussion of the theories of fermentation it was shown that acetaldehyde is formed as an intermediate and that its presence accelerates fermentation. In studying the accelerating effects of adsorbents, such as charcoal and kaolin, upon the rate of fermentation of glucose, E. Abderhalden (4) found free acetaldehyde and decided it is produced catalytically and its presence increases the rate of fermentation. H. Ikevovic (279) showed that the accelerating effect of animal charcoal is due to (1) the stimulating effect of acetaldehyde, (2) the displacement of carbon dioxide by the charcoal resulting in a lower concentration and (3) the adsorption of alcohol by the charcoal.

W. Owens (465) considered the action of charcoal as purely catalytic. B. Lampe (327) could not find an increase in aldehyde content in mash es which had been accelerated by charcoal. A. Reinhard (508) and M. Felise (174) found that charcoal accelerates fermentation of sucrose, although the products secured vary from the normal only within experimental error. R. Greig-Smith (225) showed Kieselguhr,
asbestos, precipitated silica, kaolin, agar, etc. accelerate fermentation. The electric charge on the colloid is not responsible for the accelerating effect and gels are more effective than sols. R. Greig-Smith (226) concluded "all colloids of inactive substances accelerate fermentation".

N. Schngen (565) decided the favorable influence of colloids is due to low carbon dioxide content in the liquid, caused by the formation of bubbles on the colloids through surface tension.

Oxygen causes a decrease in fermentation, but may be beneficial under certain conditions, as was shown by W. Windisch (644) who found that, in the presence of 0.001 N cyanic acid, oxygen increases the rate of fermentation three times while nitrogen decreases the rate at least 10 per cent. A. Kluuyer (304) showed that conflicting results obtained on the effect of oxygen on the rate of fermentation is due to a difference in the original concentration of oxygen and if this is corrected the results will agree.

Nitrogenous substances are necessary for the cell functions of yeast, and nitrogen in all its forms is utilisable providing the concentration is not too great. P. Petit (437) decided that the absorption of nitrogen is not limited by the lack of assimilable nitrogen, but because the yeast has need for only a small amount while large amounts may be harmful. According to W. Windisch (654), 2 parts per 100,000
of nitrates affect distillery yeasts and waters containing this concentration should be regarded with suspicion.

E. Kayser (237) found that manganous nitrate accelerates fermentation to a greater extent than potassium nitrate, but the stimulating effect varies with the strain of yeast used.

H. Zeller (667) studied the effects of the ammonium salts on fermentation and found the phosphate, oxalate, carbonate, citrate, tartrate, and formate increase the fermentation of press yeast 90 per cent to 140 per cent. The chloride, sulfate, bromide, iodide, nitrate, and thiocyanate increase fermentation 20 per cent to 30 per cent, while the benzoate is an inhibitant. He decided the action of the ammonium salts is due to the formation of compounds of these salts with the sugar. H. v. Ruler (162) found ammonium formate greatly accelerates fermentation.

According to H. Wieland (628), a 0.0002 M potassium cyanide concentration reduces the respiration to 56 per cent, while a 0.004 M. concentration is required to show the same retardation on fermentation. Sodium cyanate is twice as active as potassium cyanide and has the same action on both respiration and fermentation. M. Patterson (481) found that potassium cyanide prolongs the zymin induction period.

The rate of fermentation was found by K. Franke (184), to be increased when the proteins from normal grains are
added to a yeast-glucose mixture, while the proteins from affected grains have no accelerating effect. This effect of the proteins varies with the protein-yeast ratio.

J. Orient (457) determined the effect of amines and found that monomethylamine retards fermentation in dilute concentrations and accelerates it in high concentrations. The di- and trimethylamines stimulate fermentation in dilute as well as concentrated solutions. The intensity of fermentation decreases with increased methyl groups and oxidized radicals. J. Orient (458) found most of the free amines inhibit fermentation, while the hydrochloride compounds of these amines are generally inactive. The pharmacodynamic value of the drugs goes parallel with the degree of their action in accelerating alcohol fermentation.

E. Abderhalden (1) showed d-alanine accelerates fermentation, although he did not determine what is the role of this compound. F. Zuckerkandl (675) found the amines, such as aniline hydrogen chloride, will re activate fermentations that have been stopped by sodium fluoride or monochloroacetic acid. E. Ctero (462) showed yeast is stimulated by 0.05 milligrams of pyridine and 0.00005 milligrams of nicotine per liter. Cyanamide was found by J. Dittrick (127) to check fermentation in a concentration of 1:230.

The halogens and their compounds, due to their widespread
occurrence, have been of importance in the study of their
effects on fermentation. K. Scharrer (532) found that
.01 per cent of iodine as potassium iodide retards, 0.01
to 2.5 per cent stimulates, and higher concentrations retard
fermentation, while potassium iodate and periodate have
greater inhibitory powers than the iodide. According to
A. Sturm (532) iodine in concentrations of 0.0001 to 0.01 M
increases the rate of fermentation by 29 per cent, but this
effect depends upon the nature of the yeast, the hydrogen
ion concentration, the concentration of iodine, and the
phase of fermentation at which the iodine is added.

The inhibitory effects of the haloacetic acids are
specific to fermentation without materially affecting
respiration and growth was found by J. Cayrol (87),
A. Kluyver (305) and F. Jensen (394). E. Lundsgaard (360)
showed this inhibitory effect is dependent upon the
experimental conditions. R. Nilsson (444) explained the
action of the haloacetic acids as being due to the suppression
of the formation of hexose diphosphoric and hexose mono-
phosphoric acids. F. Cayrol (90) used a large number of
the esters of bromoacetic and iodoacetic acids and found
their effects to be similar to the acids.

The effects of chlorides on fermentation seem to depend
upon the concentration of the salt, the concentration of the
sugar, and the presence of other compounds. B. Lampe (323)
found that a 2 per cent sodium chloride concentration decreases the alcoholic yield. E. Salkowski (523) reported complete fermentation of a 12 per cent sugar in a 4 per cent sodium chloride concentration and almost complete fermentation when an 8 per cent sodium chloride concentration is used. However, with concentrations of 20 per cent sugar and 4 per cent sodium chloride the fermentation is only 90 per cent completed. A. Harden (244) found a 1 M sodium chloride solution gives a greater carbon dioxide evolution than water, although it inhibits the action of enzymes. Sodium chloride accelerates autofermentation as do all salts which plasmalize the yeast cells.

The organo-chloro compounds have effects similar to the iodo compounds. According to P. Cayrol (89) alcoholic fermentation is inhibited by 0.001 M monochloroacetic acid, but much larger quantities of the di and tri substituted acids are necessary to kill the cells. He further stated that the action of the chlorine atom depends upon its position in the molecule, and the more easily hydrolyzed derivatives are the more active inhibitors. L. Genevols (208) showed that esters of monobromoacetic acid arrest fermentation in concentration of 0.0005 N to 0.00025 N, but the similar chloro compounds require 10-15 times this concentration for the same effects. It has been shown by H. Plagge (495) that the organo-chlorides are toxic to yeast, but the effects are dependent upon the ratio of toxic substance to yeast.
The haloacetic acids inhibit fermentation and make it possible to study respiration, while E. Negelstein (420) found that 0.0001 M hydrogen sulfide stops respiration without affecting fermentation. At this concentration fermentation can be studied independent of respiration, but at concentrations of hydrogen sulfide of greater than 0.0006 M both functions are affected.

The effects of antiseptics on fermentation have been approached both from their action in preventing fermentations as preservatives and the action of the bacterial antiseptics on yeast fermentations. F. Duchacek (133), using expressed yeast juice and cane sugar, found a 0.1 per cent phenol concentration has little effect, a 0.5 per cent solution decreases fermentation about 40 per cent, and a 1.2 per cent solution destroys the enzyme action. He further found an 0.3 per cent chloroform concentration is harmless, a 3.5-4.5 per cent chloralhydrate concentration destroys, while benzoic and salicylic acids reduce fermentative activity proportional to concentrations above 0.1 per cent.

C. Neuberg (428) showed chloroform to be more harmful than toluene. Neither G. Joachimoglou (285) nor H. Dannenberg (113) could secure an acceleration of fermentative activity with low concentrations of mercuric chloride, phenol or quinine. Neither worker could substantiate the Arndt-Schulz rule by using these compounds. M. Perry (483) found the concentrations of preservatives sufficient to prevent fermentation by
Saccharomyces cerevisiae are formaldehyde 0.25 per cent, sodium bisulfite 0.25 per cent, sodium benzoate 0.5 per cent, sodium salicylate 1.0 per cent, and ethyl alcohol 20 per cent. S. Branham (76), using baker's yeast, found the greatest amount of carbon dioxide may be produced after an initial inhibition with mercuric chloride, mercuric chloride and hexylresorcinol while iodine, chloramine T, and sodium hypochlorite may cause sudden increased activity followed in 1 or 2 hours by complete inhibition.

The inorganic salts vary widely in their action on fermentations by yeasts and also in the concentration necessary to show determinable effects. E. Drabale (131) showed that in all solutions of neutral salts of the alkali metals the cells reproduce actively. Sodium and potassium chlorides and nitrates in concentrations up to 0.1 gram mole are without action on the fermentative activity. According to J. Kerk (287) low concentrations of calcium carbonate accelerate activity, while C. Richards (510) found 0.0001 M calcium sulfate is the optimum for growth, and fermentation with lower concentrations are inadequate, and greater amounts inhibit cell action. F. Schönfeld (537) showed that yeasts rich in calcium settle loosely, grow slowly, and die easily.

According to H. v. Euler (170) magnesium is necessary for yeast fermentation with the optimum concentration being
C.01 M. C. Richet (513) has shown that magnesium salts stimulate lactic acid fermentation with a maximum at 12.5 grams of magnesium chloride per liter.

There is an ion antagonism between some of the metal ions, as has been shown by G. Guanzon (230), who found calcium is antagonistic to magnesium and potassium, while magnesium is antagonistic to calcium and sodium. The sodium ions decrease efficiency; potassium ions are neutral; but calcium and magnesium ions increase the efficiency of fermentations. V. Kulikov (324) found the inhibiting effect of potassium sulfate can be lessened by adding calcium sulfate and recommended the adding of 0.4 grams of calcium sulfate to 100 c.c. of concentrated molasses solution containing potassium sulfate. By this procedure the rate of fermentation is increased 50 per cent.

The arsenates and arsenites have been reported as being stimulative towards fermentation. F. Beas (55) found yeast may become acclimated to these compounds and he observed no acceleration. A. Harden (243) found the arsenates and arsenites accelerate the fermentation of glucose with yeast juice, but these compounds do not replace the phosphates. A. Harden (240) also found sodium arsenate produces greater acceleration on yeast juice fermentations than sodium phosphate. The arsenate remains precipitable by magnesium mixture; therefore, it does not replace the phosphates in the
organic compounds. A. Harden (245) decided the stimulating action of arsenic on zymin preparations is directly on the hexosephosphate fermentation. F. Mayer (382) showed the organic arsenic compounds as well as arsenic acid promote the fermentation of hexosediphosphate esters. K. Dresel (132) found arsenous acid restricts fermentation by yeasts, but glucose has a protective effect against this compound. According to A. Moxon (407) the toxicity of 0.0000126 M solutions of some sodium salts decrease in the order of sodium selenite, vanadate, arsenite, and tellurite. The toxicity of sodium selenite, selenide, and selenate decrease in the order named. Sodium selenite in concentrations up to 0.5 per cent was found by M. Korsahov (313) to accelerate fermentation by living yeast.

The action of copper and its salts on fermentation depends largely upon the salt combination. K. Schweizer (649) showed the decrease in activity in copper vessels is due to a cuprous oxide film. This substance completely inhibits fermentation at 0.014 grams per 100 c.c., while a cleaned copper surface and cupric oxide are without effect. According to M. Van Lear (614) copper is much less toxic at 25° C.

Yeast can be acclimated to copper with some strains being more resistant than others. R. Guillemet (233) found yeast killed by copper ions had increased in copper content from 0.03 to 1.3 millegrams per gram of dried yeast.
R. Guillemet (235) found the rate of fermentation begins to decrease at a concentration of copper of 0.5 grams per liter. He concluded that yeasts absorb copper from solution. F. Hildebrandt (264) reported an increase in alcohol of 1 to 2 per cent from fermentations by yeasts that had been pretreated with copper sulfate, manganese sulfate or sodium cyanide.

Iron compounds are always found in small quantities in the raw materials used in fermentations, and P. Hodel (265) decided the accelerating action of the ferrous and ferric salts involves the formation of a ferriophosphate. A. Malkov (366) found 0.6 to 4.5 milligrams of iron per 100 c.c. stimulate, while higher concentrations retard fermentation. He found the quality of yeast grown in a media containing iron, is normal, gives a higher yield, and increases the assimilation of nitrogen and phosphorous.

F. Zuckerkandl (676) distinguished between the cyanide sensitive hemin iron which catalyzes respiration and the insensitive ferrous compound involved in fermentation. He decided iron is an essential component of halozymase. According to K. Harpuder (249), respiration of beer yeast is not affected by a ferrous sulfate concentration of 0.001 M but fermentation is reduced. Also this yeast is not affected by manganese sulfate of a concentration of 0.1 M. M. Rosenblatt (521) found 0.001 to 0.1 per cent manganese
produces a gradual increasing inhibition of alcoholic fermentation. M. Rosenblatt (522) showed some of the heavy metals paralyze yeast growth, but allow fermentation. The paralyzing action differs from the order of effect on growth and follows the order of nickel-iron-cobalt and manganese.

The presence of minute quantities of lanthanum sulfate was shown by C. Richet (514) to increase lactic acid fermentation by about 30 per cent. According to G. Gimel (220), the presence of 50 to 100 milligrams per liter of stannous chloride accelerate fermentation, while 0.01 to 0.02 grams per liter of bismuth subnitrate inhibit alcoholic and acetic fermentations and may be used to prevent the hardening of cider as well as its spoilage by acetic acid bacteria.

According to E. Kayser (286) and H. Agulhon (14) low concentrations of uranium salts stimulate alcoholic and acetic fermentations.

S. Kostuichev (317) found that zinc chloride causes the formation of acetaldehyde in "hefenol", but not in living yeasts and a part of the sugar is converted to compounds of unknown structure with an evolution of carbon dioxide. A. v. May (380) believed the observations of Kostuichev were due to the difficulty in determining sugar by reduction methods in the presence of yeast proteins. A. Zlataroff (674)
studied the effects of zinc salts individually and in mixtures with other salts. He found concentrations of zinc nitrate and sulfate of 0.002 N stop, 0.001 to 0.002 N inhibit, and less than 0.001 N do not affect fermentation. Of the salts studied, calcium nitrate has the strongest stimulating action and sodium succinate the strongest inhibiting action. The action of sodium succinate is evident in a concentration of 0.0000005 N.

F. Arloing (19) showed that the organo-metallic complexes of ascorbic acid with iron, titanium, and zinc have a slight accelerating action, while the complexes with calcium, barium, lead, and copper retard or stop fermentation.

The stimulating effects of various substances on cell functions were divided by R. Neier (386) into three groups: 1, phenol, arsenic, chloropicrin, and mustard oil inhibit respiration and stimulate fermentation; 2, mercuric chloride, chromates, iodine, eosin, and hydroxyquinoline inhibit fermentation in lower concentrations than is required to inhibit respiration; 3, salts of silver, copper, and methylarsenoxide inhibit both fermentation and respiration in the same concentrations.

The effects of the organic compounds, some of which are present in the raw materials and some of which are produced as the result of the metabolism of the yeasts, generally can be divided into classes according to molecular relationship.
Of the alcohols, ethyl alcohol is the most important and M. Kochmann (307) found it to stimulate fermentation in concentrations of 1 to 500 but higher concentrations decrease activity. This is one of the few cases where metabolic products stimulate the activity of the organism. According to A. Dorner (129) given concentrations of methyl, ethyl, propyl, butyl, phenyl, amyl, and heptyl alcohols inhibit cellular fermentation less than they affect fermentation by yeast press juice. E. Abderhalden (3) found that fermentation of 25 c.c. of glucose, yeast and phosphate mixture is accelerated by 0.01-0.02 c.c. methyl alcohol; 0.01 ethyl alcohol; 0.001 c.c. propyl alcohol and 0.001 c.c. of trimethyl carbinol. He found little, if any, acceleration by butyl, iso butyl, amyl, iso amyl, hexyl, and heptyl alcohols. C. Barthel (34) showed the fermenting power and growth of yeast can be separated by the addition of ethyl alcohol and ether which destroys growth, but has little effect on the fermenting power and synthesis of hexose diphosphate.

W. Windisch (646) found nonyl and octyl alcohols in low concentrations first act as accelerators and then as inhibitors of fermentation. H. Freundlich (187) explained the inhibition of fermentation by the higher alcohols as an accumulation of the alcohols at the surface of the cells, thereby displacing the sugars and altering the surface tension. Ethylene glycol was found by L. Young (664) to
almost completely stop fermentation at a concentration of 75 per cent, while at a concentration of 20 per cent it reduces the fermentation rate by 66 per cent.

The acids present in raw materials and those formed during fermentation by the yeasts and associated bacteria was shown by C. Neuberg (423), to retard fermentation in concentrations of 0.2 N pyroracemic, 0.5 N lactic, 0.5 N 1-malic, 0.45 N d-tartaric, and 0.15 to 0.3 N acetic acids.

The di and trihydroxy- or alkylxybenzoic acids and their esters were found by T. Sabalitschka (525) to inhibit fermentation from 0.1 to 6.8 times as much as phenol but there is no relationship between the substitution and the inhibiting power. E. Mameli (388) showed the phenoxycetic acids and their sodium salts stimulate, while methyl iodide, manitol or quinol increases fermentation about 20 per cent and phenol or resorcinol retards it.

Phenol is a strong inhibitor for yeast fermentations as was shown by E. Abderhalden (7) who found 0.0000001 to 0.0000002 grams of phenol in 25 c.c. of phosphate buffered solution accelerate while higher concentrations inhibit fermentation. According to H. v. Euler (168) the fermentation velocity is diminished 50 per cent by 0.03 to 0.05 N phenol, cell reproduction diminished 10 per cent by 0.015 N phenol, and completely inhibited by 0.017 N phenol. He found 0.04 N
phenol completely inhibits maltose fermentation but reduces glucose fermentation only 50 per cent; therefore, maltose is not fermented direct. A. Mossini (397) showed the inhibiting action of phenol is increased in the presence of surface tension reducing agents such as sodium sulfonic acid which multiplies the inhibiting action 10 times.

The effects of a large number of miscellaneous organic compounds have been determined and some have been found to be accelerators while others are inhibitors. K. Meyer (388) showed that yeast treated with olive oil or oleic acid has higher fermenting powers, although respiration decreases. The fat solvents, benzene, xylene, ether, and ethylbutyrate were shown by N. Kerr (298) to cause a marked reduction in fermentation, while chloroform, ethyl acetate, and butyl alcohol completely stop fermentation.

Methylene blue is used as a stain to distinguish dead yeast cells and according to V. Palladin (467), oxidizing action is not present in these dead cells, but in some cases the liberation of carbon dioxide is accelerated. H. Fink (181) found methylene blue inhibits fermentation, but this action is almost overcome by the action of salts with the activity of their ions following the order of the Hofmeister series.

E. Mameli (369) showed the organic compounds with double
bonds between carbon and oxygen, sulfur and nitrogen, nitrogen and oxygen, carbon and carbon catalyze fermentation.

E. Nameli (370) found fermentation is accelerated by naphthalene, indene, styrene,acenaphthene, anthracene, and phenanthrene and some of their unsaturated derivatives. He decided all unsaturated compounds accelerate alcoholic fermentation by yeasts. C. Neuberg (433-435) and co-workers found fermentation is accelerated by adenine, xanthine, guanine, adenosine, and their substituted compounds, while alloxan and inosic acid are retardants. C. Neuberg (436) also found that alcocin, cetararin, cubebin, elatein, and cholic acid and its compounds accelerate fermentation. A stimulating action is secured with abetic acid, copainic acid, bone charcoal, saponin, digitalin, keratin, etc.

According to B. Inonye (277), vanillin, xylose, uric acid, and irone accelerate while the neutral organic salts are neutral to fermentation activity.

T. Soda (564) showed fermentation is accelerated by cinnamic alcohol, allyl alcohol, crotonic acid, etc.

A. Mossini (398) found 0.0125 per cent follicolin increases carbon dioxide evolution by 83.7 per cent, while the presence of surface tension reducing substances, as sodium oleate, increases evolution of carbon dioxide 50 per cent. F. Boas (58) decided the highly active saponins retard fermentation due to destruction of the yeast plasma, while the less active
saponins increase carbon dioxide evolution due to increased permeability of the plasma membrane.

The effects of gases and increased pressures due to gases on fermentations appear to depend upon the gas and also on the physical condition of the substrate. H. Wüstenfeld (660) believed the carbon dioxide is kept at a minimum by the presence of dead yeast and grains and this prevention of supersaturation with carbon dioxide causes an accelerating effect on the fermentation. R. Kolkwitz (310) was able to secure a pressure of 40 atmospheres by carbon dioxide evolution in special equipment. O. Hofmann (268) secured fermentation in an atmosphere of hydrogen at 150 atmospheres. He concluded dissolved carbon dioxide in high concentrations inhibits fermentation.

Increased oxygen pressure inhibits fermentation as was shown by W. Libbrecht (540), who decided the effect increases according to the ease with which the substrate ferments under atmospheric pressure. H. Zikes (671) decided very low air pressures are beneficial to fermentation, while A. Rippel (516) found day to day fluctuations in loss of carbon dioxide by a fermenting mixture correspond to the variations in barometric pressure, with a low pressure favoring loss of carbon dioxide and vice versa. H. Zikes (673) showed the speed of fermentation is related to the atmospheric conditions, but the total fermentation is unaffected.
According to R. Lieske (342) fermentation ceases at a carbon dioxide pressure of 33-40 atmospheres, but if fermentation is started at 80 atmospheres no effect is observed and if started at 1000 atmospheres only 50 per cent action is secured. K. Schuster (547) found that yeast grown under pressure has larger cells, shows a stronger fermentation and is more resistant to infection.

R. Sandstedt (529) did not believe that pressure was a serious factor in regulating yeast fermentations, and R. Guillemet (234) found no difference in the rate of fermentation at partial vacuum or at a pressure of two atmospheres.

The temperature at which fermentations are carried on affects the rate and the resulting products. R. Guillemet (234) found the fermentative activity to be three times as great at 30° C. as at 20° C. Using lactic acid as an indicator, C. Richet (515) showed a temperature of 57-58° C. decreases acid from 100 to 47, while at 55° C. it is reduced from 100 to 74. Yeasts should be grown at low temperatures and stored in cold temperatures, as was shown by F. Schünfeld (536), who found these conditions cause better fermentations than when warm temperatures are used.

The viscosity of the fermenting mixture would be expected to partially control the ease with which carbon dioxide was evolved, and therefore, it would be one of the controlling
factors in the rate of fermentation. M. Minlescu (396) found that increasing the viscosity by adding agar or gelatin has no apparent effect on the rate of fermentation. The addition of surface-tension-lowering substance was found by H. Zeller (668) to have no general effect on fermentation, but individual compounds have their own specific effect; some inhibit while others stimulate the activity.

There is a difference of opinion as to the influence of electric potentials upon yeast fermentations. M. Potter (497) could find no influence of electric potentials upon the velocity of fermentations, while M. Kleiber (301) found an alternating current of 3-4 volts and 7-8 milliamps has no effect, but a direct current of the same strength and voltage diminishes fermentation. F. Stockhausen (579) found a current of 0.1-0.6 milliamps per square centimeter of liquid cross section has a deteriorating effect on fermentations, which is probably due to chemical changes and nascent oxygen produced by the electric current. E. Bendetti (41) determined the effect of an oscillating electromagnetic field on fermentation and found activity is slower than normal at a frequency of 1200, increases slightly at frequency of 500, and increases greatly at frequencies of 400 and 272.7.

The effects of light rays have received much attention. G. Guerrini (331) found the decreasing effect on fermentation
of light filtered through various colored screens to follow
the order of red, yellow, green, blue. G. Guerrini (232)
decided the light rays of long wave length stimulate the
action of saccharomyces cerevisiae on glucose. P. Murakami
(409) found a maximum production of alcohol under a red
light of greater than 6200 A.U., a maximum yield of aldehydes
at wave lengths of 3960-5400 A.U., a maximum production of
volatile acids in the dark, and a maximum production of
non-volatile acids in violet to yellow light of wave lengths
of 3900-5800 A.U. P. Murakami (410) also showed the
production of alcohol decreases gradually from 6130 A.U.,
while a large amount of acids are formed during the early
stages of fermentations exposed to light of long wave
lengths.

Ultra-violet light was found by V. Henry (253) to arrest
the acetic fermentation of wine and E. Maurain (378) found
it completely arrested the acetic fermentation of cider in
2 to 3 minutes and of white wine in 5 to 10 seconds.
R. DePazi (113) showed fermentations of figs by saccharomyces
opuntiae to be more active under ultra-violet light if
exposure is limited. He also found this treatment gives a
better yield of alcohol and a decreased acidity. According
to P. Lindner (348), fermentations of dextrose solutions by
bottom yeast, when exposed to ultra-violet light, give a
600 per cent increase in the amount of carbon dioxide evolved
in 24 hours.

R. DeFazi (116) recommended the use of ultra-violet light in industrial fermentations. He claimed the fermenting time for beer can be reduced by 25 per cent and the quality of the product can be improved. R. DeFazi (118) found that glucose solutions prepared with distilled water, previously exposed to ultra-violet light, ferment faster than those prepared with plain distilled water, and that glucose solutions irradiate after preparation are the most active. W. Owen (466) showed that exposure of yeast for 1 minute to ultra-violet light increases both the fermentation and growth, while exposure for 3 minutes acts as a retardant. V. Cronchi (227) showed exposure to ultra-violet light of fermentations by Saccharomyces cerevisiae excites fermentation, but this action ceases when irradiation is stopped and from this point on the activity is markedly diminished. W. Owen (464) found the fermentation of molasses is greatly accelerated by short exposures to ultra-violet rays and 24 per cent less yeast is needed.

Contrary to the results of other investigators, N. Sühngen (566) found exposure to ultra-violet exerts a harmful effect on the fermenting and reproductive powers of yeast.

E. Abderhalden (6) found the fermentation by yeasts is
slightly inhibited by irradiation with ultra-violet or magnesium light.

According to V. Gronchi (228) the exposure of glucose fermentations to X-rays increases the carbon dioxide proportionally to the intensity of the radiant energy and the hard rays are more effective than the soft.

Radio-active emanations have been shown, by G. Jacquemin (261), to have a stimulating effect on alcoholic fermentations resulting in a more complete utilization of sugar and a higher yield. R. DeNazi (120) reported an accelerating effect of radium emanations on yeast fermentations, but only up to 3.5 millicuries with 6.75 millicuries showing a retarding action.

COMMERCIAL FERMENTATION OF GRAIN MASHES

The commercial fermentation of grain mash is carried on in large cylindrical tubs which are divided into two classes - open and closed. The open tubs, as the name implies, are open at the top and without covers. The closed tubs have covers which are a part of the tubs.

In recent years much criticism has been aimed at the open tubs, although research and experience have shown that there is little chance of infection of an open tub if the fermenting room is kept clean and the charge is adjusted to
the correct conditions and is stocked with the correct quantity of an active yeast. The yeast rapidly develops a medium in which is not suitable for the growth and fermentation by wild yeast and bacteria. The worse criticism of the open tub is that as much as 1 per cent of the alcohol formed may be lost as well as the loss of tremendous quantities of carbon dioxide which could be utilized as dry ice. The open tub generally has a depth of about the same dimension as the diameter and is made of select cypress, although tile and metal are used.

The closed tub is generally made of iron or iron alloys and differs from the open tub in that it can be completely closed for the drawing off of the carbon dioxide formed. The closed tub is always equipped with cooling coils and air jets while the open tub may or may not have these accessories. The closed tub is always used in plants in which the fermentation is carried on rapidly (in from 30 to 60 hours) while the open tub is used in the slower processes requiring up to 96 hours. E. Lüdher (356) found that closed tubs show a rapid rise in temperature due to large volumes used and a rapid fall due to conductance of the iron sides. He also found closed tubes to give a more rapid yeast growth in the first 24 hours and a higher yield of alcohol. E. Lüdher (358) determined the efficiency of the two types of tubes and found open tubes to yield 34 to 36 per cent of the theoretical
quantity of alcohol, while the closed tubs gave 90 to 92 per cent. F. Harden (246) found the loss from open tubs was only about 0.001 per cent, while E. Mariller (373) found a loss of 1 per cent from the tubs and almost as much from the still.

The general operations involved in the fermentation using the open tubs are; (1) sterilizing; (2) charging; (3) fermenting.

1. The sterilization of an open tub is done by means of steam and chemical disinfectants. After the tub has been washed free of the previous charge, it is steamed for 45 minutes after which the sides, bottom and coils (if any) are sprayed with a chemical disinfectant such as a chlorite solution or one of the fluorine compounds such as hydroflosilic acid, etc. The tub is then steamed again for a short time and then allowed to stand for about 4 hours when it is again ready to be charged.

The sterilization of closed tubs is generally done by steam. After the charge is dropped to the beer well, the tub is thoroughly washed with cold water, and is then steamed under pressure or is sprayed with superheated water until a steam pressure develops in the tub. The steam pressure is maintained for about one hour after which time it is released and the tub is ready for recharging.
2. The charging of the tub depends upon the type of product desired and the materials used. There are three different charges that are used in the grain fermenting industries (a) yeast, grain mash and water, (b) yeast, grain mash, water and mineral acids and (c) yeast, grain mash, and cutback or thin strained dealcoholized beer from a previous fermentation.

The first charge, i.e., yeast, grain mash and water is the basis for what is known as the sweet mash method. In this charge there is generally two-thirds grain mash and one-third water. Sweet yeast is added shortly after the start of the addition of the mash and the water is added last to finish the charge.

The second charge, i.e., yeast, grain mash, water, and acid is essentially the same except enough of a mineral acid, usually sulfuric, is added to bring the entire charge to a pH of 5. Strict attention should be paid to the addition of this acid as under no conditions should more acid be added than is necessary to liberate sufficient organic acids to bring the pH to the desired value. Sweet yeast is generally used in this method.

The third charge, i.e., yeast, grain mash, and cutback is used instead of water, and sour yeast is used in preference to sweet yeast. By adding the cutback to the yeast and grain
mash a pH value of 4 is secured. This pH value is lower than that to which a sweet yeast is accustomed and an acclimating period is necessary before the yeast reaches the active stage of growing and fermenting. Sour yeast, grown at a pH value of 3.8 to 3.9, requires no such acclimating and begins its functions immediately. In this method, there is a gradual building up of the nitrogen, acid and mineral content of the charge. Therefore, the outback must be analyzed regularly and sufficient water added to maintain a concentration which is the optimum for the charge being used.

In any of the above methods, the final charge must be mixed by blowing with air or agitation.

3. The fermentation procedures vary widely depending upon plant equipment and past experiences of the operators. The main point to be watched is the temperature to which the charge is allowed to rise. It is common knowledge that the early period of fermentation progresses best at from 80 to 85°F. This is the period of maximum yeast growth and requires 6 to 10 hours. The second period or active fermentation should be run at temperatures of 85 to 88°F.

It can be seen from this that tempering coils are essential if the fermentation is to be run under the best conditions. In plants that are not equipped with tempering coils in the fermenting tubs, the fermentation must be carried
on in what may be termed a left handed manner. The tubs
are set at varying temperatures depending upon the charge
and weather conditions. For water washes the setting
temperatures are usually 70° F. during winter and 72° F.
during summer. For sour washes the temperatures used are
70 to 72° F. during the winter and 65 to 67° F. during
summer. Even with these low setting temperatures, the
final temperature reached will be from 93 to 96° F.

The use of these low setting and high finishing temperatures
is not desirable. Original low temperatures do not allow
an early development of the yeast, while the high finishing
temperatures inhibit the actions of the culture yeast, but
allow the growth of acetic and lactic acid bacteria, which
are present in the grain washes. These high temperatures
always cause the formation of secondary products which are
not desirable. The combination of temperatures used in tubs
not equipped with tempering coils also cause a loss in yield
of from 1 to 2 per cent. This loss is due to the fermentation
not being active in the early stages and the "after fer-ven-
tation" being at a temperature too high for best efficiency,
as well as part of the sugars being changed to products other
than alcohol.

The complete charges of the fermenter generally have a
gravity of 10 to 11.5 Balling. For a 96 hour mixed grain
mash fermentation, this gravity should be 6.0 at 24 hours, 2.0 at 48 hours, 1.5 at 72 hours and 1.0 at 96 hours. If the fermentation is progressing slower than is desired it may be accelerated by bubbling air through the beer. This procedure causes acceleration by driving out some of the carbon dioxide and by growing more yeast. However, it also causes a loss in alcohol which is carried out with the air.

Many factors affect commercial fermentations such as, acidity of mashes and beers, nitrogen content of beers, physical condition of grain used, and infection, as well as the disinfectants used. Of these probably the most important and the hardest one to control is infection.

Of the grains, corn is the only one heated to a temperature high enough to kill the bacteria present, while the rye and malt, if highly infected, carry this infection into the set beer. The common bacteria found in fermentations are: Lactobacillus, pediococcus, clostridium, staphylococcus, acetobacter, and micro coci, chiefly sarcina. Any infection of the beers may become general throughout the entire plant, on walls and in the pipe lines. Many disinfectants have been tried with varying experiences by the investigators.

Hop is the oldest disinfectant known to have been used in the fermenting industries. M. Fays (179) found the quality
of hops could be judged by their antiseptic power and there
is a considerable loss of this property during fermentation.
Infection of the yeast has caused much trouble and
W. Henneberg (256) tried washing the yeast with a number of
different acid solutions. He found sulfuric, hydrochloric,
nitric, lactic, tartaric, and citric acids are adaptable to
this use while hydrofluoric, formic, acetic, butyric and
oxalic acids are not satisfactory. G. Feuerstein (178)
found nitric acid to be the best for freeing yeast of torulae,
sarcinae, lactic, and acetic acid bacteria, but it is not very
effective for wild yeast. According to P. Petit (484)
washing yeast with phosphoric acid is not the same as with
other acids as it acts upon the functions of the yeast and
renders it immune to disease. The treatment is with 2 grams
of acid per liter for 30 minutes and the resulting mixture
is used for pitching.

T. Chrzaszcz (94) used lacto-formol made from 1 part
formalin and 2 parts of milk and when used as a 0.06 per cent
solution it causes more complete fermentation, increases the
yield, and suppresses acid formation. Sulfurous acid inhibits
the growth of wild yeasts, molds, and bacteria as was shown
by W. Cruess (111) who also found it has no effect on
Saccharomyces ellipsioidus. G. Thavenot (598) recommended
washing infected yeast with a 0.4 per cent solution of ammonium
persulfate but F. Schönfeld (538) could not repeat Thevenot's work and doubted its efficiency. G. Benys (124) secured increases in alcohol yields of 20 to 40 per cent by using 1 part of salicylic acid in 50,000 parts of wort. It decreases the activity of foreign organisms and stimulates the yeast fermentation. N. Rasoumoff (507) tested a large number of compounds including sodium carbonate, lime, sodium hypochlorite, ammonium fluoride, formalin, and chloramine, but found chlorine compounds have the strongest bacterial value. T. Chrzaszc (95) showed lactic and sulfuric acids and Bauer-Kuess solution are better for yeast control than lactoformal and formalin.

L. Vetter (617-618) showed ozone to be an efficient sterilizing and antiseptic agent for brewery use. It kills bacteria and weak cells and leaves the surviving cells in a resistant condition. Long periods of treatment with low ozone concentration are better than short periods with high concentrations.

H. Wooldridge (658) used a 12 per cent solution of hydrogen peroxide and secured good results. He found this solution penetrated deeply into the wood and left no bad results. A 2 per cent solution of sodium fluoride was recommended by P. Petit (485) as being a good disinfectant. Ammonium bifluoride has been used widely in some countries and to a lesser extent in the United States.
Chemical acidification for fermentations was studied by R. Bauer (35) and he found lactic acid unreliable, while sulfuric and hydrofluoric gave good results. F. Emslander (149) found the higher the litmus acidity in proportion to the phenolphthalein acidity in the wort, the better the resulting beers. E. Rudiger (594) recommended Sucheler's process of acidification with concentrated sulfuric acid in such quantities that only sufficient of the organic salts are decomposed to give the desired acidity without leaving any free mineral acid. Abietic acid is recommended by L. Terry (594), who claimed its use decreases the amount of sulfuric acid that must be used and that it gives an active fermentation which requires little attention. W. Owen (463) found the addition of 1 to 1 1/2 gallons of concentrated sulfuric acid per 1000 gallons of cane molasses accelerates the rate of fermentation and increases the yield of alcohol.

The pH value of mashes has no connection with the acid number determined by titration as was shown by I. Horvath (272) who also found fermentation can be controlled by determining both the pH value and acid number. W. Lamont (326) recommended a pH of 4.0 on filling the tub and 3.7 after fermentation is complete. By controlling the pH value he secured 6.5 per cent greater yield than from an uncontrolled standard. K. Taxner (592) found that both the pH value and the titratable acidity must be determined. He believed the
titratable acidity served as an index of sensitivity to infection.

The addition of nitrogen bearing compounds to fermentations has been recommended by W. Bierberg (51) who found it does not increase the yeast crop, but stimulates fermentation. This accelerating action of ammonia and ammonium salts is also shown by P. Kubsik (322) and H. Müller (408). According to G. Ellrodt (145), the addition of ammonium salts stimulate growth and gives yeast richer in proteins. The cause of low yields of alcohol from cane molasses was shown by N. Taketomi (590-591) to be due to an insufficient nitrogenous yeast nutrient and the presence of 4.5 to 6 per cent glucose, an unfermentable hexose. He increased the yield from 82-83 per cent to 97-98 per cent of the theoretical by adding 0.1 gram of ammonium sulfate or phosphate per 100 c.c. of the liquid to be fermented.

H. Harman (248) found the factors affecting fermentation are alkalinity of water, temperature of conversion of the grain and of fermentation, the race and temperature of pitching yeast, aeration, change in nitrogen content and salt content of the mash. He found the potassium-phosphate ratio is important and although there is plenty in the grains, it may not be present in an assimilable form.

The yield of alcohol appears to partially depend on the
race and condition of yeast as was shown by J. Magne (365), who secured the following from sugar fermentations: 40-60 per cent of theoretical yield with natural yeasts present, 50-75 per cent with pressed yeast, 70-85 per cent with pure culture yeast. G. Staiger (570) found the average yield from rye to be 34.4 c.c. and from wheat to be 34.9 c.c. of alcohol per 100 grams of grain. According to L. Buhot (81) the alcohol yield with pure culture yeast is 20 to 40 per cent greater than with natural yeasts. P. Lindner (347) found bacteria in beer worts reduces the alcoholic content by 50 per cent. K. Schuster (546) showed malt dust and too finely ground malt cause sluggish fermentations with a resulting decrease in yield.

A slow development during the first fermentation of corn mashes followed by a rapid rise in temperature during the principal fermentation and low yields was shown by G. Poth (183) to be due to an insufficient supply of nutriment for the yeast.

Another appearance of incorrect fermentation is excessive frothing. N. Kiihp (291) suggested this may be due to the nitrogen compounds and advised reducing the malt content, using higher mashing temperatures, and higher cooking pressure. If frothing still continued he advised using molten neutral fats, oils or petroleum. W. Windisch (638) showed that increased yields from the mashing under pressure
are derived mainly from the proteins and pentosans and only slightly from the starch. W. Windisch (637-641) also showed premashing increases the alcoholic yield and that it is a cure for sluggish fermentations instead of the cause. F. Wendel (626) varied the percentage of malt from 4 to 12 per cent and found the alcohol yield was no higher with the high diastatic content. The percentage of malt needed was found to depend upon the character of the grain and the procedure used. E. Lühder (357) reported that increasing the gravity above 18° Balling and shortening the fermentation time cause decreased yields which more than offsets the reduction in overhead costs.
PRODUCTS OF ALCOHOLIC FERMENTATIONS
When the term "alcoholic fermentation" is used, the uninitiated thinks that only alcohol, carbon dioxide and yeast are produced. Many different fermentation compounds are present in the finished beer and some are volatile, coming over with the alcohol, while others are not volatile at still conditions and remain in the spent beer. Of the many compounds that have been found in yeast fermentations, the more important are ethyl alcohol, acetaldehyde, glycerol, acids, esters and fusel oils or the higher alcohols. There are other groups of substances formed but generally in traces only.

In the fermentation of grain mashses, in which rye and barley malt are used and cooked at temperatures below sterilization, there are present many compounds which owe their origin to bacterial action. The combination of products of yeast and bacterial fermentations is little understood and it is doubtful if all of the compounds, which may be present, have been even qualitatively determined. Many attempts have been made to analyse the spirituous beverages for their individual compounds, but in each case the analyst has finally concluded that his analysis does not represent the complete division of the groups present. The investigators have found widely varying percentages of the principal compounds in products belonging to the same class. This is to be expected as no two industrial plants are operated alike nor do they produce exactly the same product. Due to
the inaccuracies in the methods of determining some of
the individual compounds and the difficulty of separating
others, the standard methods of analyses are those that
determine groups of compounds, and some of the methods used
for these determinations give results difficult to duplicate.

The wide variations in secondary products secured in
fermentation require a constant chemical control over pro-
duction and analyses of raw materials and finished products.
G. Staiger (573) pointed out the importance of chemical
analyses for acidity, pH value, diastatic power, starch,
alcohol, condition of yeast, and attenuation. It was shown
by E. Fulser (201) that the chemical and physical changes are
influenced by the nature of the changes of the carbohydrates
and the environment. He concluded that fermentation products
are the results of synthesis as well as degradation. The
production of volatile compounds during fermentation was shown
by E. Kayser (291) to be connected with the entire life cycle
of the yeast. He found an increase in yeast causes an
increase in higher alcohols, and decided that esters are
formed endogenously by the yeasts.

L. Lindet (346) found the quantity of by-products from
fermentation is related to the composition of the substrate
and the rate of fermentation. With sucrose, salts, protein,
and carbohydrates as the substrate and rapid fermentation, the
quantity of by-products is small, while with ammoniacal
nitrogen in place of the protein and slow fermentation, the quantity of by-products is large. L. Lindet (345) found that 5 per cent of the sugar used during alcoholic fermentation goes to glycerol, succinic acid, higher alcohols, etc. He concluded that the formation of by-products is influenced by the amino acids.

A large number of acids have been reported as being present in the volatile products of fermentations. Among these are formic, acetic, propionic, butyric, valeric, caproic, caprylic, palargonic, capric, and capranthylic as well as the fatty acids palmitic, oleic, linolic, and lauric. The formation of these acids during fermentation was found by F. Boas (56) to depend upon the source of nitrogen in the medium and a final pH of 2.94 to 3.80 is secured. F. Ehrlich (134) showed succinic acid is formed by yeast from the amino compounds of the grain or of yeast itself with glutaminic acid being the parent body.

H. Franzen (186) found formic acid is formed during fermentation of sugar by yeast and decided it is the result of an enzymic process as the acid is also decomposed by yeast.

F. Thomas (597) found the amount of formic acid produced in an acetamide media is not related to the weight of yeast nor the amount of sugar consumed. Acetic acid has long been known to be one of the products of fermentation and E. Friedmann (189) found this acid, in the presence of sugars
is changed to $\beta$-hydroxybutyric acid by fermenting yeasts. K. Jacobsen (280) showed that yeasts cause an equilibrium to be reached at 20 to 40 per cent fumaric acid and 60 to 80 per cent malic acid regardless of which acid is used at the start.

Calcium compounds have been considered as aiding acid formation, but E. Kayser (293) could determine little or no effect on the formation of volatile acids with yeasts rich in calcium. J. Kerb (296) found that the addition of calcium carbonate gives normal alcoholic fermentations but increases the amounts of acetaldehyde and volatile acids formed, especially acetic. S. Kostuichev (319) secured appreciable quantities of succinic, acetic and malic acids by adding calcium carbonate to fermentations having a large amount of yeast and an absence of nitrogenous nutrients. S. Kostuichev (318) secured acetic and malic acids from a medium containing calcium carbonate and succinic acid. He decided that acetic acid is probably produced from acetaldehyde by oxidation and succinic acid from glutaminic acid according to the equation

$$\text{CH}_2\text{COOH} \cdot \text{CH}_2 \cdot \text{CH} \cdot (\text{NH}_2)\text{COOH} + \text{O}_2 = (\text{CH}_2\text{COOH})_2 \text{ CO}_2 + \text{NH}_3$$

From a fermentation, by a yeast and a bacteria, of sugar containing an infusion of tea, H. Valentine (610) secured lactic, malic, tartaric, citric, oxalic, malonic, acetic, succinic, and gluconic acids. J. Weichberz (623) identified valeric acid and probably capric acid in yeast and stated
there are other unidentified non-volatile acids present. W. Windisch (644) showed that agitation increases the acidity during fermentation. W. Windisch (648) also found there is no regular correlation between the composition of the wort and acid formation. He determined that yeast does not produce enough acid to form the optimum pH value without outside help by the addition of mineral or lactic acids.

The method of formation of the esters has been a point of controversy. E. Kayser (290-292) has shown that distillation of yeasts always gives esters, and the addition of acetic acid to fermentations does not increase the ester content of the distillate. He concluded esters are formed in the yeast cell and the loss of esters during fermentation is due to the escaping carbon dioxide. G. Ordonneau (456) found formic, acetic, butyric, crotonic, caproic, and cenanthic acids and the ethylic esters of these acids in brandy. He showed that a slow distillation gives higher yields of esters than rapid distillations as the prolonged ebullition causes esterification of the acids. H. Zikes (671) found high temperatures during fermentation favor the production of acids and esters.

Acetaldehyde and glycerol have received much attention as products of yeast fermentation due to their presence as intermediate compounds. In ordinary fermentations, W. Connstein (108) found 0.3 per cent of the sugar is
converted to glycerol, while the addition of alkaline salts as sodium acetate and bicarbonate increases the glycerol to 5-10 per cent and sodium sulfite further increases the amount of glycerol formed. C. Neuberg (432) found calcium sulfite better than sodium sulfite for stopping fermentation at the acetaldehyde-glycerol stage. According to Y. Tomoda (599) sodium bisulfite is injurious to the yeast cell and glycerol fermentation due to the bisulfite ion, but sodium carbonate can regenerate the yeast activity. E. Abderhalden (5) increased the formation of glycerol by removing the acetaldehyde by charcoal. The aeration of alcoholic fermentations was shown by Y. Tomoda (600) to depress the alcohol formation, but it increases the amount of acetaldehyde and glycerol to 10 per cent of the sugar fermented. According to C. Neuberg (427) the fermentation of magnesium salts of fructose and diphosphate, as the substrate, yields 100 per cent of the theoretical amount of glycerol and pyruvic acid. When yeast juice is used, these two compounds are in equal amounts, but if live yeast is used, this equivalence disappears. P. Levine (339) obtained 50 to 60 per cent of optically active glycols by adding ketones and hydroxyaldehydes to fermenting mixtures of yeast and sugar.

Fusel oil or the higher alcohols are always present as products of fermentation. Their formation by yeast has been
explained by A. Dox (130) as, first the addition of one molecule of water with the subsequent loss of one molecule of ammonia and carbon dioxide. This produces an alcohol of the same chemical structure as the acid but with one less carbon atom. According to J. Effront (137) some amino acids are decomposed by an enzyme, amidase into ammonia and volatile acids but not alcohols. H. Pringsheim (500) showed that normal butyl and iso-propyl alcohols are produced by butyric acid bacteria. F. Ehrlich (141) found fusel oil is not formed by the bacteria and if the nitrogen is present in a readily assimilable form, the yeast does not decompose the amino acid and less fusel oil is produced. H. Pringsheim (499) found the addition of ammonium sulfate reduces the amount of fusel oil formed. According to G. Heinzelmann (255) the addition of asparagine, ammonium salts or autolyzed yeast decreases the production of higher alcohols. He also found the use of small amounts of pitching yeast gives more fusel oil than pitching with large amounts. M. Yamada (662), using sake yeast in Hayduck's solution found leucine, glycine, and alanine increase fusel oil formation, while asparagine, glutamic acid, and ammonium sulfate give no fusel oil. The amino acids in autolyzed yeast were determined by J. Meisenheimer (387) who found glycocoll, alanine, valine, leucine, proline, phenylamine, aspartic, and glutaminic acids to be present. E. Luce (354) found, on analysis of a fusel oil, that it contained 3 per cent pelargonic acid, 9.5 per cent
capric acid but no acids lower than C_{18}H_{36}O_2. The fusel oil from corn whisky was found by H. Stevenson (577) to contain 75 per cent isopropyl alcohol, 13 per cent normal propyl alcohol, 4 per cent isobutyl alcohol, 2 per cent amyl alcohol, and 1 per cent undetermined.

Sulfurous acid has been shown to be present in yeast fermentations, and M. Matsuyama (375) found its production depends upon the race of yeast used and the quantity of sulfates in the substrate.

G. Warcollier (620) found 0.12 to 1.66 grams per liter of acrolein in distilled liquor from ciders. He attributed its presence to the organism B. welchii. He also showed acrolein can be determined colorimetrically by using the Schiff-Gayon reagent.

A. Windhaus (634) secured the same sterol, ergosterol, from yeast as has been obtained from ergot and N. Meland (627) identified episterol, anasterol, and zymosterol in the sterol fraction of yeast. I. McLean (383) reported a sterol, resembling ergosterol, made up 20 per cent of the yeast fat which also contains palmitic, oleic, linolic, and probably lauric and arachidic acids.

In working on the relation of chemical composition to the taste and aroma of spirits, T. Chrzaszcz (97) found that
the materials of construction used in stills, tanks and
tubs have no effect on the compounds present in the finished
product. Continuous systems were shown to give better and
more uniform products than batch systems. He found poor grade
raw materials, bad fermentations, and infection give off-
tastes. He found no relation between the acid, aldehyde and
ester contents and the flavor, but high fusel oil content
and a rapid reduction of potassium permanganate have a direct
relation to bad taste. T. Chrzaszcz (86) later found that
furfural has the strongest influence towards causing poor
taste, while aldehydes and acidity have a slight effect.
S. Haste (251) decided the "character" or palate flavor of
whisky is affected by furfural, alcohols, acids, esters and
aldehydes. He found furfural itself has little effect on
the final "character" when present in normal quantities.

The method of distillation used has been shown to affect
the final products. S. Haste (250), found the quantity of
secondary products in whisky, as acids, esters, aldehydes,
etc., depends upon the distillation rate and the time of
change from foreshots to feints. A. Adams (9) decided
practically all of the higher alcohols from whisky distillery
beers are found in the finished products, while the quantity
of acids is decreased during distillation.

Wherever low wines of a whisky distillery are allowed to
stand in a copper container a deposit is formed which is
known as "whisky verdigris". R. Baker (30) analyzed this deposit and found it contained ethyl caprate, laurate, palmitate, oleate, and linolate and palmitic, oleic, and linolic acids.
EXPERIMENTAL
The products secured from a fermentation are the results of all the various factors involved and this investigation required the study of the following before any fermentations could be attempted: waters normally used in fermentations; grains and methods of processing them; yeast, its selection and growth; and methods of analyses of the finished products.

Since the main object of this investigation was to determine the effects of the minerals normally found in limestone waters upon the products of fermentation, it was first necessary to secure and analyze some of these waters. Samples of water were secured from eight different sources, which were being used in fermentations. These samples were analyzed and the results of these analyses are given in Table I.

The methods used in the analyses of these waters were the standard procedures found in the "Mineral Analysis of Water", Volume II, Scott's "Technical Methods of Analysis", published by D. Van Nostrand Co.

An attempt was made to evaluate the grains used for fermentation by chemical analysis. Although the percentage of starch, sugars, etc. are determined, no definite relationship could be established between the percentages of these compounds and the yields of alcohol and by-products secured. It was decided the only test of any value was the use of blank fermentations run on the grains under the same conditions
as were used in the experimental runs.

Different yeasts were tried and the one finally selected was a strain of distiller's bottom yeast belonging to the Saccharomyces cerevisiae type. This yeast was selected because it had been used in whisky distilleries for a period of at least thirty-five years and had a good reputation for yield and quality of the products secured from its use.

**APPARATUS**

The apparatus used will be divided according to the operations involved, namely: yeast growing, grinding of grain, mashing, fermenting, distilling, redistilling and analyzing.

Glass apparatus was used in growing and testing the yeast for runs 1 through 7. The stock yeast was propagated in a 500 c.c. Erlenmeyer flask. The yeast mash was soured, sterilized and grown in a 2000 c.c. flask. The yeast for runs 8 through 13 was grown in plant equipment. In this case, a 50 gallon dona mash was made and stocked with jug yeast. This dona yeast was then transferred to a yeast mash of 700 gallons in a tin lined copper yeast tub of 950 gallons.

The microscopic examination and counting of the yeast was done in a Howard Counting Cell and on slides, with a magnification of 550 X.
The grinding of the grain was done in a three high roll mill of commercial capacity.

The mashing of the grain for runs 1 through 7 was done in a 10 gallon open crockware container which was equipped with one inch steam and cold water coils. The runs 8 through 13 were mashed on commercial equipment. The cooking of the corn was done in a 300 bushel, 8$\frac{1}{2}$ ft. by 25 ft. pressure cooker followed by the mashing of the rye and the malting in a 21 ft. diameter mash tub equipped with cooling coils.

The fermenters for runs 1 through 7 were 2 gallon glazed jars, while 1 gallon glass jars were used for runs 8 through 13.

The distillation of the beers of runs 1 through 7 was done in a 1$\frac{1}{2}$ gallon pot still equipped with steam heating coils at the bottom. The still had a 2$\frac{1}{2}$ by 6 inch dephlegmator column from which the vapors passed into a copper condenser equipped with tin condensing coils.

The distillation of the beers of runs 8 through 13 was done in 1 gallon batch live steam stills. The vapors passed through a reflux and then into glass condensers.

The redistillation of all samples was done in 1000 c.c. glass distilling flasks and condensed in glass condensers.

The apparatus used in analyzing the products was standard
laboratory equipment and will be mentioned under the methods of analysis.

PROCEDURES

The first step in making a test run was the propagation of the yeast. A supply of jug yeast was grown by stocking a malt-hops mash liquor with a pure culture. The mash liquor was prepared by adding 8 grams of hops to 300 c.c. of water. The mixture was boiled for 30 minutes and strained through cheese cloth. The temperature of the liquid was adjusted to 55° F. and 75 grams of barley malt were added. The temperature was held at 140° F. for 1 hour and the mash again strained through cheese cloth into a sterile 500 c.c. flask. The liquid was brought to 15 pounds steam pressure and sterilized for 1 hour. It was allowed to cool to 80° F. and stocked with 10 c.c. of pure culture from a previous jug yeast and the mass allowed to stand for 24 hours at 80° F. It was then placed in sterile pressure bottles and kept in a refrigerator until needed.

The next step in growing the yeast was making the dona yeast. This was done by soaking 3 grams of hops in 125 c.c. of water at 180° F. for three hours. The hops were strained off and the temperature adjusted to 155° F. 15 grams of barley malt were added and a temperature of 140° F. maintained for 1½ hours. The mash was then boiled for 15 minutes, cooled
to 80° F. and stocked with 10 c.c. of jug yeast. The dona was then held at 80 to 85° F. for 12 hours and then pitched to the main yeast mash.

The main yeast mash was prepared by adding 60 grams of rye to 400 c.c. of water at a temperature of 160° F. This temperature was maintained for 20 minutes and then allowed to drop to 150° F. 60 grams of barley malt were then added and the temperature held at 140° F. for 1 hour. The mash was then boiled for 30 minutes, cooled to 80° F., and the dona yeast added. This main or day yeast was allowed to propagate at 80-85° F. for 6 hours at which time it had a cell count of 150 X 10⁶ to 200 X 10⁶ per c.c. of mash and was ready for pitching to the fermenters.

The grain mash for runs 1 through 7 was made with 6.12 pounds of ground corn meal, 0.36 pounds of coarse ground rye, and 0.72 pounds of coarse ground malt, or a total charge of 7.2 pounds of grain. This made a charge of 85 per cent corn, 5 per cent rye, and 10 per cent barley malt.

The mashing of this grain was done by running 3 gallons of distilled water into the mash tub and heating it to 150° F. The corn was added with constant agitation and the temperature slowly raised to boiling. The corn mash was boiled for 30 minutes and then cooled to 165° F. and the rye added. This temperature was maintained for 15 minutes and the mass
cooled to 150° F. and the malt added. The temperature was held at 145° F. for 1 hour and then dropped to 80° F., when sufficient day yeast was added to give a cell count of $8 \times 10^6$ per c.c. on a volume of 6 gallons.

The mash was then divided into four equal parts and run into the fermenters. The salt solutions were added to make the desired concentrations and distilled water added to make a total volume of $1\frac{1}{2}$ gallons in each fermenter. The temperature was held below 90° F. and the fermentation allowed to proceed for 72 hours. At the end of this time the beer was distilled in the pot still. Each fermentation was distilled until the temperature of the vapors leaving the still was that of the boiling point of water at the existing barometric pressure.

The mashing for the runs 8 through 13 was done on large plant scale equipment. The mash was made from a grain charge of 80 per cent corn, 8 per cent rye, and 12 per cent barley malt. The cooking of the corn was done with live steam in a pressure cooker.

The cooker was charged with 5500 gallons of well water and the temperature adjusted to 150° F. To this was added 15,400 pounds of ground corn meal and 300 pounds of barley malt. The temperature was slowly raised to 300° F. and held for 5 minutes. The purpose of the malt was to give a partly
liquified mash which would pump easily. The pressure was released and the corn mash pumped to the mash tub where it was cooled to 160° F. and 1540 pounds of ground rye added. This temperature was maintained for 10 minutes and the temperature then dropped to 150° F. when 2010 pounds of ground barley malt were added. A temperature of 145° F. was maintained for 45 minutes and the mash then cooled to 30° F. Enough mash was taken from this tub to make the charges for runs 8, 9 and 10. Another mash of the same composition was made in the same manner, and part of it was taken for runs 11, 12 and 13.

1666 c.c. of this mash were taken for each fermentation. To it was added sufficient yeast to make a cell count of \(8 \times 10^6\) per c.c. in a volume of 2000 c.c. The salt solutions were added to give the correct concentration and the volume made up to 2000 c.c. by adding distilled water. The fermentations were set at 80° F. and kept at 80 to 88° F. for 72 hours. At this time, the beers were steam distilled until the temperature of the vapor was that of the boiling point of water at the existing barometric pressure.

The distillates secured from the two methods of distilling the beer were then redistilled in a 1000 c.c. distilling flask equipped with a three bulb distilling head and a glass condensor. The distillate secured was adjusted to approximately 100 proof for analysis.
ANALYSIS OF THE DISTILLATES

The analysis of the final redistilled distillate involved the determination of acidity, expressed as acetic acid; esters, expressed as ethyl acetate; aldehydes, expressed as acetaldehyde; furfural; gravity of the original distillate and proof of the final distillate; (pH values on some runs and fusel oil on some runs).

The methods used for analysis of the distillates were those accepted by United States Internal Revenue Department. They are as follows:

ACIDS - Total - as Acetic Acid

50 c.c. of the distillate at 100 proof was rapidly titrated with 0.1 N sodium hydroxide solution to an end point with phenolphthalein. From the number of c.c. of standard base required, the grains of acetic acid per 100,000 c.c. were determined.

ESTERS - as Ethyl Acetate

50 c.c. of the 100 proof distillate was exactly neutralized with 0.1 N NaOH to phenolphthalein. Then 25 c.c. of 0.1 N base was added in excess and the mixture boiled for 1 hour under a reflux having a mercury vapor seal. The liquid was cooled and the excess base rapidly titrated with 0.1 N H₂SO₄. From the data secured the grams of ethyl...
acetate per 100,000 c.c. were calculated.

**ALDEHYDES - As Acetaldehyde**

The solutions necessary for the determination of acetaldehyde are aldehyde-free alcohol (50 per cent), sulfite-fuchsin solution and a standard acetaldehyde solution.

The aldehyde-free alcohol was prepared by redistilling 95 per cent alcohol from sodium hydroxide. Three grams of meta-phenyleneamine hydrochloride were added and the alcoholic solution digested under a reflux for 5 hours. It was then slowly distilled and the middle part of the distillate collected and tested to be certain it did not give an aldehyde test.

The sulfite-fuchsin solution was prepared by dissolving 0.5 gram of fuchsin in 500 c.c. of distilled water. Sulfur dioxide was bubbled into the solution until the solution was colorless. This solution was kept in a refrigerator as it decomposes rapidly at ordinary temperatures.

The standard acetaldehyde solution was prepared by dissolving 1.386 grams of purified aldehyde ammonia in 50 c.c. of 95 per cent alcohol. 22.7 c.c. of 1 N alcoholic H$_2$SO$_4$ solution were added and the solution made up to 100 c.c. with 95 per cent alcohol. 0.8 c.c. of alcohol were added and the solution allowed to stand over night and then filtered. This solution contained 1 gram of acetaldehyde in 100 c.c.
Standards were prepared by diluting 2 c.c. of this solution to 100 c.c. with a 50 per cent alcohol-water solution. 1 c.c. of this diluted solution contained 0.0002 grams of acetaldehyde.

The determination of aldehydes was made by diluting 10 c.c. of the distillate to 50 c.c. with a 50 per cent alcohol-water solution and adding 25 c.c. of the sulfite-fuchsin solution. This mixture was allowed to stand at 15°C for 15 minutes and its color was then compared in a colorimeter with the standard aldehyde solution which had been run in the same way as the unknown. The aldehydes were then calculated as acetaldehyde in grams per 100,000 c.c.

**FURFURAL**

A standard furfural solution was prepared by dissolving 1 gram of redistilled furfural in 100 c.c. of 95 per cent alcohol. Working standards were prepared by diluting 1 c.c. of this concentrated solution to 100 c.c. with a 50 per cent alcohol-water solution. 1 c.c. of this dilute solution contained 0.1 milligram of furfural.

The determination of furfural was made by diluting 10 c.c. of the 100 proof distillate to 50 c.c. with a 50 per cent alcohol-water solution. To this was added 2 c.c. of aniline and 0.5 c.c. of HCl (sp. gr. 1.125) and the mixture kept at 15°C for 15 minutes. It was then
compared in the colorimeter with the standard furfural solution which had been run simultaneously. The furfural was then calculated in grams per 100,000 c.c.

**PH VALUE**

The pH value on the distillate was determined with the glass electrode using a Kasstner Titrometer.

**GRAVITY OF FIRST DISTILLATE**

The specific gravity of the first distillate was determined by the Westphal balance and the result calculated to c.c. of 100 proof alcohol.

**PROOF OF SECOND DISTILLATE**

The proof of the second distillate was determined with a Bureau of Standards certified alcohol hydrometer.

The results of the various runs and the partial analysis of the products secured are shown in Tables II through XV and Figures I through XIII.
TABLES
TABLE NUMBER I

ANALYSES OF SOME KENTUCKY WATERS USED IN FERMENTATIONS

<table>
<thead>
<tr>
<th></th>
<th>No. 1 Scott County</th>
<th>No. 2 Fayette County</th>
<th>No. 3 Nelson County</th>
<th>No. 4 Jefferson County</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>10</td>
<td>6</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Fe</td>
<td>trace</td>
<td>1.5</td>
<td>0.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Al</td>
<td>1.0</td>
<td>0.3</td>
<td>6.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Mn</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Ca</td>
<td>72.1</td>
<td>10.3</td>
<td>332</td>
<td>110</td>
</tr>
<tr>
<td>Mg</td>
<td>23.6</td>
<td>9.6</td>
<td>170</td>
<td>18</td>
</tr>
<tr>
<td>I</td>
<td>8</td>
<td>7</td>
<td>620</td>
<td>22</td>
</tr>
<tr>
<td>PO$_4$</td>
<td>19</td>
<td>14</td>
<td>68</td>
<td>16</td>
</tr>
<tr>
<td>SO$_4$</td>
<td>40</td>
<td>49</td>
<td>428</td>
<td>48</td>
</tr>
<tr>
<td>HCO$_3$</td>
<td>284</td>
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<td>Total Solids</td>
<td>326</td>
<td>438</td>
<td>1960</td>
<td>448</td>
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</tbody>
</table>

Above units in parts per million.
### TABLE NUMBER I (continued)

ANALYSES OF SOME KENTUCKY WATERS USED IN FERMENTATIONS

<table>
<thead>
<tr>
<th></th>
<th>No. 5 Fayette County</th>
<th>No. 6 Franklin County</th>
<th>No. 7 Nelson County</th>
<th>No. 8 Harrods County</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>11.2</td>
<td>2.3</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Fe</td>
<td>trace</td>
<td>1.2</td>
<td>trace</td>
<td>2.1</td>
</tr>
<tr>
<td>Al</td>
<td>2.9</td>
<td>0.6</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Mn</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Ca</td>
<td>81</td>
<td>6.3</td>
<td>32</td>
<td>78</td>
</tr>
<tr>
<td>Mg</td>
<td>7.4</td>
<td>26</td>
<td>19.2</td>
<td>22</td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>592</td>
<td>61</td>
<td>11.8</td>
</tr>
<tr>
<td>PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>8</td>
<td>38</td>
<td>26</td>
<td>6.2</td>
</tr>
<tr>
<td>SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>15</td>
<td>184</td>
<td>62</td>
<td>23</td>
</tr>
<tr>
<td>HCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>240</td>
<td>63</td>
<td>219</td>
<td>286</td>
</tr>
<tr>
<td>Total Solids</td>
<td>263</td>
<td>1630</td>
<td>344</td>
<td>304</td>
</tr>
</tbody>
</table>

Above units in parts per million
### TABLE NUMBER II A

**RUN NO. 1**

Added Salt Concentration of Set Beers

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>CALCIUM SULFATE</th>
<th>DISODIUM PHOSPHATE</th>
<th>SODIUM CHLORIDE</th>
<th>MAGNESIUM SULFATE</th>
<th>SODIUM BICARBONATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water - no salts added</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Water - no salts added</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>.000185 M</td>
<td>.000038 M</td>
<td>.00022 M</td>
<td>.0000105 M</td>
<td>.000017 M</td>
</tr>
<tr>
<td>4</td>
<td>.000185 M</td>
<td>.000176 M</td>
<td>.00022 M</td>
<td>.0000105 M</td>
<td>.000017 M</td>
</tr>
<tr>
<td>5</td>
<td>.000185 M</td>
<td>.000254 M</td>
<td>.00022 M</td>
<td>.0000105 M</td>
<td>.000017 M</td>
</tr>
<tr>
<td>6</td>
<td>.000185 M</td>
<td>.000352 M</td>
<td>.00022 M</td>
<td>.0000105 M</td>
<td>.000017 M</td>
</tr>
<tr>
<td>7</td>
<td>.000185 M</td>
<td>.000440 M</td>
<td>.00022 M</td>
<td>.0000105 M</td>
<td>.000017 M</td>
</tr>
<tr>
<td>8</td>
<td>.000185 M</td>
<td>.000528 M</td>
<td>.00022 M</td>
<td>.0000105 M</td>
<td>.000017 M</td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>100 PROOF SPIRIT</td>
<td>ACIDS AS ACETIC</td>
<td>ESTERS AS ETHYL ACETATE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>secured in c.c.</td>
<td>grams per 100,000 c.c.</td>
<td>total grams secured</td>
<td>grams per 100,000 c.c.</td>
<td>total grams secured</td>
</tr>
<tr>
<td>1</td>
<td>358</td>
<td>7.29</td>
<td>0.026</td>
<td>26.2</td>
<td>0.093</td>
</tr>
<tr>
<td>2</td>
<td>369</td>
<td>19.6</td>
<td>0.070</td>
<td>18.7</td>
<td>0.067</td>
</tr>
<tr>
<td>3</td>
<td>384</td>
<td>50.4</td>
<td>0.193</td>
<td>31.5</td>
<td>0.117</td>
</tr>
<tr>
<td>4</td>
<td>362</td>
<td>32.2</td>
<td>0.116</td>
<td>22.3</td>
<td>0.080</td>
</tr>
<tr>
<td>5</td>
<td>371</td>
<td>43.1</td>
<td>0.160</td>
<td>43.5</td>
<td>0.187</td>
</tr>
<tr>
<td>6</td>
<td>366</td>
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<td>0.077</td>
<td>43.9</td>
<td>0.160</td>
</tr>
<tr>
<td>7</td>
<td>380</td>
<td>13.6</td>
<td>0.051</td>
<td>51.8</td>
<td>0.197</td>
</tr>
<tr>
<td>8</td>
<td>373</td>
<td>12.9</td>
<td>0.046</td>
<td>47.4</td>
<td>0.177</td>
</tr>
</tbody>
</table>
## TABLE NUMBER III A

### RUN NO. 2

Added Salt Concentration of Set Beers

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>CALCIUM SULFATE</th>
<th>POTASSIUM CARBONATE</th>
<th>MAGNESIUM SULFATE</th>
<th>DISODIUM PHOSPHATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 a</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.00001 M</td>
</tr>
<tr>
<td>1 b</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.00001 M</td>
</tr>
<tr>
<td>2 a</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.00001 M</td>
</tr>
<tr>
<td>2 b</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.00001 M</td>
</tr>
<tr>
<td>3 a</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0005 M</td>
</tr>
<tr>
<td>3 b</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0005 M</td>
</tr>
<tr>
<td>4 a</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.001 M</td>
</tr>
<tr>
<td>4 b</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.001 M</td>
</tr>
<tr>
<td>5 a</td>
<td>Distilled water only - no salts added</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 b</td>
<td>Distilled water only - no salts added</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE NUMBER III B

ANALYSES OF DISTILLATES FROM RUN 2

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>100 PROOF SPIRIT</th>
<th>ACIDS AS ACETIC</th>
<th>ESTERS AS ETHYL ACETATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>secured in c.c.</td>
<td>grams per 100,000 c.c.</td>
<td>total grams secured</td>
</tr>
<tr>
<td>1 a</td>
<td>233</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>1 b</td>
<td>246</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>1 avg.</td>
<td>239</td>
<td>6.1</td>
<td>0.014</td>
</tr>
<tr>
<td>2 a</td>
<td>347</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>2 b</td>
<td>354</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>2 avg.</td>
<td>350</td>
<td>5.2</td>
<td>0.018</td>
</tr>
<tr>
<td>3 a</td>
<td>365</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>3 b</td>
<td>361</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>3 avg.</td>
<td>363</td>
<td>6.0</td>
<td>0.021</td>
</tr>
<tr>
<td>4 a</td>
<td>374</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>4 b</td>
<td>368</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>4 avg.</td>
<td>371</td>
<td>6.7</td>
<td>0.024</td>
</tr>
<tr>
<td>5 a</td>
<td>245</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>5 b</td>
<td>232</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>5 avg.</td>
<td>237</td>
<td>5.9</td>
<td>0.014</td>
</tr>
<tr>
<td>Time (h)</td>
<td>0.001</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>---------</td>
<td>-------</td>
<td>-----</td>
<td>------</td>
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<td>0.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
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<td>0.0</td>
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<td>0.0</td>
</tr>
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<td>0.0</td>
</tr>
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<td>4.95</td>
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</tr>
</tbody>
</table>

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**Sample Analysis of Distillates From Run 2**

<table>
<thead>
<tr>
<th>DISTILLATES</th>
<th>Acetic Acetate</th>
<th>Acetate Peroxide</th>
<th>Acetate Alcohol</th>
<th>Acetates</th>
<th>Acetate Value</th>
<th>Acetates + Acetate Peroxide</th>
<th>Acetates + Acetate Peroxide + Acetate Alcohol</th>
<th>Acetates + Acetate Peroxide + Acetate Alcohol + Acetate Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Table Number III (Contd.)**
TABLE NUMBER IV A

RUN NO. 3

Added Salt Concentration of Set Beers

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>CALCIUM SULFATE</th>
<th>POTASSIUM CARBONATE</th>
<th>MAGNESIUM SULFATE</th>
<th>DISODIUM PHOSPHATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>2</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>3</td>
<td>0.0005 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>4</td>
<td>0.001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>5</td>
<td>Distilled water - no salts added</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>100 PROOF SPIRIT</td>
<td>ACIDS AS ACETIC</td>
<td>ESTERS AS ETHYL ACETATE</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------------------</td>
<td>------------------</td>
<td>-------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>secured in c.c.</td>
<td>grams per 100,000 grams</td>
<td>total grams secured</td>
<td>grams per 100,000 grams</td>
</tr>
<tr>
<td>1</td>
<td>352</td>
<td>13.4</td>
<td>0.047</td>
<td>14.9</td>
</tr>
<tr>
<td>2</td>
<td>338</td>
<td>12.1</td>
<td>0.040</td>
<td>14.2</td>
</tr>
<tr>
<td>3</td>
<td>345</td>
<td>15.6</td>
<td>0.053</td>
<td>16.8</td>
</tr>
<tr>
<td>4</td>
<td>356</td>
<td>15.3</td>
<td>0.054</td>
<td>18.4</td>
</tr>
<tr>
<td>5</td>
<td>311</td>
<td>12.8</td>
<td>0.039</td>
<td>15.2</td>
</tr>
</tbody>
</table>
TABLE NUMBER IV B (continued)

ANALYSES OF DISTILLATES FROM RUN 3

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>FURFURAL grams per 100,000 c.c.</th>
<th>ALDEHYDES AS ACETALDEHYDE grams per 100,000 c.c. total grams secured</th>
<th>PH VALUE OF DISTILLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.03</td>
<td>0.7</td>
<td>0.002</td>
</tr>
<tr>
<td>2</td>
<td>0.06</td>
<td>0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>0.07</td>
<td>0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>5</td>
<td>0.02</td>
<td>0.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>
### TABLE NUMBER V A

**RUN NO. 4**

**Added Salt Concentration in Set Beer**

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>CALCIUM</th>
<th>POTASSIUM</th>
<th>MAGNESIUM SULFATE</th>
<th>DIAMMONIUM PHOSPHATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.00001 M</td>
</tr>
<tr>
<td>2</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>3</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0005 M</td>
</tr>
<tr>
<td>4</td>
<td>Distilled water - no salts added</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAMPLE SPIRIT</td>
<td>100 PROOF SPIRIT IN C.C.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>288</td>
<td>341</td>
<td>370</td>
<td>231</td>
</tr>
</tbody>
</table>

**ANALYSES OF DISTILLATES FROM RUN 4**

<table>
<thead>
<tr>
<th>ESYTERS AS ETHYL ACETATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>grams per total 100,000 c.c. secured</td>
</tr>
<tr>
<td>grams secured 100,000 c.c.</td>
</tr>
</tbody>
</table>

**ACIDS AS ACETIC**

| grams per total 100,000 c.c. secured | 0.014 | 0.014 | 0.018 | 0.015 |
| grams secured 100,000 c.c.           | 6.2   | 4.2   | 5.1   | 6.0   |
TABLE NUMBER V B (continued)

ANALYSES OF DISTILLATES FROM RUN 4

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>FURFURAL grams per 100,000 c.c.</th>
<th>ALDEHYDES AS ACETALDEHYDE grams per 100,000 grams c.c. secured</th>
<th>PH VALUE OF DISTILLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.8</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>0.8</td>
<td>0.002</td>
</tr>
<tr>
<td>3</td>
<td>0.08</td>
<td>0.8</td>
<td>0.002</td>
</tr>
<tr>
<td>4</td>
<td>0.00</td>
<td>0.2</td>
<td>0.0005</td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>CALCIUM SULFATE</td>
<td>POTASSIUM CARBONATE</td>
<td>MAGNESIUM SULFATE</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------</td>
<td>---------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>1</td>
<td>0.00001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>2</td>
<td>0.00005 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>3</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>4</td>
<td>Distilled water - no salts added</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE NUMBER VI B

**ANALYSES OF DISTILLATES FROM RUN 5**

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>100 PROOF SPIRIT</th>
<th>ACIDS AS ACETIC</th>
<th>ESTERS AS ETHYL ACETATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>secured in c.c.</td>
<td>grams per 100,000 grams secured</td>
<td>grams per total 100,000 grams secured</td>
</tr>
<tr>
<td>1</td>
<td>366</td>
<td>12.3</td>
<td>0.045</td>
</tr>
<tr>
<td>2</td>
<td>322</td>
<td>14.6</td>
<td>0.047</td>
</tr>
<tr>
<td>3</td>
<td>356</td>
<td>11.7</td>
<td>0.041</td>
</tr>
<tr>
<td>4</td>
<td>261</td>
<td>6.0</td>
<td>0.015</td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>FURFURAL</td>
<td>ALDEHYDES AS ACETALDEHYDE</td>
<td>PH VALUE OF DISTILLATE</td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>--------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td></td>
<td>grams per 100,000 c.c.</td>
<td>grams per total 100,000 c.c.</td>
<td>grams secured</td>
</tr>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.07</td>
<td>0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>0.00</td>
<td>0.2</td>
<td>0.0005</td>
</tr>
</tbody>
</table>
### TABLE NUMBER VII A

**RUN NO. 6**

**Added Salts Concentration in Set Beer**

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>CALCIUM SULFATE</th>
<th>POTASSIUM CARBONATE</th>
<th>MAGNESIUM SULFATE</th>
<th>DIAMMONIUM PHOSPHATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>2</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.00005 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>3</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>4</td>
<td>Distilled water - no salts added</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>100 PROOF SPIRIT</th>
<th>ACIDS AS ACETIC</th>
<th>ESTERS AS ETHYL ACETATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>secured in c.c.</td>
<td>grams per 100,000 c.c.</td>
<td>total grams secured</td>
</tr>
<tr>
<td>1</td>
<td>278</td>
<td>17.3</td>
<td>0.048</td>
</tr>
<tr>
<td>2</td>
<td>230</td>
<td>19.4</td>
<td>0.056</td>
</tr>
<tr>
<td>3</td>
<td>260</td>
<td>20.3</td>
<td>0.052</td>
</tr>
<tr>
<td>4</td>
<td>261</td>
<td>6.0</td>
<td>0.015</td>
</tr>
</tbody>
</table>
### TABLE NUMBER VII B (continued)

**ANALYSES OF DISTILLATES FROM RUN 6**

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>FURFURAL grams per 100,000 c.c.</th>
<th>ALDEHYDES AS ACETALDEHYDE grams per 100,000 c.c.</th>
<th>total grams secured</th>
<th>PH VALUE OF DISTILLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>1.0</td>
<td>0.002</td>
<td>4.18</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>1.0</td>
<td>0.002</td>
<td>4.20</td>
</tr>
<tr>
<td>3</td>
<td>0.19</td>
<td>1.0</td>
<td>0.002</td>
<td>4.19</td>
</tr>
<tr>
<td>4</td>
<td>0.00</td>
<td>0.2</td>
<td>0.0005</td>
<td>4.45</td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>CALCIUM SULFATE</td>
<td>M 0.00001</td>
<td>M 0.00001</td>
<td>M 0.00001</td>
<td>M 0.00001</td>
</tr>
<tr>
<td>M 0.00001</td>
<td>M 0.00001</td>
<td>M 0.00001</td>
<td>M 0.00001</td>
<td></td>
</tr>
<tr>
<td>DISTRIBUTED WATER - NO SALT ADDED</td>
<td>0.00001</td>
<td>0.00001</td>
<td>0.00001</td>
<td>0.00001</td>
</tr>
</tbody>
</table>
### TABLE NUMBER VIII B
**ANALYSES OF DISTILLATES FROM RUN 7**

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>100 PROOF SPIRIT</th>
<th>ACIDS AS ACETIC</th>
<th>ESTERS AS ETHYL ACETATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>secured in c.c.</td>
<td>grams per 100,000 c.c.</td>
<td>total grams secured</td>
</tr>
<tr>
<td>1</td>
<td>339</td>
<td>11.2</td>
<td>0.037</td>
</tr>
<tr>
<td>2</td>
<td>342</td>
<td>11.8</td>
<td>0.040</td>
</tr>
<tr>
<td>3</td>
<td>362</td>
<td>13.9</td>
<td>0.053</td>
</tr>
<tr>
<td>4</td>
<td>261</td>
<td>6.0</td>
<td>0.015</td>
</tr>
</tbody>
</table>
### TABLE NUMBER VIII B (continued)

**ANALYSES OF DISTILLATES FROM RUN 7**

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>FURFURAL</th>
<th>ALDEHYDES AS ACETALDEHYDE</th>
<th>PH VALUE OF DISTILLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grams per 100,000 c.c.</td>
<td>grams per total 100,000 grams secured</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.17</td>
<td>2.3</td>
<td>0.007</td>
</tr>
<tr>
<td>2</td>
<td>0.19</td>
<td>1.0</td>
<td>0.003</td>
</tr>
<tr>
<td>3</td>
<td>0.21</td>
<td>2.9</td>
<td>0.011</td>
</tr>
<tr>
<td>4</td>
<td>0.00</td>
<td>0.2</td>
<td>0.0005</td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>MAGNESIUM SULFATE CONCENTRATION</td>
<td>100 PROOF SPIRIT secured in c.c.</td>
<td>ACIDS as ACETIC grams per 100,000 c.c.</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>0.002 M</td>
<td>155</td>
<td>24.5</td>
</tr>
<tr>
<td>2</td>
<td>0.005 M</td>
<td>158</td>
<td>24.2</td>
</tr>
<tr>
<td>3</td>
<td>0.01 M</td>
<td>144</td>
<td>24.8</td>
</tr>
<tr>
<td>4</td>
<td>0.1 M</td>
<td>152</td>
<td>24.5</td>
</tr>
<tr>
<td>Control</td>
<td>no salts</td>
<td>167</td>
<td>25.2</td>
</tr>
</tbody>
</table>
**TABLE NUMBER IX (continued)**

**RUN No. 8**

**Salt Used - Magnesium Sulfate**

<table>
<thead>
<tr>
<th>SAMPLE No.</th>
<th>MAGNESIUM SULFATE CONCENTRATION</th>
<th>ESTERS AS ETHYL ACETATE</th>
<th>ALDEHYDES AS ACETALDEHYDE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grams per 100,000 c.c. secured</td>
<td>grams per total grams</td>
<td>grams per total grams</td>
</tr>
<tr>
<td>1</td>
<td>0.002 M</td>
<td>21.8</td>
<td>96.4</td>
</tr>
<tr>
<td>2</td>
<td>0.005 M</td>
<td>18.7</td>
<td>116.0</td>
</tr>
<tr>
<td>3</td>
<td>0.01 M</td>
<td>19.2</td>
<td>90.5</td>
</tr>
<tr>
<td>4</td>
<td>0.1 M</td>
<td>20.5</td>
<td>51.0</td>
</tr>
<tr>
<td>Control</td>
<td>no salts</td>
<td>21.4</td>
<td>88.8</td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>DIPOTASSIUM PHOSPHATE CONCENTRATION</td>
<td>100 PROOF SPIRIT secured in c.c.</td>
<td>ACIDS AS ACETIC grams per 100,000 c.c.</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>0.001 M</td>
<td>172</td>
<td>27.2</td>
</tr>
<tr>
<td>2</td>
<td>0.005 M</td>
<td>185</td>
<td>26.8</td>
</tr>
<tr>
<td>3</td>
<td>0.01 M</td>
<td>183</td>
<td>26.0</td>
</tr>
<tr>
<td>4</td>
<td>0.1 M</td>
<td>155</td>
<td>39.2</td>
</tr>
<tr>
<td>Control</td>
<td>no salts</td>
<td>167</td>
<td>25.2</td>
</tr>
</tbody>
</table>
# TABLE NUMBER X (continued)

## RUN NO. 9

**Salt Used** - Dipotassium Phosphate

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>DIPOTASSIUM PHOSPHATE CONCENTRATION</th>
<th>ESTERS AS ETHYL ACETATE</th>
<th>ALDEHYDES AS ACETALDEHYDE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grams per 100,000 total grams secured</td>
<td></td>
<td>grams per 100,000 total grams secured</td>
</tr>
<tr>
<td>1</td>
<td>24.2</td>
<td>0.041</td>
<td>76.2</td>
</tr>
<tr>
<td>2</td>
<td>21.8</td>
<td>0.040</td>
<td>103.0</td>
</tr>
<tr>
<td>3</td>
<td>23.7</td>
<td>0.043</td>
<td>88.9</td>
</tr>
<tr>
<td>4</td>
<td>22.8</td>
<td>0.035</td>
<td>45.4</td>
</tr>
<tr>
<td>Control</td>
<td>21.4</td>
<td>0.035</td>
<td>88.8</td>
</tr>
</tbody>
</table>
TABLE NUMBER XI

RUN NO. 10

Salt Used - Calcium Carbonate

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>CALCIUM CARBONATE CONCENTRATION</th>
<th>100 PROOF SPIRIT secured in c.c.</th>
<th>ACIDS as ACETIC grams per 100,000 c.c.</th>
<th>total grams secured</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.005 M</td>
<td>172</td>
<td>26.0</td>
<td>0.048</td>
</tr>
<tr>
<td>2.</td>
<td>0.01 M</td>
<td>166</td>
<td>25.2</td>
<td>0.041</td>
</tr>
<tr>
<td>3.</td>
<td>0.1 M</td>
<td>157</td>
<td>25.2</td>
<td>0.039</td>
</tr>
<tr>
<td>4.</td>
<td>0.5 M</td>
<td>120</td>
<td>19.3</td>
<td>0.023</td>
</tr>
<tr>
<td>Control</td>
<td>no salts</td>
<td>167</td>
<td>25.2</td>
<td>0.042</td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>CALCIUM CARBONATE CONCENTRATION</td>
<td>ESTERS AS ETHYL ACETATE</td>
<td>ALDEHYDES AS ACETALDEHYDE</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------</td>
<td>-------------------------</td>
<td>---------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>grams per 100,000 c.c. secured</td>
<td>grams per 100,000 c.c. secured</td>
<td>grams per 100,000 c.c. secured</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.005 M</td>
<td>23.7</td>
<td>0.040</td>
<td>108.7</td>
</tr>
<tr>
<td>2</td>
<td>0.01 M</td>
<td>21.9</td>
<td>0.036</td>
<td>92.5</td>
</tr>
<tr>
<td>3</td>
<td>0.1 M</td>
<td>24.6</td>
<td>0.036</td>
<td>10.7</td>
</tr>
<tr>
<td>4</td>
<td>0.5 M</td>
<td>18.2</td>
<td>0.021</td>
<td>1.8</td>
</tr>
<tr>
<td>Control</td>
<td>no salts</td>
<td>21.4</td>
<td>0.035</td>
<td>88.8</td>
</tr>
<tr>
<td>SAMPLE NO.</td>
<td>AMMONIUM BIFLUORIDE CONCENTRATION</td>
<td>100 PROOF SPIRIT SECURED in c.c.</td>
<td>ACIDS AS ACETIC grams per 100,000 c.c.</td>
<td>total grams secured</td>
</tr>
<tr>
<td>------------</td>
<td>----------------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>1</td>
<td>0.00005 M</td>
<td>169</td>
<td>26.2</td>
<td>0.044</td>
</tr>
<tr>
<td>2</td>
<td>0.0005 M</td>
<td>164</td>
<td>16.1</td>
<td>0.026</td>
</tr>
<tr>
<td>3</td>
<td>0.001 M</td>
<td>164</td>
<td>14.3</td>
<td>0.023</td>
</tr>
<tr>
<td>4</td>
<td>0.01 M</td>
<td>150</td>
<td>5.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Control</td>
<td>no salt</td>
<td>158</td>
<td>30.2</td>
<td>0.047</td>
</tr>
</tbody>
</table>

TABLE NUMBER XII

RUN NO. 11

Salt Used - Ammonium Bifluoride
TABLE NUMBER XII (continued)

RUN NO. 11

Salt Used - Ammonium Bifluoride

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>AMMONIUM BIFLUORIDE CONCENTRATION</th>
<th>ESTERS AS ETHYL ACETATE</th>
<th>ALDEHYDES AS ACETALDEHYDE</th>
<th>CELL COUNT OF FINISHED BEER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>grams per 100,000 c.c.</td>
<td>grams secured</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.00005 M</td>
<td>22.7</td>
<td>0.038</td>
<td>28.8</td>
</tr>
<tr>
<td>2</td>
<td>0.0005 M</td>
<td>18.6</td>
<td>0.030</td>
<td>43.5</td>
</tr>
<tr>
<td>3</td>
<td>0.001 M</td>
<td>19.1</td>
<td>0.031</td>
<td>99.0</td>
</tr>
<tr>
<td>4</td>
<td>0.01 M</td>
<td>8.2</td>
<td>0.012</td>
<td>98.0</td>
</tr>
<tr>
<td>Control</td>
<td>no salt</td>
<td>24.9</td>
<td>0.039</td>
<td>10.1</td>
</tr>
</tbody>
</table>
TABLE NUMBER XIII

RUN NO. 12

Salt Used - Salicylic Acid

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>SALICYLIC ACID CONCENTRATION</th>
<th>100 PROOF SPIRIT secured in c.c.</th>
<th>ACIDS AS ACETIC grams per 100,000 c.c.</th>
<th>total grams secured</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00005 M</td>
<td>154</td>
<td>29.7</td>
<td>0.045</td>
</tr>
<tr>
<td>2</td>
<td>0.00001 M</td>
<td>168</td>
<td>28.0</td>
<td>0.047</td>
</tr>
<tr>
<td>3</td>
<td>0.0005 M</td>
<td>166</td>
<td>23.3</td>
<td>0.038</td>
</tr>
<tr>
<td>4</td>
<td>0.001 M</td>
<td>170</td>
<td>15.5</td>
<td>0.026</td>
</tr>
<tr>
<td>Control</td>
<td>no salt</td>
<td>158</td>
<td>30.2</td>
<td>0.047</td>
</tr>
</tbody>
</table>
### TABLE NUMBER XIII (continued)

**RUN NO. 12**

**Salt Used - Salicylic Acid**

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>SALICYLIC ACID CONCENTRATION</th>
<th>ESTERS AS ETHYL ACETATE</th>
<th>ALDEHYDES AS ACETALDEHYDE</th>
<th>CELL COUNT OF FINISHED BEER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00005 M</td>
<td>21.0</td>
<td>0.032</td>
<td>38.2</td>
</tr>
<tr>
<td>2</td>
<td>0.00001 M</td>
<td>18.7</td>
<td>0.031</td>
<td>40.0</td>
</tr>
<tr>
<td>3</td>
<td>0.00005 M</td>
<td>17.3</td>
<td>0.028</td>
<td>59.4</td>
</tr>
<tr>
<td>4</td>
<td>0.001 M</td>
<td>14.7</td>
<td>0.025</td>
<td>53.4</td>
</tr>
<tr>
<td>Control</td>
<td>no salt</td>
<td>24.9</td>
<td>0.039</td>
<td>10.1</td>
</tr>
</tbody>
</table>
### TABLE NUMBER XIV

**RUN NO. 13**

**Salt Used - Arsenous Acid**

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>ARSENOUS ACID CONCENTRATION</th>
<th>100 PROOF SPIRIT secured in c.c.</th>
<th>ACIDS AS ACETIC grams per 100,000 c.c.</th>
<th>total grams secured</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0005 M</td>
<td>145</td>
<td>23.6</td>
<td>0.033</td>
</tr>
<tr>
<td>2</td>
<td>0.001 M</td>
<td>147</td>
<td>25.4</td>
<td>0.037</td>
</tr>
<tr>
<td>3</td>
<td>0.005 M</td>
<td>11</td>
<td>26.8</td>
<td>0.002</td>
</tr>
<tr>
<td>4</td>
<td>0.01 M</td>
<td>9</td>
<td>22.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Control</td>
<td>no salt</td>
<td>158</td>
<td>30.2</td>
<td>0.047</td>
</tr>
</tbody>
</table>
### TABLE NUMBER XIV (continued)

**RUN NO. 13**

Salt Used - Arsenous Acid

<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>ARSENOUS ACID CONCENTRATION</th>
<th>ESTERS AS ETHYL ACETATE</th>
<th>ALDEHYDES AS ACETALDEHYDE</th>
<th>CELL COUNT OF FINISHED BEER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grams per 100,000 c.c. total grams secured</td>
<td>grams per 100,000 c.c. total grams secured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0005 M</td>
<td>9.1</td>
<td>0.013</td>
<td>9.8</td>
</tr>
<tr>
<td>2</td>
<td>0.001 M</td>
<td>11.8</td>
<td>0.017</td>
<td>16.0</td>
</tr>
<tr>
<td>3</td>
<td>0.005 M</td>
<td>6.8</td>
<td>0.0007</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.01 M</td>
<td>4.7</td>
<td>0.000</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>no salt</td>
<td>24.9</td>
<td>0.039</td>
<td>10.1</td>
</tr>
</tbody>
</table>
### Table Number XV

**Acid, Ester and PH Values of Some 100 Proof Distillates**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Acids as Acetic (grams per 100,000 c.c.)</th>
<th>Ethers as Ethyl Acetate (grams per 100,000 c.c.)</th>
<th>PH Value of Distillate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.4</td>
<td>16.7</td>
<td>4.88</td>
</tr>
<tr>
<td>2</td>
<td>7.3</td>
<td>13.3</td>
<td>5.04</td>
</tr>
<tr>
<td>3</td>
<td>8.2</td>
<td>18.6</td>
<td>4.98</td>
</tr>
<tr>
<td>4</td>
<td>9.2</td>
<td>25.2</td>
<td>4.90</td>
</tr>
<tr>
<td>5</td>
<td>9.4</td>
<td>25.2</td>
<td>4.97</td>
</tr>
<tr>
<td>6</td>
<td>12.1</td>
<td>21.2</td>
<td>4.59</td>
</tr>
<tr>
<td>7</td>
<td>9.5</td>
<td>25.2</td>
<td>4.26</td>
</tr>
<tr>
<td>8</td>
<td>9.7</td>
<td>21.8</td>
<td>4.25</td>
</tr>
<tr>
<td>9</td>
<td>9.2</td>
<td>18.0</td>
<td>4.30</td>
</tr>
<tr>
<td>10</td>
<td>8.1</td>
<td>21.3</td>
<td>4.43</td>
</tr>
<tr>
<td>11</td>
<td>13.8</td>
<td>12.9</td>
<td>4.71</td>
</tr>
<tr>
<td>12</td>
<td>11.9</td>
<td>19.1</td>
<td>4.73</td>
</tr>
<tr>
<td>13</td>
<td>10.7</td>
<td>19.6</td>
<td>4.72</td>
</tr>
<tr>
<td>14</td>
<td>12.8</td>
<td>22.6</td>
<td>4.70</td>
</tr>
<tr>
<td>15</td>
<td>11.2</td>
<td>25.9</td>
<td>4.71</td>
</tr>
<tr>
<td>16</td>
<td>11.6</td>
<td>30.6</td>
<td>4.78</td>
</tr>
<tr>
<td>17</td>
<td>9.7</td>
<td>22.6</td>
<td>4.61</td>
</tr>
<tr>
<td>18</td>
<td>9.8</td>
<td>26.0</td>
<td>4.58</td>
</tr>
<tr>
<td>19</td>
<td>9.8</td>
<td>25.1</td>
<td>4.62</td>
</tr>
<tr>
<td>20</td>
<td>7.3</td>
<td>21.1</td>
<td>4.72</td>
</tr>
</tbody>
</table>
SAMPLE CALCULATIONS
Acid Value.
\[ \text{c.c. of } 0.1 \text{ N NaOH} \times 2 \times 0.006 \times 100,000 = \text{Acid Value} \]

Example. Run No. 5: Sample 2
\[ 1.22 \times 2 \times 0.006 \times 100,000 = 14.6 \text{ grams per 100,000 c.c.} \]

Ester Value.
\[ (25 - \text{c.c. } 0.1 \text{ N } H_2SO_4) \times 2 \times 0.0088 \times 100,000 = \text{Ester Value} \]

Example. Run No. 5: Sample 2
\[ (25-24.04) \times 2 \times 0.0088 \times 100,000 = 16.9 \text{ grams per 100,000 c.c.} \]

Aldehydes.
\[ \frac{\text{M.M. reading of standard}}{\text{M.M. reading of sample}} \times 0.0002 \times 100,000 = \text{Aldehydes as acetaldehyde} \]

Example. Run No. 12: Sample 2.
\[ \frac{40}{20} \times 0.0002 \times 100,000 = 40 \text{ grams per 100,000 c.c.} \]

Furfural.
\[ \frac{\text{M.M. reading of standard}}{\text{M.M. reading of sample}} \times 0.00001 \times 100,000 = \text{Furfural} \]

Example. Run No. 7: Sample 1.
\[ \frac{4.2}{20} \times 0.00001 \times 100,000 = 0.21 \text{ grams per 100,000 c.c.} \]
Distillate in terms of 100 proof alcohol

c.c. of original distillate \times \text{sp. gr.} \times \text{per cent by weight of alcohol} \times \frac{1}{\text{sp. gr. of 100 proof alcohol}} = \text{c.c. of 100 proof alcohol}

Example. Run No. 5; Sample 4.

\[ 415 \times 0.961 \times 27.75 \times \frac{1}{0.9344} = 261 \text{ c.c. of 100 proof alcohol} \]
Calcium Sulphate = 0.000185 M
Sodium Chloride = 0.00022 M
Sodium Bicarbonate = 0.00017 M
Magnesium Sulphate = 0.000105 M
Disodium Phosphate = Varies

FIGURE 1. THE EFFECT OF VARYING DISODIUM PHOSPHATE RUN 1.
FIGURE 2. THE EFFECT OF VARYING DISODIUM PHOSPHATE ON ACIDS, ESTERS, ALDEHYDES, AND ALCOHOLS.
FIGURE 3
THE EFFECT OF VARYING CALCIUM SULPHATE
RUN 3.

Grams of Acids - Esters - Aldehydes

Potassium Carbonate = .0001 M
Magnesium Sulphate = .0001 M
Disodium Phosphate = .0001 M
Calcium Sulphate varies

0.03 0.06 0.09 0.12 0.15 0.18

0.0001 0.0001 0.0001

Aldehydes
Esters
Acids

pH values

0.0 0.2 0.4 0.6

c.c. of 100 Proof Ethanol or Spirits
Potassium Carbonate - .0001 M
Magnesium Sulphate - .0001 M
Calcium Sulphate - .0001 M
Diammonium Phosphate - Varies

FIGURE 4. THE EFFECT OF VARYING DIAMMONIUM PHOSPHATE RUN 4.
FIGURE 5. THE EFFECT OF VARYING CALCIUM SULPHATE. RUN 5.
FIGURE 6. THE EFFECT OF VARYING MAGNESIUM SULPHATE RUN 6.
FIGURE 7. THE EFFECT OF VARYING POTASSIUM CARBONATE. RUN 7.
grams of Acids - Esters - Aldehydes

FIGURE 8.
THE EFFECT OF VARYING MAGNESIUM SULPHATE 
RUN 8.

grams of Acids - Esters - Aldehydes

cc. of 100 Proof Ethanol or Spirits
Grams of Acids - Esters - Aldehydes

FIGURE 11.
THE EFFECT OF VARYING AMMONIUM BIFLUORIDE on:

- Acids
- Esters
- Aldehydes

c.c. of 100 Proof Ethanol or Spirits

0.00001

0.0001

0.001

0.01

0.1

0.5

1.0

0.03

0.06

0.09

0.12

0.15

0.18
FIGURE 12. THE EFFECT OF VARYING SALICYLIC ACID (RUN 12).
INTERPRETATION OF RESULTS
The interpretation of the results secured during the preliminary study and the actual runs must be divided into the individual topics which were considered.

During the preliminary investigation a large number of samples of corn and rye were analyzed for total carbohydrates, starch, protein, fat, and moisture. The results secured from actual fermentation of these grains showed that no one constituent or combination of constituents can be used to accurately forecast the results which will be secured in fermentation. Preliminary laboratory fermentations are necessary to give an estimate on the yield of alcohol which will be secured.

In the past, the waters of certain sections have been considered as having the correct chemical composition necessary for fermentations. Table I shows the wide variance in both the percentages of total minerals as well as the individual chemical compounds present in waters used in the fermenting industry. Although some of the waters analyzed were secured from wells in close proximity to each other, they showed considerable variation in their chemical analyses.

In order to estimate the effects of different concentrations of various added salts, control fermentations were made using distilled water as the mashing medium. From the
results secured on Sample 1, Table II; Sample 5, Table III; Sample 5, Table IV; and Sample 4, Table V, it can be seen that the yields of alcohol are always lower with distilled water than when salts are added. The distillate secured after fermentation always shows a smaller amount of determined by-products when distilled water is used in preparing the mash instead of water to which mineral salts have been added. In the fermentations of the mashes made with distilled water the only salts present were those secured from the grains. Since these fermentations gave lower yields of alcohol than when the added salts were present, it shows that the salts present in grains do not satisfy the conditions necessary for efficient fermentations. The chemical analyses of the ash from the grains show a mash has an excess of certain chemicals above the actual requirements for fermentation. Therefore, part of these chemicals must be in a form not utilized by the yeast.

The added salts used in the fermentations represented by Tables II through VIII were magnesium sulfate, calcium sulfate, disodium and diammonium phosphates, sodium bicarbonate and potassium carbonate. The concentration of each of the salts added to a mash was kept at 0.0001 M, excepting the one salt being varied in that run. This concentration was selected as the preliminary fermentations showed it to give satisfactory fermentations and products. The effects on
the fermentation of each salt will be considered separately except the phosphates of sodium and ammonium which will be grouped together since it was the phosphate radical which was being studied and not the cation.

The action of the phosphates on the fermentations of grain washes can be seen from Figures 1, 2, 4, and 9. The yield of alcohol increases up to a phosphate concentration of 0.01 M. without the cation with which it is combined showing any appreciable action. Above 0.01 M. the yield of alcohol showed a gradual decrease. In Run 1, Figure 1, the amount of acids formed reached a maximum at a concentration of 0.0001 M., while in Run 2, Figure 2; Run 4, Figure 4; and Run 9, Figure 9, the formation of acids seemed to remain fairly constant regardless of the salt concentration, except at 0.1 concentration of dipotassium phosphate. In this case, the alkali concentration was high enough to effect the enzymes, thereby causing the reduction shown.

The amount of esters formed appear to reach a maximum at a phosphate concentration of 0.0001 M. However, in Run 1, Figure 1, the esters showed an increase with concentrations up to 0.00055 M.

The formation of aldehydes appear to be little affected by the concentrations of the phosphates salts used in the experiments.
The pH values of the distillates show a gradual increase with increased phosphate concentrations. These values are approximately the same for both the ammonium and sodium phosphates.

Calcium sulfate appears to cause a slight increase in the yields of alcohol secured, as is shown by Run 3, Figure 3, and Run 5, Figure 5. A concentration of 0.00001 M gives the best results.

The acids and esters show a slight increase with calcium sulfate up to a concentration of 0.001 M, while the amount of aldehydes remain constant through the entire range of salt concentrations used. The pH values of the distillates show a gradual reduction up to a concentration of 0.0001 M.

Magnesium sulfate shows a maximum yield as well as a higher production of acids and esters at a concentration of 0.0001 M, as is shown by Run 6, Figure 6 and Run 8, Figure 8. Above this concentration there is a gradual decrease in the constituents which were determined.

Increased concentration of potassium carbonate up to 0.0001 M shows an increase in alcohol produced as well as in the amount of acids, esters, and aldehydes formed. Run 7, Figure 7 shows that the optimum concentration of this salt may not have been reached. Contrary to expectations the
pH values of the distillates slightly decrease with increased concentrations of the salt.

The distillates show a gradual decrease in amounts of alcohol, acids, and esters with increased concentrations of calcium carbonate from 0.001 M to 0.5 M. Run 10, Figure 10, shows 0.1 and 0.5 M concentrations of this salt greatly reduce the amount of aldehydes formed. This decrease in aldehydes appears to be the reverse of the action of the sulfites which arrest fermentation at the aldehyde stage.

As can be seen from Run 11, Figure 11, the action of ammonium bifluoride on the products secured is considerably different than that shown by any of the other salts tried. Increasing concentrations of this salt up to 0.01 M cause a gradual reduction in the amount of acids, and esters, but little effect is shown on the yield of alcohol. The quantity of aldehydes formed shows a gradual increase with an increase in the salt and a decided increase at 0.001 M salt concentration. This shows a result somewhat similar to the sulfites and may be due to a poisoning action on the reducing enzymes of the yeast.

At low concentrations salicylic acid has little effect upon the products of fermentation excepting the aldehydes which show a gradual increase with increasing concentrations of the salt up to 0.001 M. This shows an effect upon the
reducing enzymes present with a partial stopping of the fermentation at the aldehyde stage. From Run 12, Figure 12, it can be seen that the amounts of acids and esters drop sharply between the 0.0005 and 0.001 M. concentrations.

Concentrations up to 0.001 M. of arsenous acid have little effect upon the products secured from the fermentation of the grain mashes. As can be seen from Run 13, Figure 13, a general poisoning of all the yeast and enzymic functions takes place between the concentrations 0.001 and 0.005 M. When concentrations above this are used, they seem to have no further action. The ester formation was more affected by the lower concentrations than any of the other products determined.

During the preparation of the mashes and the yeast charge, precautions were taken to prevent the contamination by foreign yeasts and bacteria. The products secured in the distillates can be considered as being produced by the culture yeast during its action upon the grain mashes.

The pH value of a number of distillates is shown in Table XV. From this it can be seen that there is no relation between the amount of acids or esters in the distillate and its pH value. This shows there is some other constituent which controls the pH, probably the fusel oil which tends to act as a buffer.
CONCLUSIONS
From the preliminary work done and the data reported, the following conclusions can be drawn as to the evaluation of grains and the effects of certain chemical compounds upon the fermentation of grain mashes and the products which are present in the distillates that are secured by distilling the fermented mashes.

1. The chemical analysis does not give sufficient information for the complete evaluation of the grains. It must be supplemented by bacteriological and fermentation analyses for a true value to be placed upon its adaptability to the fermenting industries.

2. The grains do not contain the necessary inorganic compounds in the correct proportions or combinations to give good fermentations.

3. The addition of small amounts of the mineral salts normally found in limestone waters is beneficial to the fermentation of grain mashes.

4. The addition of phosphates increases the total fermentation as well as the secondary products, acids and esters. Since the grains contain considerable phosphates, this shows that part of the phosphates in the grains are present in forms not readily utilizable by the yeast and its enzymes.
5. The addition of calcium sulfate in concentrations up to 0.0001 M. causes a decrease in the pH value of the distillate secured without materially affecting the products formed.

6. A concentration of 0.0001 M. magnesium sulfate appears to be the optimum for best results in fermenting grain mashos.

7. Concentrations of potassium carbonate up to 0.0001 M. are beneficial to fermentations both in the amount of alcohol and secondary products which are secured.

8. Calcium carbonate in concentrations greater than 0.1 M., causes a marked reduction in the amount of aldehydes formed. High concentrations of calcium carbonate also reduce the total alcohol production but only to a slight extent.

9. The addition of ammonium bifluoride in concentrations greater than 0.001 M. causes a decided increase in the amount of aldehydes in the final distillate secured. The concentration of ammonium bifluoride used does not materially effect the amount of alcohol produced. Therefore, the increase in aldehydes is not due to a retardation of the enzymic action of zymase.

10. Salicylic acid in concentrations of 0.0005 to 0.001 M.
is detrimental to the fermentation of grain mash as it causes an increase in the amount of aldehydes without materially increasing the yield of alcohol.

11. Arsenous acid in concentrations greater than 0.0001 M, rapidly poisons the yeast and reduces the enzyme functions.

12. Fermentations which give distillates high in aldehydes are not necessarily infected with wild yeast and bacteria as yeast under incorrect conditions will give this result.
BIBLIOGRAPHY
1. Abderhalden, E.

2. Abderhalden, E.
The behavior of yeast towards different sugars at various concentrations and the influence of addition of alanine on fermentation. J. Soc. Chem. Ind. 36:1283. 1917.

3. Abderhalden, E. and Schausmann, E.

4. Abderhalden, E.

5. Abderhalden, E. and Glaubach, L.

6. Abderhalden, E.

7. Abderhalden, E.
18. Agrait, J. and Gaim, E.


20. Asheshov, I.N. and Giaja, J.

21. Atwood, W. B.

22. Bach, A.

23. Bach, A.

24. Bachmann, F. M.

25. Bachrach, E. and Roche, J.

26. Backhaus, A. A.

27. Baetje, R.

28. Baily, C.
29. Baker, J. L. and Hulton, H. F. E.

30. Baker, R. H. and Barkenhus, C.

31. Ball, A.
Water (for brewing). Modern Brewer 16 No. 6:45, 72-5. 1936.

32. Bancroft, W. D. and Richter, G. H.


34. Barthel, G., Euler, H. v. and Nilsson, R.
Gärung und Wachstum in getrockneten Hefezellen. lII.

35. Bauer, E.
Fermentative or chemical acidifications in the distillery. Chem. Zeit. 31: 627-33. 1907.

36. Baumgarten, A. and Luger, A.

37. Beauverie, J.

38. Becker, G.
Abstracted in C.A. 20: 3535. 1926.

39. Beetlesone, N. C.
40. Bekaert, E.
1915-6. 1913. Original not seen. Abstracted in
C.A. 7: 4040. 1913.

41. Bendotti, E.
Some changes in the process of alcoholic fermentation
by the action of an oscillating electromagnetic field
1928.

42. Benedek, K.
Über den Einfluss der Temperatur auf das pH-Optimum
der Diastase beim Maischen. Hochschr. Brau. 46:
345-9. 1929.

43. Berczeller, L. and Freud, J.
Über die Wirkung der Halogen auf Diastasen.

44. Bergheim, O. and Hawk, P. B.
Inhibition of enzyme action by lime softened water.

45. Bertrand, G.
Influence des acides sur l'action de la laccase.

46. Bertrand, G. and Rosenband, M.
The paralyzing action of certain acids upon

47. Bertrand, G. and Rosenband, M.
Action des acides sur la per oxydiastase. Compt.

48. Bertrand, G. and Rosenblatt, Hm.
Action de la chloropéctrine sur la levure et sur

49. Biedermann, W. and Rueba, A.
Enzyme studies VIII. Conditions for the activity
of amylases. Fermentforschung. 5:56-83. 1921.

50. Biedermann, W.
Über die Wirkung von Pepsin und Trypsin auf Diastase.
51. Bierberg, W.
Addition of ammonium salts in fermentation of wine.

52. Bierema, S.
The assimilation of ammonium compounds, nitrates, and
II. Abt. 23:672-676. 1907. Original not seen.

53. Bishop, L. R.
Institute of brewing research scheme. I. The
1930.

54. Blich, M. J. and Sandstedt, R. M.
Biocatalytic activators specific for the yeast
1937.

55. Boas, F.
Action of arsenic compounds on yeast. J. Inst.
Brew. 24:76. 1917.

56. Boas, F. and Leberle, H.
Untersuchungen über Säurebildung bei Pilzen und

57. Boas, F.
Über die Abhängigkeit von Hefewachstum und Hefegärung
von physikalisch-chemischen Erscheinungen. Biochem. Z.

58. Boas, F.
Action of saponins on yeast cells. Ber. deutsch. botan.
in C.A. 16:3525. 1922.

59. Boas, F. and Neumüller, G.
Action of bile salts on some fermenting organisms.
Arch. Mikrobiol. 1:35-59. 1930. Original not

60. Bode, G.
Significance of sucrose in barley. Fortschr.
Landw. 4:545-7. 1929. Original not seen. Abstracted

61. Bokorny, T.
Einswirkung des freien Ammoniaks auf die Hefe; Vergleich
62. Bokorny, T.
Action of salts of metals upon yeast and other fungi.

63. Bokorny, T.

64. Bokorny, T.

65. Bokorny, T.

66. Bokorny, T.

67. Bokorny, T.

68. Bokorny, T.

69. Bokorny, T.
The nutritional physiology of alcohol and acids in yeasts and other widely distributed fungi. 1917.

70. Bokorny, T.
91. Cernovodame, Mlle. and Henri, V.
Action des rayons ultra violents sur les microorganismes et sur différentes cellules. Etude microchimique.

92. Chapman, A. C.
The reproduction of yeast in copper fermenting vessels.

93. Charmandarjan, M.C. and Tjutjumnikowa, A.V.
Einfluss von Salzen auf die Tätigkeit der Salzkatalase.

94. Chrzaszcz, T. and Pituozek, S.
Die Anwendung des Lakoformols und Formalins in der Brennerei.
Z. Spiritusind 32:23-6, 1908.

95. Chrzaszcz, T.
Ein Vergleichsstudium über die Hefeführung in der Brennerei.

96. Chrzaszcz, T. and Joscht, A.
Über die Verschiebung einzelner amylolytischer Kräfte der Malzamylase und deren Verhalten beim Aufbewahren in Gegenwart verschiedener Reagentien.


98. Chrzaszcz, T. and Keszetnick, J.

99. Chrzaszcz, T. and Sawicki, J.

100. Church, Margaret B.
The sugar tolerance of yeasts expressed in atmospheres.
Science 74:492-3, 1931.
101. Classen, H.  
Das Wachstum der Hefe und die Zunahme ihrer  
Bestandteile bei dem Luftheferfahren. Biochem.  

102. Clark, N. A.  
The rate of formation and the yield of yeast in  

103. Clark, N. A.  
The influence of certain chemicals on the rate  
26:221-31. 1924.

104. Clark, R. H., Fowler, L. and Black, P. L.  
(3) 725:99-105. 1931.

105. Colin, H. and Chandin, A.  
The inhibitory action of certain products on the  
enzymic hydrolysis of sucrose. J. Chim. phys. 28:  
546-55. 1931. Original not seen. Abstracted in  

106. Colin, H.  
The laws of sugar inversion. Effect of the  
concentration of the medium. Chimie and Industrie  

107. Collier, H. B. and Wasteneys, R.  
Action of radiation on enzymes. Australian J.  

108. Connstein, W. and Lédecki, K.  
1919. Original not seen. Abstracted in C.A. 14:  
88. 1920.

109. Crooke, A.  
Phosphates and fermentation. Australian Chem.  

110. Crowther, E. M.  
Note on phosphoric acid content of barley grain.  
111. Cruess, W. V.

112. Cruess, W. V., Richtert, F. H. and Irish, J. H.

113. Dannenberg, E.

114. De Fazi, Romolo and De Fazi, Remo.

115. De Fazi, Romolo and De Fazi, Remo.

116. De Fazi, Remo.

117. De Fazi, Remo.

118. De Fazi, Remo.

119. De Fazi, Remo.
120. De Fazi, Rono.
Alcoholic fermentation of glucose solutions with beer yeast in the presence of radium emanation.

121. De Groot, C.

122. Dehnice, J. and Kilp, W.
Der Einfluss der Wassermenge-Konzentration (pH) auf die Schnelligkeit der Vergärung und Ausbeute an Alkohol. Z. Spiritusind 50: 69-70. 1927.

123. Delenac, N. T. and Bezincacco, E. D.
Action of methyl and ethyl alcohols on soluble and fixed enzymes. 1. Action on beer yeasts and (the lipase of) the cytoplasm of Ricinus seeds.

124. Denys, G.

125. Deplanque, N.

126. Deplanque, P.
Mlnige über das Betriebswasser in der Brennerei.
Z. Spiritusind 60: 110. 1937.

127. Dittrick, J.

128. Dobbs, C. and Hibbard, R. F.
Nutrient ions of plants and the ion activation of plant enzymes. J. Biol. Chem. 73:405-16. 1927.

129. Donner, A.
130. Dox, W.

131. Drabale, E. and Scott, D. G.

132. Dresel, K.

133. Ducháček, F.

134. Duncan, W.

135. Eck, K.

136. Eckhardt, K.

137. Effront, J.

138. Effront, J.

139. Effront, J.

140. Ehrlleb, F. and Pischel, F.
141. Ehrlich, F.
Conditions of formation of fusel oil. Mon. Sci.
32:603-10. 1914.

142. Ehrlich, F.
The formation of succinic acid in alcoholic

143. Ehrlich, B.
Role of phosphorus in the life of yeast and in
alcoholic fermentation. Thesis présentées a la
faculté des sciences de l'université de Paris.
1927-137 pp. Original not seen. Abstracted in

144. Ellrodt, G.
Nitrogen content of ( beet) molasses and yield of

145. Ellrodt, G.
Superphosphates as yeast nutriment. Brennerei-
Zeit. 36:8239. 1919. Original not seen. Abstracted in

146. Elwelyem, C. A.
The role of iron and copper in the growth and
1931.

147. Emslander, F.
The influence of the containing surface on
diastatic fermentation. Z. Chem. Ind. Kolloid 2:
308-10. 1908. Original not seen. Abstracted in
C.A. 222. 1909.

148. Emslander, F.
Water in the brewery. Z. Chem. Ind. Kolloid 5:
25-30. 1909. Original not seen. Abstracted in

149. Emslander, F.
Künstliche Säuerung von Malische und Würze?

150. Ender, H. v. and Ohlson, H.
Über den Einfluss der Temperatur auf die Wirkung
151. EULER, H. V. and KULLBERG, S.
Um das Verhalten freier und an Protoplasma
gebundener Katalysen. Z. physiol. Chem. 73:
85-100. 1912.

152. EULER, H. V. and CRASER, H.
Uber die Anpassung von Mikroorganismen und Gifts.

153. EULER, H. V. and JOHANSSON, D.
The reaction phases of alcoholic fermentation.

154. EULER, H. V. and SAHLEN, J.
The activation of yeast. Z. Gärungsphysiol. 3:
225-34. 1913. Original not seen. Abstracted in
C.A. 7: 3988. 1913.

155. EULER, H. V. and BÄDLIN, K.
Uber die alkoholische Gärung bei verschiedenen
OH'-Konzentrationen. Z. physiol. Chem. 100:
69-73. 1917.

156. EULER, H. V. and LÖWENBAM, E.
Untersuchungen über die chemische Zusammensetzung
und Bildung der Enzyme. XII. Z. physiol. Chem. 97:
279-90. 1916.

157. EULER, H. V. and THOLIN, T.
Uber die Phosphatwirkung auf die alkoholische Gärung
bei verschiedenen OH'-Konzentrationen. Z. physiol.

158. EULER, H. V. and HEINTZE, S.
Uber die Rolle der Phosphate bei der alkoholischen

159. EULER, H. V. and EULER, B.
Poisoning of enzymes in the living cell.
Fermentforschung 1: 465. J. Chem. Soc. 112:
172. 1917. Original not seen. Abstracted in C.A. 11:
14. 1917.

160. EULER, H. V. and HEINTZE, S.
Über die pH-Empfindlichkeit der Gärung einer Oberhefe.
161. Euler, H. v., Svanberg, G., and Heintze, S.

162. Euler, H. v.

163. Euler, H. v. and Elix, R.

164. Euler, H. v. and Emberg, F.

165. Euler, H. v. and Florell, N.

166. Euler, H. v. and Svanberg, G.

167. Euler, H. v. and Wyrbaek, K.

168. Euler, H. v. and Sandberg, V.

169. Euler, H. v. and Nilsson, R.

170. Euler, H. v. and Nilsson, R.
171. Euler, H. v. and Nilsson, R. and Anhagen, F.
Uber die Funktion des Magnesiums beim enzymatischen

172. Euler, H. v. and Myrbäck, K.

173. Euler, H. v. and Philipson, T.

174. Felice, Sister Mary.
Study of the action of surface-active substances.

175. Fernbach, A. and Schoen, M.
The role of acetaldehyde in alcoholic fermentation.

176. Fernbach, A. and Niculan, S.

177. Feuer, B. and Tanner, F. W.
Action of ultra violet rays on the Saccharomycetes.

178. Feuerstein, G.
Versuche uber den Einfluss von Säure auf infizierte
Brauereihefe im Laboratorium und in der Praxis.

179. Feys, M.
The antiseptic power of hops. Bull. 1 assoc. eleves
inst. super fermentations Gand. 22:403-10; 429-34.

180. Ficker, M. and Szücs, S.

181. Fink, H.
Beiträge zur Methylenblaufärbung der Hefezellen und
Studien über die Permeabilität der Hefe zellmembran.
182. Pink, H. and Kukles, R.
Beiträge zur Methylenblaufärbung der Hefezellen und
Studien über die Permeabilität der Hefezellmembran.

183. Foth, G.
Lange runde Angärung und starke Erwärmung der Zellmasse
während der Kulturdauer. Z. Spiritusind 44:104-5. 1921.

184. Frank, K. and Moxon, A.
Effect of proteins on yeast fermentation.

185. Frankel, S. and Scharf, A.
Über Vitamine. III. Über grünsbeschleunigende
Extrakte aus Pflanzen und die Wirkung von
Z. 186:227-36. 1921.

186. Franzen, H.
Formation and fermentation of formic acid by yeast.
Abstracted by C.A. 6:1017. 1912.

187. Freundlich, H.
The importance of colloid chemistry to the fermentation
industry. Z. angew. Chem. 35:517-9. 1922. Original

188. Frey, C. N.
History and development of the modern yeast industry.

189. Friedmann, E.
Quantitative Untersuchung über Acetessigsäureumsatzes

190. Fulmer, E. I.
The acclimatization of yeast to ammonium fluoride
and its reversion in wort. J. Phys. Chem. 25:
455-72. 1921.

191. Fulmer, E. I.
The effect of alcohol on the toxicity of phenol

Nutritional requirements of yeast. I. The role of
vitamines in the growth of yeast. J. Am. Chem.
Soc. 43:186-91. 1921.
193. Fulmer, E. I. and Nelson, V. E.
Studies on yeast V. Is bio a single substance?

194. Fulmer, E. I.
Utilization of atmospheric nitrogen by

195. Fulmer, E. I. and Buchanan, R. E.
Studies on toxicity. J. Gen. Physiol. 6:77-89.
1923.

196. Fulmer, E. I., Sherwood, F. F., and Nelson, V. E.
Effect of ammonium chloride upon the growth of
yeast and the hydration of gluten in beer wort.

197. Fulmer, E. I.
The effect of ammonium salts upon the swelling of
colloids and upon the growth of yeast at various
temperatures. Second Colloid Symposium Monograph.
1925:204-8. 1925.

198. Fulmer, E. I., and Christensen, L. K.
The fixation of atmospheric nitrogen by yeast as
a function of the hydrogen-ion concentration. J.

199. Fulmer, E. I. and Heresselman, B.
The production of a yeast-growth stimulant by
heating media under pressure. Iowa State Coll.

200. Fulmer, E. I.
The chemical approach to problems of fermentation.

201. Fulmer, E. I., Williams, A. L. and Werkman, C. H.
The effect of sterilization of media upon their
growth-promoting properties towards bacteria.

Effect of the composition of the medium upon
the growth of yeast in the presence of bios
preparations. I. The effect of magnesium salts.

203. Funk, C. and Freedman, L.
Can yeasts grow in chemically pure medium. Proc.
204. Carino, E. and Asti, C.
Amino acids in wine and their biological
1919. Original not seen. Abstracted in C.A.
14:794. 1920.

205. Geiger-Huber, M.
Influence of neutral red on yeast respiration.

206. Geiger-Huber, M.
The influence of the concentration of respiration
material upon the rate of respiration of plants.

207. Genaud, P.
Researches in ionic interchange between yeast cells
G:240-330. 1930. Original not seen. Abstracted in

208. Genevois, L., Cayrol, P. and Nicolaieff, T.
Action de divers dérivés halogénés sur la cellule
des champignons inférieure. (Lévures et monisures)

209. Genevois, L.
Substances which can stop alcoholic fermentation.
Ann. fermentations 1:86-100. 1935. Original not

210. Gerber, C.
Action des sels des métaux du groupe aurique sur
la saccharification de l'empois d'amidon par les

211. Gerber, C.
Action des sels des métaux alcalins sur la
saccharification de l'empois d'amidon par les ferments
amyloymes. I. Sels a acides minéraux. Compt.

212. Gerber, C.
Action des sels des métaux du groupe aurique sur la
saccharification de l'empois d'amidon par les ferments
amyloymes. IV. Chlorure de zinc et oxalate de
potassium acidules. Compt. rend. soc. biol. 70:
213. Gerber, C.
Action des composés du chrome sur la saccharification
de l'empois d'amidon par les ferment amylolytiques.

214. Gerber, C.
Action des sels des métaux alcalins sur la
saccharification de l'empois d'amidon par les ferment
proteolytiques. IV. Sels neutres ammoniacaux à
acides minéraux. Compt. rend. soc. biol. 70:
822-4. 1910.

215. Gerber, C.
Action des sels de métaux alcalins sur la
saccharification de l'empois d'amidon par les
ferments amylolytiques. VII. Sels ammoniacaux
acides organiques. Compt. rend. soc. biol. 71:
41-3. 1910.

216. Gerber, C.
Action des alcaloïdes et de leurs sels sur la
saccharification de l'empois d'amidon par les
ferments amylolytiques. I. Sels basiques de

217. Gerber, C.
Action de quelques sels sur la saccharification de
l'amidon soluble de Fernbach-Wolff par les ferment
1910.

218. Gibbs, R. D.
The toxicity of normal aliphatic alcohols. I.

219. Giez, W. J.
Experiment with salts of aluminium and beryllium.
J. Pharmacol. 2:403. 1911.

220. Gimel, G.
Influence of certain inorganic salts and especially
of stannous chloride and of bismuth subnitrate upon
fermentation. Bull. assoc. chim. sucr. dist. 31:
1289. 1914. Original not seen. Abstracted in
C.A. 8:2594. 1931.

221. Gioja, J.
Is fermentation of sugar by yeast due solely to
1921.
222. Giri, K., and Subrahmanyan, V.

223. Gorboch, G. and Russ, H.

224. Green, H. H. and Kestell, N. N.

225. Greig-Smith, R.

226. Greig-Smith, R.

227. Gronchi, V.

228. Gronchi, V.

229. Gruss, J.


231. Guerrini, G.
232. Guerrini, G.

233. Guillemet, R.

234. Guillemet, R. and Schell, C.

235. Guillemet, R.

236. Haarmann, W. and Folsche, O.

237. Hägglund, E.

238. Hägglund, E., Soderblom, A. and Troberg, B.

239. Hahn, A.

240. Harden, A. and Young, W. J.
241. Harden, A. and Young, W. J.
The alcoholic ferment of yeast juice. Part III.

242. Harden, A. and Young, W. J.

243. Harden, A. and Young, W. J.

244. Harden, A. and Paine, S. G.

245. Harden, A.

246. Harden, E.

247. Hardt, A.

248. Harman, H.W. and Oliver, H. J.

249. Harpuder, K.

250. Haste, S.S.
251. Hastie, S. S. and Dick, W. D.

252. Hawkins, L. A.
The effect of certain chlorides singly and combined in pairs on the activity of malt

253. Hayduck, F.
Über einen Hefegiftstoff in Hefe.

254. Hayduck, F.
Yeast poisons in grains, worts and yeasts and
the dependence of their action on mineral
salts. Allgem. Z. Bierbrau. Malzfabr. 36:505-7;
613-6. 1910. Original not seen. Abstracted

255. Heinzelman, G. and Dehnicke, J.
Über Versuche zur Anreicherung des Gehalt des
Kohlespiritus an höhere Alkoholen durch die
Lebensfähigkeit der Hefe. Z. Spiritusind 38:
316,328. 1915.

256. Henneberg, W.
Versuche über die Widerstandsfähigkeit der
verschiedenen Kartoffelsorten gegen Pflanzenbakterien.
Z. Spiritusind 29:52-5. 1905.

257. Henneberg, W. and Bohrer, M.
Die Widerstandsfähigkeit der Presshefe und
Bierhefe gegen grössere Schwefelsäuremengen in
ihrer Abhängigkeit vom Innenzustand der Hefezellen.

258. Henri, V. and Schnitzler, J.
Action des rayons ultra-violets sur la fermentation

259. Herbert, A.
On the toxicity of some rare metals. Their
influence on different kinds of ferment action.
Bull. soc. chim. 35:1299-1304. 1924. Original

260. Hermann, E.
Der Einfluss der Carbonate in Brauwasser auf die
261. Hermann, S. and Neiger, R.

262. Heron, H.

263. Heyl, R. C.

264. Hildebrandt, F. M. and Boyce, F. F.

265. Hodel, P. and Neuenschwander, N.

266. Hoffman, C.H.

267. Hoffmann, C.

268. Holderer, M.

269. Hoop, L. de and Van Lear, J. A.

270. Hopkins, R. H.

271. Hopkins, R. H.
272. Horvath, I.
ph values of lactic acid fermentation produced
with bacillus Delbrücki. Kísérletügyi Koziemenyek.

273. Hurre, R.
1906.

274. Hughes, A.H., Yudkin, J., Kemp, J. and Rideal, E.K.
1105-12. 1934.

275. Hulton, H. F. E.
The relation of nitrogenous matter in barley to

276. Inonye, E.
Heat produced during the action of enzymes.

277. Inonye, Dr.
1922.

278. Isaiew, K.

279. Ivecovic, H.
Beitrag zur Kenntnis der alkoholischen Hefegärung
und deren Beschleunigung durch Tierkohle. Biochem.
Z. 183:451-60. 1927.

280. Jacobschm, K. P.
Über die biochemische Hydratisierung der Fumarsäure
durch pflanzliche Zellen und Hefe. Biochem. Z. 234:
401-18. 1931.

281. Jacquemin, G. and Guirel, G.
The influence of radio active emanations on yeasts
and alcoholic fermentations. Bull. Agr. Intelligence
5:1505. 1914.

282. Jalowetz, E.
Technology of malting. IV. Cold and warm flooring
method. Brau. und Malzinö 1:1-5. V. Influence of
the composition of steep water. Ibid. 2:25-70. 1930.
283. James, W. O. and Cottle, E.

284. Jensen, F.

285. Joachimoglou, G.

286. Katagiri, H.

287. Kayser, E.

288. Kayser, E.

289. Kayser, E.

290. Kayser, E.

291. Kayser, E. and Demolon, A.

292. Kayser, E. and Demolon, A.
293. Kayser, E. and Demolon, A.
Influence of calcium salts on the ageing of
wine in the presence of wine yeasts. Rev. vit.
36:6509. 1911. Original not seen. Abstracted

294. Kayser, E. and Marchand, H.
Influence des sels de manganèse sur les levures

295. Kelly, H.E. and Brenner, P. S.
Some factors affecting the solubility of nitrogenous
substances in mashing II. The effect of various
salts in the mashing liquor. J. Inst. Brewing 39:
622-39. 1933.

296. Kerb, J.
Course of alcoholic fermentation in the presence
1930.

297. Kerb, J. and Leckendorf, K.
Weiteres über den Verlauf der alkoholischen Gärung
bei Gegenwart von kohlensäurem Kalk. Biochem. Z.

298. Kerr, E. G. and Young, W. J.
Action of certain fat solvents on alcoholic

299. Kilp, W.
Die Bekämpfung der Schaumgärung. Z. Spirituusind.
34:31-2. 1931.

300. Kitza, G.
Influence of calcium salts on the starch liquifying
Kyoto Imp. Univ. 2:1-5. 1918.

301. Kleiber, H.
Beitrag zur Frage der Einwirkung elektrischer Ströme

302. Klinkenberg, G. A.
The separation and action of the two malt amylases.
Amsterdam 34:883-905. 1931. Original not seen.
303. Kluger, W.
Clarification and sterilization of plant water
53:54-61. 1931. Original not seen. Abstracted

304. Kluyskens, A. J. and Hoogerheide, J. C.
L'influence de L'oxygen sur la fermentation

305. Kluyskens, A. J. and Hoogerheide, J. C.
Influence of monochloacetic acid on the respiration
and the fermentation of yeast. Proc. Acad.
Sci., Amsterdam. 56:596-605. 1933. Original not

306. Knaysi, G. and Gordon, M.
Disinfection II. The manner of death of certain
bacteria and yeasts when subjected to mild
chemical and physical agents. J. Infectious
Diseases 47:303-17. 1930.

307. Kochmann, H.
Die Einfluss der Athylalkohols auf die Hefegärung.

308. Kohler, E.
Untersuchungen über den Gang der alkoholischen Gärung

309. Kolbach, P. and Haussman, G.
Über den Einfluss der Erdalkali:ulfate und-
chloride des Brauwassers auf die Zusammensetzung der

310. Kolkwitz, R.
Pressure developed by alcoholic fermentation.

311. Kopaczewski, W.
Einfluss verschiedener Säuren auf die Hydrolyse
der Maltose durch Maltase. Z. Physiol. Chem. 50:
182-93. 1912.

312. Kopaczewski, W.
L'influence des acides sur l'activité. Compt. rend.
158:940-2. 1913.
313. Korsahov, M.

314. Kosieradski, K.

315. Kossowicz, A.

316. Kossowicz, A. and Loew, W.

317. Kostuichev, S. and Frey, L.

318. Kostuichev, S.

319. Kostuichev, S. and Frey, L.

320. Kostuichev, S. and Medvedev, G.

322. Kubsik, P., Klumpf, T., Hadrich, E. and Killer, D.
The addition of ammonia salts in the fermentation

323. Kufferath, H.
Culture of yeasts in concentrated solutions of
organic acids. Ann. Zymol. Ser. II. 1. No. 1,
9-29. 1931. Original not seen. Abstracted in
C.A. 27:3029. 1933.

324. Kulikov, V. and Popova, A.
Ion antagonism and activation in alcohol
1934.

325. Labes, R.
Toxicity of noscent elementary tellurium and

326. Lamont, N. E.
Influence of pH on alcoholic recovery in cane
1930.

327. Lampe, B.
Weitere Untersuchungen über den Einfluss von
Adsorbentien auf den Verlauf der Gärung bei Melasse-

328. Lampe, B. and Kilp, W.
The influence of sodium chloride upon fermentation
by the biological estimation of starch with
1935. Original not seen. Abstracted in C.A. 26:
250. 1932.

329. Lampe, B. and Kilp, W.
Über Maischverfahren und künstliche Säuerung bei

330. Lange, H.
Über den physiologischen Zustand der Hefe.
331. Larson, W., F., Hartzell, T. B., and Diehl, H.S.
The effect of high pressures on bacteria.

332. Lasnitski, A., and Szőrnyi, E.
Influence of different cations on the growth of

333. Lebedoff, A.
Ueber Hexosphosphorsäureester.

334. Lejhanac, G., Parfentjev, I.A., and Sokoloff, B.
The influence of quinine on cell. J. Pharmacol.

335. Lemmermann, O., Fischer, O., and Husek, E.
The influence of different bases on the
transformation of ammonia and nitrates. Landw.
Abstracted in C.A. 5:1684. 1911.

336. Lennisterand, A.
Über die Inaktivierung der Co-Zymase in mit
Fluorid vergiftetem Apo-Zymase-System und die

337. Lennisterand, A.
Über die Wirkung von Phosphat auf Oxidation und
Phosphorylierung in durch Fluorid vergiftetem

338. Lepeshkin, V.
The influence of vitamins upon the development of

Phytochemical reductions. The configurations
of glycols obtained by reduction with fermenting

340. Libbrecht, W.
Influence of the substrate on the respiration of
and fermentation by yeast cells. Naturw.
341. Lieben, F., and Laszlo, D.
Über den Einfluss einiger Ionen auf die
Zuckerassimilation durch sauerstoffgeschuttete

342. Lieske, R., and Hofman, E.
Untersuchungen über Hefegärung bei hohen

343. Lindberg, E.
Über Gärungsaktivatoren.
Biochem. Z. 132:110-34. 1922.

344. Lindermann, M.
Der Arsengehalt des Hopfens bestimmt den

345. Lindet, L.
Le dechet de la fermentation alcoolique.

346. Lindet, L.
The by-products of alcoholic fermentation.
1918.

347. Lindner, P.
Bakterien als Alkoholverdorger im Bier und anderen
alkoholischem Flüssigkeiten. Wochschr. Brau. 38:
206-8. 1921.

348. Lindner, P.
Zur Wirkung ultravioletter Strahlen auf die
alkoholische Gärung und auf Hefe. Wochschr.
Brau. 39:186-7. 1922.

349. Lindner, P. and Schmidt, O.
Die Widerstandsfähigkeit eines bei verschiedenen
Temperaturen herangezüchteten Hefen materials
gegenüber verschiedenen Desinfektionsmitteln und
der Einfluss der Temperatur während Einwirkung

350. Ling, A. R., and Nanje, D. R.
Longevity of certain species of yeasts. Proc.
351. Loewenthal, S. and Wohlgemuth, J.
Untersuchungen über die Diastasen. VIII.
Über den Einfluss der Radioaktivität auf die
Wirkung des diastatischen Ferments. Biochem.

352. Lohmann, K.
Notiz über das Verhalten der Phosphatase in
Gegenwart von Glutathion und Monojodessigsäure.
Biochem. Z. 262:157-60. 1933.

353. Lövgren, S.
Alteration in the inverting power of top yeast
by pretreatment. Fermentforschung 3:221-40.
1920. Original not seen. Abstracted in C.A. 15:
1731. 1921.

354. Luce, E.
Composition of a fusel oil, and origin of acids
in alcoholic fermentation. J. pharm. chim. 22:
136-8. 1920. Original not seen. Abstracted in

355. Lüers, H. and Jacobson, S.
Der Einfluss Kiesel Säure-haltigen Brauwassers
1935.

356. Lünder, E.
Die Gärung in geschlossenen und in offenen Gärbottichen.

357. Lünder, E.
Wann ist betriebstechnisch ein stärkeres Maisbrennen

358. Lünder, E.
The efficiency of closed fermentation vats.
Abstracted in C.A. 16:1830. 1922.

359. Lünder, E. and Lampe, B.
Mangelhafte Endvergärung der reifen Maischen.
Z. Spiritusind. 51:335-6. 1923.

360. Lundsgaard, E.
Weitere Untersuchungen zwischen den natürlichen
Uronsäuren, Pentosen und Pentosanen. Biochem. Z.
250:61-88. 1932.
361. Lutndin, H.
Über den Einfluss des Sauerstoffes auf die
assimilatorische und dissimilatorische
Tätigkeit der Hefe. III. Verhalten zugesetzten
Alkohols in Hefesuspensionen. Biochem. Z. 142:
454-62. 1923.

362. Macfarlane, M. G.
Action of arsenate on hexosephosphate. Biochem.

363. Macfarlane, M. G.
Fermentation by yeast preparations. I. Effect
of monolactate on the fermentation of
hexosediphosphate. II. Action of arsenate on
the induction period of zymin. Biochem. J. 25:
822. 1931.

364. Maclean, T. S. and Hoffert, D.
Carbohydrate and fat metabolism of yeast II.
The influence of phosphates on the storage of
fat and carbohydrate in the cell. Biochem. J.
18:1273-8.

365. Magne, J.
Theoretical alcohol yield. Louisana Planter

366. Malkov, A.
Influence of iron on aerobic alcoholic fermentation.
1933.

367. Malkov, A. M.
Zur Frage nach der Rolle der Phosphate bei der
alkoholischen Gärung und Atmung der Hefe.
Biochem. Z. 262:185-95. 1933.

368. Mameli, E.
Action of certain organic substances on alcoholic
1927.

369. Mameli, E. and Nossini, A.
The action of organic substances on alcoholic
fermentation II. Olifinoc substances. Giorn. chim.
370. Mameli, E. and Mossini, A.

371. Manford, E.

372. Manolow, E.

373. Mariller, E.

374. Maschaupt, J. G.

375. Matsuyama, M. and Nakamura, H.

376. Matsuyaman, M.

377. Maurain, E. and Warcollier, C.

378. Maurain, E. and Warcollier, C.

379. Maurer, K. and Steinacker, F.
Über die Einwirkung von Metallsalzen auf den
Verlauf der alkoholischen Garung. Biochem. Z.
141:447-57. 1923.

381. Mayer, P.
Über den Einfluss von Mineralwasser auf den
Kohlenhydratumsatz durch Hefen. Biochem. Z.
131: 1-5. 1921.

382. Mayer, P.
Vergleichende Untersuchungen über die Wirkung von
Arsenat und von organischen Arsenverbindungen
auf die alkoholische Zuckerspaltung. Ein
Beitrag zur Analyse der Arsenwirkung. Biochem. Z.

383. McKern, J. S.
The cause of deterioration and spoiling of corn

384. McKern, J. S. and Calfee, R. K.
Effect of manganese, copper and zinc on the growth

385. McLean, I. M. and Thomas, B. M.
1920.

386. Meier, R.
Stimulation in individual cells. Arch. exptl.

387. Meisenheimer, J.
Nitrogenous constituents of yeast. Z. physiol.

388. Meyer, K.
Experiments on fermentations produced by yeast
cells whose permeability has been modified.
Ann. brasserie and distillerie 1931 no. 22.
1932.

389. Meyerhof, O. and Iwaski, K.
Über Beeinflussung der Garungsgröße und des
Oxidationsquotienten der Hefe. Biochem. Z. 226:
18-31. 1930.
390. Meyerhof, O. and Kiessling, W.
Über die phosphorilierten Zwischenprodukte und
die letzten Phasen der alkoholischen Garung.

391. Mezzadro, G. and Vareton, E.
Effect of short electromagnetic waves on the
characteristics of the enzymes of the seeds.
Chimie und Industrie, Special No. 337-42
March 1931. Original not seen. Abstracted in

392. Michaelis, L. and Rona, F.
Die Wirkungsbedingungen der Maltaese aus Bierhefe.
III. Über die Natur der verschiedenartigen
Hemmungen der Fermentwirkungen. Biochem. Z. 66:
62-70. 1914.

393. Miller, E. W.
The effect of certain stimulating substances on
the invertase activity of yeast. J. Biol. Chem.

394. Mitia, S. K.
Toxic and antagonistic effects of salts on wine

395. Minulescu, M.
Influence of the viscosity of the medium on

396. Morii, G.
The inhibitory action of the salts of silver and
some other heavy metals on amylase. J. Biochem.
(Japan) 2:117-22. 1922.

397. Mossini, A.
Antifermentative action of the phenols in presence
of substances which decrease the surface tension.

398. Mossini, A.
Effect of organic substances on alcoholic ferment-
tion. V. Action of follicol in. chimica e
industria (Italy) 17:524-7. 1935. Original not
399. Moufang, E.

400. Moufang, E.

401. Moufang, E.

402. Moufang, E.

403. Moufang, E.

404. Moufang, E.

405. Moufang, E.

406. Moufang, E. and Vetter, L.


408. Müller, H., Thurgan, C. and Osterwalder, A.
409. Murakami, P.

410. Murakami, P.

411. Murakami, P.

412. Myrbäck, K.

413. Myrbäck, K. and Nilsson, K.

414. Mysztkowski, E. M. and Landau, M.

415. Nadson, G. and Stern, C.A.

416. Nakamura, H.

417. Nakamura, H.

418. Nayasart, E.
419. Navaz, A. E. and Rubenstein, B. B.
Starch hydrolysis as affected by light. II.

420. Negelín, E.
Über die Wirkung des Schwefelwasserstoffs auf
chemische Vorgänge in Zellen. Biochem. Z. 165:

Some organic acids in barley, maize, oats and

422. Neuberg, C.
Über eine allgemeine Beziehung der Aldehyde zur
alkoholischen Gärung nebst Bemerkung über das

423. Neuberg, C. and Czapski, L.
Über Einfluss einiger biologisch wichtigen Säuren
auf die Vergärung des Traubenzuckers. Biochem. Z.
37:51-5. 1914.

Zur Nomenclatur der an der alkoholischen Gärung

425. Neuberg, C. and Hirsch, J.
Über den Verlauf der alkoholischen Gärung bei
alkalischer Reaktion. II. Gärung mit lebender Hefe

426. Neuberg, C. and Hofmann, E.
The heat source of anaerobic energy-producing
1932.

427. Neuberg, C. and Kobel, M.
Die Zerlegung von nicht phosphoryliertem höheren
Pflanzen. Die Bildung von Methylglyoxal durch
1930.

428. Neuberg, C. and Nord, F.F.
Über die Gärwirkung frischer Hefen bei Gegenwart
429. Neuberg, C. and Nord, F. F.
Anwendung der Absorptionsmethode auf die Bakterien-
Gärungen. 1. Acetaldehyd als Zwischenstufe bei der
Vergärung von Zucker, Hannit und Glycerin durch
Bacterium coli, durch Erregen der Ruhr und des

430. Neuberg, C. and Reinfurth, E.
Die Festlegung der Aldehydstufe bei der alkoholischen

431. Neuberg, C. and Reinfurth, E.
Naturliche und gezwangene Glycerinbildung bei der

432. Neuberg, C. and Reinfurth, E.
Further investigations on the correlative formation
of acetaldehyde and glycerol in the scission of
sugar and new contributions to the theory of
alcoholic fermentation. Ber. 52 B:1677-703. 1919.

433. Neuberg, C., Reinfurth, E. and Sandberg, M.
Neue Klassen von Stimulatoren der alkoholischen

434. Neuberg, C. and Sandberg, M.
Weitere Mitteilungen über chemisch definierte
Katalysatoren der alkoholischen Gärung. Biochem. Z.

435. Neuberg, C. and Sandberg, M.
Von den Stimulatoren der alkoholischen Zuckerspaltung.

436. Neuberg, C. and Sandberg, M.
Über Stimulatoren der alkoholischen Zuckerspaltung.
IX. Biochem. Z. 126:152-78. 1921.

437. Neuberg, C. and Schwenk, E.
Kofermentartige Wirkung von Salzen der a-Ketosäuren.

Über die Hexosediphosphorsäure, ihre Zusammensetzung
und die Frage ihrer Rolle bei der alkoholischen
Gärung sowie über das Verhalten der Dreikohlenstoff-
zucker zu Hefen. Biochem. Z. 83:244-52. 1917.
439. Neuberg, C. and Ursum, W.
Die dritte Vergärungsform des Zuckers als allgemeine
 Folge anorganischer und organischer Alkaliformen.

440. Neuschloz, S. M.
Influence of neutral salts on enzyme action.
 Arch. ges Physiol. (Pflüger) 181:45-64. 1920.
 1921.

441. Newton, Wm. and Edwards, H. I.
Chemical compounds lethal to yeasts and bacteria.

442. Nicolau, S.
 Distillerie, 42:49-65-61-97. 1924. Original

443. Niethammer, A.
The influence of nickel compounds and of cyanides
 on the germination of grains. Miss. Arch. Landw.

Zur Kenntnis der Monojod- und Monbromessigsäure-
 vergiftungen beim Kohlenhydratabbau. Z. physiol.
 Chem. 194: 53-68. 1931.

445. Noni, I.
Growth and enzymic activity of Saccharomyces
 cerevisiae in presence of sodium and potassium
 1935.

446. Norris, R. J. and Ruddy, Sister Veronita
A study of stimulation of growth, respiration and
 fermentation by bios and bios-like substances.
 Studies Inst. Divi. Thomas 1: 53-64. 1937. Original

447. Nottin, P.
Influence of mercury on alcoholic fermentation.
 Abstracted in C.A. 8:1442. 1914.
448. Newark, C. A.

449. Celsner, A. and Koch, A.

450. Ohlsson, E.

451. Ohlsson, E.

452. Ohlson, E.

453. Olsson, U.

454. Olsson, U.

455. Oparin, A. and Kurssenow, A.

456. Ordonneau, C.

457. Orient, J.
458. Orent, J.
Über die Wirkung der Oxymethyllanthraclinon enthaltenden Drogen auf die Gärung. Biochem. Z. 144:353-60. 1924.

459. Ornstein, L. S. and Meyer, J. W.

460. Oster, R. H.
Results of irradiating Saccharomyces with monochromatic ultra violet light. I. Morphological and respiratory changes. J. Gen. Physiol. 18: 71-86. 1934.

461. Oster, R. H.

462. Otero, W. J.

463. Owen, W. L.

464. Owen, W. L.
Ultra violet irradiation stimulates yeast activity. Food Ind. 5:252-4. 1923.

465. Owen, W. L. and Denson, W. P.

466. Owen, W. L. and Mobley, R. L.
467. Palladin, V. I. and Lovchinknokhaya, E. I.
Influence of alcohol and methylene blue on the liberation of carbon dioxide by killed yeast.

468. Palladin, W.

469. Palmieri, G. G. and Giordano, G.

470. Pankrat, O.
Endvergarung und Salzanalyse.

471. Pantanelli, E.
The mechanism of the secretion of enzymes. I.
Influence of colloids on the secretion of invertase.

472. Panzer, T.
Einzirkung von Chlorwasserstoffgas auf Diastase. I.

473. Panzer, T.
Einwirkung von Ammoniakgas auf Diastase III.

474. Panzer, T.

475. Panzer, T.

476. Panzer, T.
Einwirkung von Stickoxyd auf Diastase. VII.
477. Panzer, T.
Einwirkung von Stickstoff auf Diastase, VIII.

478. Panzer, T.
Einwirkung von Ammoniakgas auf eine durch
Erhitzen unwirksam gewordene Diastase. X. Z.
physiol. Chem. 86:401-6. 1913.

479. Paris, G. and Maisiglia, T.
The reduction of nitrate during alcoholic

480. Pascu, E.
Alcoholic fermentation of d-glucose in
1934.

481. Patterson, M. B.
Effects of cyanide and some other salts on
fermentation by yeast preparations. Biochem. J.
25:1595. 1931.

482. Pavy, F. W. and Rywaters, E. W.
The effect of activators on enzyme action. J.
Physiol. 41: 168-93. 1911.

483. Perry, E. C. and Beal, G. D.
The quantity of preservatives necessary to
inhibit and prevent alcoholic fermentation and
1920.

484. Petit, P.
Obergelege Hefe und Aziditat.

485. Petit, P.
Treatment of vats and casks in brewery.
Brasserie et malterie 6:369. 1917. Original

486. Petit, P.
Troubles encountered with new malts (of 1928).
487. Petit, F. and Raux, J.

488. Philipson, T.

489. Philipson, T.

490. Philipson, T.

491. Piretto, F.

492. Piretto, F.

493. Pfeiffer, H.

494. Plante, F.

495. Popp, N. and Contzen, J.

496. Pott, F.
497. Potter, M. C.
    The influence of electric potential upon the

498. Pringsheim, H.
    Über die Stickstoffernährung der Hefe. Biochem.
    Z. 3:121-286. 1907.

499. Pringsheim, H.
    Über die Unterdrückung der Fuselölbindung und die
    Mitwirkung von Bakterien an der Bildung höherer

500. Pringsheim, H.
    Bemerkungen zur Mitwirkung von Bakterien an der

501. Pringsheim, H., Borchardt, H. and Hupfer, H.
    Activator der fermentativen Stärkeverzuckerung.

502. Pringsheim, H., Borchart, H. and Hupfer, H.
    Über Glutathion als Komplement der Amylased

503. Pronin, S.
    Zur Frage des Grenzabbaues der Stärke durch

504. Rahn, O.
    Der Einfluss der Temperatur und der Gifte auf
    Enzymwirkung, Gärung und Wachstum. Biochem. Z. 72:
    351-77. 1913.

505. Rahn, O.
    The decreasing rate of fermentation. J. Bact. 13:
    207-26. 1929.

506. Rahn, O.
    The order of death of organisms larger than

507. Rasoumov, N. and Rubinstein, V.
    Influence of disinfecting substances on the
    microorganisms of the fermentation industries.
508. Reinhard, A. and Cbrastzova, V.

509. Reinhard, A. W.

510. Richards, O. W.

511. Richards, O. W.

512. Richards, O. W. and Haynes, F. W.
Oxygen consumption and carbon dioxide production during the growth of yeast. Plant Physiol. 7:139-44. 1932.

513. Richet, C.

514. Richet, C. and Braumann, L.

515. Richet, C. and Cardot, H.

516. Rippel, A.

517. Roger, M.
518. Rona, P. and Michaelic, L.
Die Wirkungsbedingungen der Maltase aus Bierhefe.
II. Die Wirkung der Maltase auf α-methylglucosid
und die Affinitätsgrösse des Ferments. Bioehem. Z.
58:148-57. 1914.

519. Rona, P. and Grasshein, K.
Studien zur Zellatmung. II. Die Wirkung von Chinin
auf die Atmung lebender Hefezellen. Biochem. Z.
140:490-516. 1923.

520. Rona, P., Eweyk, C. v. and Tennenbaum, M.
Über die Wirkung der Alkaloiden aus der Atropin-
Cocain- und Morphingruppe auf die Hefe-Invertase.
Biochem. Z. 144:490-519. 1923.

521. Rosenblat, M. and March, A. J.
Über die Wirkung des Mangans auf die alkoholische

522. Rosenblat, M. and March, A. J.
Über den Einfluss katalytischen Elemente auf die

523. Rosenow, L. P.
Über die Wirkung von Thyreoidea, Gerebin und Cordin
auf die anaerobe Atmung der Hefe. Biochem. Z. 159:

524. Rüdiger, M.
Introduction of Buchelers process for the
preparation of yeast in the distilling industry.

525. Sabalitschka, T. and Tietz, H.
Relation between chemical constitution and anti-
microbial action. XI. Di and trihydroxy-
alkyloxy benzoic acids and esters. Arch. Pharm.
265:545-66. 1931. Original not seen. Abstracted

526. Sage, C.

527. Salkowski, E.
Über Zuckerbildung und andere Fermentationen in der

528. Salkowski, E.
The carbohydrate content of lichens and the influence
of chlorides upon alcoholic fermentation. Z. physiol.
Chem. 104:105-23. 1919. Original not seen. Abstracted
529. Sandstedt, R. M. and Elish, M. J.

530. Sanzo, L. and Pizzone, F.

531. Satterfield, G. and Jones, S. C.

532. Scharrer, K. and Claus, G.

533. Scharrer, K. and Schwartz, W.

534. Scharrer, K. and Schwartz, W.

535. Schneidewind, W., Meyer, D. and Münster, F.

536. Schönfeld, F.

537. Schönfeld, F.

538. Schönfeld, F. and Hardeck, M.
539. Schönfeld, F. and Korn, M.

540. Schönfeld, F., Krumhaar, H. and Korn, M.

541. Schönfeld, F., Krumhaar, H. and Korn, M.

542. Schönfeld, F. and Krumhaar, H.

543. Schönfeld, F. and Krumhaar, H.

544. Schopmeyer, H. J.

545. Schopmeyer, H. and Fulmer, E. I.

546. Schuster, K.

547. Schuster, K.

548. Schwarz, O.
549. Schweitzer, K.
Influence of copper on alcoholic fermentation.

550. Schwerzer, C.

551. Sechi, E.

552. Sherman, H. C., Thomas, A. W., and Baldwin, M. E.

553. Sherman, H. C. and Walker, F.
Influence of aspartic and asparagine upon the enzymic hydrolysis of starch. J. Am. Chem. Soc. 41:1866-73. 1919.

554. Sherman, H. C. and Walker, F.

555. Sherman, H. C. and Walker, J. A.
Influence of certain electrolytes upon the course of the hydrolysis of starch by malt amylase. J. Am. Chem. Soc. 39:1476. 1917.

556. Sherwood, F. F. and Pulmer, E. L.

557. Sherwood, H. C., Caldwell, M. L. and Cleaveland, M.

558. Silbereisen, K.
559. Sidersky, D.
Alcoholic fermentation of saccharine materials.

560. Slator, A.

561. Slator, A.

562. Sobotka, H., Holzman, M. and Reiner, M.

563. Sobotka, H. and Reiner, M.
Selective fermentation. I. Alcoholic fermentation of glucose, fructose, and mannose mixtures.

564. Sode, T.

565. Söhngen, N. L.
Influence of some colloids on alcoholic fermentation.

566. Söhngen, N. L. and Coolhaus, C.

567. Sonogri, R.
Wirkung von Säuren auf die Befegärung. Biochem. Z. 120:100-2. 1921.

568. Sorensen, S. F.

569. Speak, H. B., Gee, A. K. and Luck, J. N.
570. Staiger, G.
The alcohol yield from rye and wheat.

571. Staiger, G., and Glaubitz, M.
Über Beeinflussung der Gärkraft der Hefe bei
Vorbehandlung mit wechselnden Mengen Schwefelsäure.
Z. Spiritusind 52:270-1, 1929.

572. Staiger, G. and Glaubitz, M.
How much alcohol is produced by yeast? II.
Checking of alcohol production, Brennerei Zeit,
47:127, 1930. Original not seen, Abstracted in

573. Staiger, G. and Glaubitz, M.
Report from the laboratories of the experiment
station for grain distillers and institute for
fermentation industries, Brennerei Zeit. 48:76,
1931. Original not seen. Abstracted in C.A. 26:
250, 1932.

574. Staker, E. V. and Gortner, R. A.
Physicochemical studies on proteins. II. A
comparative study of the peptization of the protein
complex in various seeds and grains. J. Phys.
Chem. 35:1565-805, 1931.

575. Starkenstein, E.
Über Fermentwirkung und deren Beeinflussung durch

576. Steudel, H. and Wahnitz, R.
Über die Struktur einfachen Nucleinsäuren II.

577. Stevenson, H., M.
Corn whisky and its constituents. Texas State
J. Med. 20:562-6, 1925.

578. Stier, T. J. B.
The rate of oxygen utilization by yeast as related

579. Stockhausen, F. and Koch, R.
Wirken elektrische Ströme auf die Gärung?
580. Stoward, F.
The influence exercised by certain acids on the
inversion of saccharose by sucrose. Biochem. J. 6:
151-40. 1912.

581. Stricht, G.
Effect of the alkalinity of water in brewing.
Bull. assoc. élves. Inst. super. fermentations.
Gand 31:288-44. 1930. Original not seen.

582. Sturm, A. and Schulz, J.
Beeinflussung glykolytischer Stoffwechselvorgänge
durch Jod unter besonderer Berücksichtigung der
Hefegrührung, zugleich ein Beitrag zur Monojodessig-
saurewirkung auf die Hefegrührung. Biochem. Z. 265:
193-218. 1933.

583. Sugiiura, K. and Benedict, S.
The action of radium emanation on the vitamins

584. Svanberg, O.
The multiplying power of yeast at different degrees

585. Swooboda, F. K.
Nitrogen nutrition of yeast. J. Biol. Chem. 52:
91-109. 1922.

586. Sym, E. A.
Einfluss des kolloidalen Zustandes der Stärke und
der Amylasezüng auf die Geschwindigkeit der

587. Szego, E.
Influence of the size of starch grains on their
yield (in brewing). Ann. brasserie et distillerie

588. Takahashi, T.
Esters of Phenethyl alcohol with organic acids I.
Action as the antiseptic. J. Agr. Chem. Soc. Japan
10:970-4. 1934. Original not seen. Abstracted in
589. Takahashi, T., Gunke, M. and Yamazaki, T.
Detection of methyl alcohol in alcoholic beverages and its formation by the several kinds of yeast.

590. Taketomi, M. and Hanamura, S.
The cause of the low yields of alcohol in the fermentation of Formosan cane molasses. I. J. Soc.

591. Taketomi, M. and Matsumoto, T.

592. Taxner, E.

593. Taylor, A. F.
On the inversion of cane sugar and malts by ferments. J. Biol. Chem. 5:399-403, 1907.

594. Terry, L.

595. Thomas, A. W.

596. Thomas, P.

597. Thomas, P.

598. Thevenot, G.
A new substance for washing infected yeast. Am.
Brewer. 42:397-8. 1908.
599. Tomoda, Y.
Production of glycerol by fermentation V.
Effects of sulfites on yeast cells and fermentation.

600. Tomoda, Y.
The effect of aeration upon alcoholic fermentation.

601. Tracy, R. L., Jr.
Lethal effect of alternating current on yeast cells.

602. Trautwein, K.
Influence of aluminum on reproduction, respiration, and fermentation of yeast.

603. Trautwein, K.

604. Trillat, A.
The use of aluminum in brewing, milk and cheese industries, etc.

605. Trillat, A. and Sauton, B.
Circumstances which favor the formation and disappearance of acetaldehyde in alcoholic media.

606. Tumang, G.
Adventitious versus pure yeast fermentation.

607. Tyutyunnikova, A. W.
Der Einfluss von Giften auf die Gerstenmalskatalase VI.

608. Vahlen, E.
Über Metabolin und Antibolin aus Hefe.
609. Valentine, H.

610. Valentine, H.

611. Van Lear, M. H.

612. Van Lear, M. H.

613. Van Lear, M. H.

614. Van Lear, M. H.

615. Van Lear, M. H. and Duminage, R.

616. Veibel, S.

617. Vejtor, L.
618. Vetter, L. and Moufang, E.

619. Waldschmidt-Leitz, E., Weil, E. and Furr, A.

620. Warcollier, G. and Le Moal, A.

621. Warcollier, G. and Le Moal, A.

622. Weichherz, J. and Asmus, R.

623. Weichherz, J. and Memlender, R.

624. Weichherz, J. and Nord, F. F.

625. Wels, P. and Osann, Z.

626. Wendel, F.

627. Wiecland, H. and Gough, G. A.
628. Wieland, H., Rauch, K. and Thompson, A. F.
Mechanism of the oxidation process XLIII.
Several observations on the inhibition of
respiration and fermentation processes of yeast.

629. Wilenko, G. F.

630. Willstätter, R. and Barann, E.
Über direkte Maltosegärung durch maltasereiche Hefe.
Achte Mitteilung über Maltase. Z. physiol. Chem.

631. Willstätter, R. and Barann, E.
151:242-72. 1926.

632. Willstätter, R. and Lowry, C. D.
Invertinverminderung in der Hefe. Elfte Abhandlung
zur Kenntnis des Invertins. Z. physiol. Chem. 150:
287-305. 1926.

633. Willstätter, R. W. and Steibelt, W.
Über die Gärungswirkung maltasearmer Hefen. Z.
physiol. Chem. 115:211-34. 1921.

634. Windhaus, A. and Grosskopf, W.
Über das Ergosterin der Hefe.

635. Windisch, F.
Die Bedeutung des Sauerstoffs für die Hefe und ihre
1932.

636. Windisch, K. and Jetter, W.
Über die Verbreitung der Diastase in der
Stärkemehlhaltigen Rohstoffen der Brannt wein
brennerei (Herstellung von Brannt wein aus
Stärkemehlhaltigen Rohstoffen ohne Verwendung

637. Windisch, W.
Über die Mehrausbeute bei Brauerei und
Vormalsichverfahren und die Mehrausbeute
1909.
638. Windisch, W.

639. Windisch, W.

640. Windisch, W.

641. Windisch, W.

642. Windisch, W.

643. Windisch, W.

644. Windisch, W.

645. Windisch, W. and Goldacker, R.

646. Windisch, W., Henneberg, W. and Dietrich, W.

647. Windisch, W. and Kolbach, P. and Schild, K.
648. Windisch, W., Kolbach, R. and Illies, R.
Ueber die Säurebildung bei der Gärung von Bierwürze.

649. Windisch, W., Kolbach, P. and Schild, E.
Ueber den Eiweissabbau beim Maischen. Wochschr.
Brau. 48:253-60. 1931.

650. Windisch, W., Kolbach, P. and Schild, E.
Ueber den Stärkeabbau beim Maischen.

651. Windisch, W. and Krumbaar, H.
Beiträge zur Brauwasserfrage. V. Über die Vorgänge
bei der Entkarbonisierung des Wassers beim Gewärmen
und Kochen nebst praktischen Nutsanwendungen.

652. Windisch, W. and Bogelsang, W.
Ueber die Art der Phosphorsäureverbindungen in der
Gerste und deren Veränderungen Während des weich-,
Malz-, Darr-, und Maisch prozesses. Wochschr.
Brau. 23:516. 1906.

653. Windisch, W. and Wellman, R.
Vergleichende Ausbeuteversuche mit verschiedenen
Maischverfahren, unter besonderer Berücksichtigung
des Druckmaisch-verfahrens. Wochschr. Brau. 27:
1-3. 1910.

654. Windisch, W. and Windisch, F.
Ueber die gärungsbioiogische Bedeutung der Nitrate

655. Winslow, C. E. and Haywood, E. T.
The specific potency of certain cations with
reference to their effect on bacterial mobility.

656. Winslow, C. E. and Upton, M. F.
The electrophoretic migration of various types

657. Woodrow, J. W., Bartly, A. C. and Fulmer, E. I.
The effect of ultra violet radiation upon yeast

658. Wooldridge, H. B.
Use of hydrogen peroxide in the brewery. J. Inst.
Brewing. 22:137. 1916.
659. Woold, B.
Addition-compound theory of enzyme action.

660. Wusterfeld, H.
Gärbeschleunigung durch gewisse Stoffe--bewegte Gärung--

661. Wyatt, F. and Schlichting, E.
Do the raw cereals products used in brewing contribute any yeast food to beer wort. J. Soc.
Chem. Ind. 28:733. 1909.

662. Yamada, M.
Decomposition of amino acids by yeast. I. J.

663. Yamazaki, I.
Über die Wirkung von mono-jod-essigsäuren Natrum
auf die Teilsfärmente der Zymase und die Vergärung

664. Young, L. M. and Trimble, H. M.
The influence of ethylene glycol upon some reactions.

665. Young, W. J.
The organic phosphorus compounds formed by yeast

666. Zeller, H.
Wirkung von Arzneimitteln und Strahlen auf Hefe.
I Versuche über die Grundlage des Arndt-Schulzschen

667. Zeller, H.
Wirkung von Ammonsalzen auf die Kefagärung IV.

668. Zeller, H.
Wirkung von oberflächenaktiven Stoffen auf die
Kefagärung. VII. Biochem. Z. 183:369-68. 1927.

669. Zöllner, E. and Hamburger, R.
Über die Einwirkung von Silberverbindungen auf
670. Zikes, H.
Influence of aluminium on yeast and beer.

671. Zikes, H.
Influence of air pressure on fermentation.

672. Zikes, H.

673. Zikes, H. and Wagner, F.

674. Zlataroff, A., Andreitschewa, M. and Kaltschewa, D.

675. Zuckerhandl, F. and Messiner-Klebermass, L.

676. Zuckerhandl, F. and Messiner-Klebermass, L.
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