YEAST

ITS CHARACTERISTICS, GROWTH, AND FUNCTION IN BAKED PRODUCTS

—a Symposium

ADVISORY BOARD on QUARTERMASTER RESEARCH and DEVELOPMENT

Committee on Foods
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YEAST—Its Characteristics, Growth, and Function in Baked Products

A symposium held at

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Edited by
Charles S. McWilliams and Martin S. Peterson
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Advisory Board on Quartermaster Research and Development
Committee on Foods
Subcommittee on Cereal and Baked Products
NATIONAL ACADEMY OF SCIENCES—
NATIONAL RESEARCH COUNCIL
Washington, D. C.
January 1957

SURVEYS OF PROGRESS ON MILITARY SUBSISTENCE PROBLEMS

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1. Contributions of Browning Research to Ration Item Stability
2. Stability of Shortenings in Cereal and Baked Products
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1. Establishing Optimum Conditions for Storage and Handling of Semiperishable Subsistence Items.

Opinions expressed in the symposium on *Yeast—Its Characteristics, Growth, and Function in Baked Products* are those of the individual participants and do not necessarily represent the views or policies of the Department of Defense.

Preface

MILITARY FEEDING SYSTEMS depend for their success on the degree to which the problems of supply and the problems of food preparation and use in the field are solved. The failure of one component, such as bread, can be critical. Advances beyond those normally expected by commercial users are often necessary to assure that a ration item will be "foolproof" in areas of the globe where commercial facilities and experience are not available. The leavening agents used in bread and other baked products are therefore of definite military subsistence concern. Upon their performance can depend good bread for the troops or no bread at all.

The symposia, cosponsored by the Committee on Food of the Advisory Board on Quartermaster Research and Development and the Quartermaster Food and Container Institute for the Armed Forces, are now well established features of research counseling. To look over past and current progress in order to plot out rewarding directions for future research is good management. The symposium on the characteristics, growth, and function of yeast in baked products contains expert analyses by some of the leaders in this field regarding the present position of yeast research. The articles and discussion furnish a vantage ground for the guidance of future yeast investigations. Those who conceived, organized, and administered this symposium are to be congratulated on its excellence.

S. C. LUTZ, *vice president*
National Yeast Corp.,
Belleville, New Jersey

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YEAST—Its Characteristics, Growth, and Function in Baked Products—A Symposium

I. INTRODUCTION

THE SYMPOSIUM ON YEAST, Its Characteristics, Growth, and Function in Baked Products, National Academy of Sciences, National Research Council, Advisory Board on Quartermaster Research and Development, Quartermaster Food and Container Institute for the Armed Forces, convened at 9:40 o'clock in room 14, Palmer House, Chicago, Ill., with Mr. Robert F. Light, Standard Brands, Inc., presiding

CHAIRMAN ROBERT F. LIGHT:

On behalf of the Quartermaster Food and Container Institute for the Armed Forces and the Subcommittee on Cereal and Baked Products of the Committee on Foods, Advisory Board on Quartermaster Research and Development, who are sponsoring this symposium on yeast, I welcome you and call this meeting to order. Members of the subcommittee join me in expressing our appreciation to the speakers and to those who will be participating in this symposium.

We are honored to have with us today our host, Col. J. D. Peterman, Commandant of the Quartermaster Food and Container Institute for the Armed Forces. Colonel Peterman has taken time from his busy schedule to join us and to officially open the meeting with a word of welcome. I would like to present Colonel Peterman at this time.

Welcome to the Symposium

COLONEL J. D. PETERMAN

Commandant, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

IT IS A PLEASURE to welcome you to this symposium on "Yeast—Its Characteristics, Growth, and Function in Baked Products." The large attendance at this meeting is gratifying. It is certainly indicative of the interest in and the importance of the subject to be discussed.

We are very fortunate in having an eminent and well-qualified panel of speakers today—speakers who are widely known for their studies in this field. That these very busy research people are willing to devote the time and effort and travel the distances necessary to be with us today speaks well for the appeal of this symposium and for the public spirit of these men.

Baking bread for issue to troops is one of the few manufacturing

operations in which the Armed Forces engage. Why the Armed Forces conduct such baking operations is easily understood when one considers the firm position of fresh bread in the American diet. Usually consumed at every meal of the day, bread is one of the few food items

Figure 1. Bread, usually consumed at every meal of the serviceman's day, is one of the few ration items which does not become monotonous.

which does not become monotonous. Truly, fresh bread in the American diet is the "staff of life" whether the consumer be a serviceman or a civilian.

Yeast—the essential ingredient

Essential to the successful manufacture of bread is the yeast which leavens it and which contributes to bread a part of its characteristic flavor. With the Nation's many yeast manufacturing plants and extensive and rapid transportation system, the supply of compressed leavening yeast to the Armed Forces within the United States poses no problem. But beyond the continental limits of the United States where distances

are tremendous and where refrigerated transport is slow and scarce, the problem of supplying such yeast is difficult indeed. Under conditions of warfare, it is an impossible situation. This was clearly recognized in the early days of World War II. I well remember an occasion in Africa where we had plenty of flour on hand, had this plentiful supply increased by a daily issue of flour—but had no yeast with which to turn that flour into bread.

The commercial development of active dry yeast was without doubt one of the signal accomplishments in the food field during World War II. The Armed Forces can justly claim credit for fostering and stimulating this commercial development. That fresh bread could be served to our troops contributed much to the morale that was so typical of the American serviceman.

The problem before us

Our purpose today, however, is not to recite the accomplishments of the past but to consider the problems of the future. The use of active dry yeast in the overseas baking operations of the Armed Forces is commonplace. All is not harmonious, however, and occasional complaints come back from the field that active dry yeast has undergone storage deterioration.

As a cooperative research project the Industry Advisory Committee on Active Dry Yeast and the Quartermaster Food and Container Institute for the Armed Forces have determined the storage stability of active dry yeast under a number of specific storage temperatures. These investigations showed that active dry yeast is not as tolerant to storage at elevated temperatures as would be desirable. To illustrate, active dry yeast still produces satisfactory bread after storage at 70° F. for 21 months. At 90° F. the satisfactory storage life is only 7 months; and, at 115° F. only 7 days. These data have established our current problem as one of thermostability. The solution of this thermostability problem is a research objective of the Quartermaster Food and Container Institute and is also the motivation for this symposium.

The task of planning and executing a symposium such as this requires the time and effort of a number of people. It is fitting that recognition should be given to these individuals. Therefore, I should like to express the appreciation of the Quartermaster Food and Container Institute to our speakers, to Mr. Robert F. Light of Standard Brands, Inc., and chairman of the National Research Council Subcommittee on Cereal and Baked Products, and to Dr. W. George Parks, executive director, Advisory Board on Quartermaster Research and Development, National Research Council.

CHAIRMAN LIGHT:

Thank you very much, Colonel Peterman.

Dr. Tressler, scientific director of the Quartermaster Food and Container Institute, our next speaker, has had a broad experience in many

phases of food research and technology. He was a Research Fellow at Mellon Institute from 1920 to 1929, was with Birdseye Frozen Foods, 1929–33, was head of the Chemical Division of the New York Agricultural Experiment Station in Geneva, N. Y., 1933–42, and directed his own consulting laboratory, Donald K. Tressler Associates, from 1942 to 1950. In 1950 he was selected to head the work of the Quartermaster Food and Container Institute.

Scope and Purpose of the Symposium

D. K. TRESSLER
Scientific Director, Quartermaster Food and Container Institute for the Armed Forces

IT IS A PLEASURE for me to come here. I want to add my words of welcome to those of Colonel Peterman. We are very glad that you are here, and we hope that you will get something out of the papers presented before this symposium. We hope also that the Quartermaster Food and Container Institute will obtain some good ideas arising out of the discussion of these papers which will be of help to us in solving our yeast problems.

Colonel Peterman has indicated to you the importance of bread in the life of the men of the Armed Forces. He has also indicated the need for yeast and the importance of active dry yeast, and has indicated to you some of the difficulties that have come about because of the instability of active dry yeast at elevated temperatures.

We have had a program at the Institute in that general field—a program of improving the thermostability of active dry yeast. It has been in effect for the past 4 years, and it is being pushed and pushed ever faster. I am sure that Mr. McWilliams and Dr. Mitchell will tell you that we have made great strides. Some of our advances we can talk about; but most of them we can't talk about yet.

We had an adviser in this overall program of improving the thermostability of active dry yeast, Dr. Mrak, head of the Food Technology Department at the University of California at Davis. He made some good suggestions, and Dr. Mitchell is going to tell you something about the results of this work.

We are not ready to talk about all of our advances, but we have made great strides, and I am sure that in the next year or two years you will be quite astonished when the whole story has unfolded.

Capt. Robert S. Yare, whom I see in the audience here, worked at the Institute for some time and made considerable progress. He had certain ideas on the importance of trehalose as an index of quality or thermostability in active dry yeast, the amount of trehalose supposedly being an index of the probable storage life.

Much work has gone on elsewhere in that same field, and I hope that at this particular meeting there will be a very real discussion of the question of whether or not the actual trehalose content of the

active dry yeast is a good index of the probable storage life of the yeast.

This meeting, however, is not to be limited to just a discussion of the work on active dry yeast and possible solutions to thermostability problems. Rather, there will be an opportunity to review the overall last work, to consider in general the characteristics and also the best means of growing yeast, and to discuss its use in bread and other baked products.

We feel that if the papers are merely presented and not discussed, perhaps little will be gained. However, if each of you who is an expert in his field or who is a first-class chemist or bacteriologist or microbiologist—and has a knowledge of yeast—will add your ideas and discuss the problems brought up by the speakers, we will all get much valuable assistance from this meeting.

II. BACKGROUND AND BASIC PRINCIPLES

History of the Development of Active Dry Yeast

CHARLES N. FREY
Scarsdale, N.Y.

THE ACTION OF YEAST in the fermentation of wine and beer and in leavening of doughs has been known for 4,000 to 5,000 years or perhaps more. But the nature of the active factor in these fermentations did not become established until the 19th century. The relation of living yeast cells to fermentation was not fully accepted by scientists until about 1870.

In 1680 Leeuwenhoek described and illustrated bodies existing in a fermenting fluid, and his illustrations indicate that they were yeast cells, but he did not relate these cells to fermentation.

During the years 1835–37 Kützing, Cagniard de la Tour, and Schwann pointed out that fermentation of beer was caused by living organisms. "All life from previous life" is a statement that summed up the philosophy of those who opposed spontaneous generation and believed in Schleidan and Schwann's cell theory.

The first mention of yeast in technology is found in the work of Krunitz who published an encyclopedia on yeast in 1775. He stated that yeasts are those bodies existing in the liquid during fermentation and which are driven to the surface, and later settle to the bottom, when the fermentation is completed. He also stated that good results in preservation were obtained if the yeast which had been produced during the course of fermentation was added to wheat flour, a small amount of sugar, and some malt wort, and the mixture placed in a warm place. He recognized the importance of culturing the yeast.

The statements I have just cited indicate that scientific men 150 years ago were dimly perceiving that another series of activities was going on in nature not explained by their scientific theories of transmutation.

Paracelsus, 1493–1541, described putrefaction and fermentation, but no experimental work is cited to substantiate his theories. During the 10th century hops were added to beer, and it would be of interest to know whether they were added to protect beer from contaminating ferments or whether the bitter taste appealed to the consumer.

Albertus Magnus, 1193–1280, and Roger Bacon, 1214–84, defined fermentation and digestion as analogous, but Krunitz may have had a more technical point of view. Practical men were ahead of the scientists until about 1870.

Von Justo, who lived from 1720 to 1771, described methods of pre-

paring "Kunsthsefe," and Westrumb, 1751–1810, advanced the art of propagating stock yeast by improving the method of preparing the "sauerteig." He described the preparation of wort from malt flour by properly malting the barley, by the addition of hops, and by control of the temperature. He also advised the use of the thermometer.

Hermstadt, 1760–1833, advocated the use of low temperatures for the mash. He developed good technical methods. He differentiated between top and bottom yeast, preferring the former. He described a yeast test which consisted of dropping a spoonful of good yeast into boiling water. If the yeast floated on the surface, similar to lard, it was a good yeast. If it sank to the bottom, however, he considered it of poor quality.

Another test consisted in taking a quart of yeast suspension, adding a teaspoonful of brandy, some sugar, and a spoonful of flour, and allowing the mixture to stand. If fermentation began promptly, the yeast was good. He also introduced a saccharimeter.

Fremy and Boutron about 1839 investigated the souring of wort. Scheele had observed that an acid was produced in the fermentation of milk and during the processing of sauerkraut. The acid was believed to be acetic acid. Fremy and Boutron added fluids from the stomach of dogs and stomach contents of calves to worts and observed the formation of lactic acid. Liebig was interested in these experiments because he thought the results supported his idea of the nature of fermentation.

Paupie in 1771 was carrying on a method of preparing dry yeast by means of ashes. He wrapped the ashes and yeast in a cloth and dried the material in the sun. Von Plonitz about 1780 and Aubry froze yeast and stored it in the frozen state. The first dry yeast was manufactured by Burka in Vienna in 1822.

Thénard in 1803 stated that yeast is undoubtedly the cause of fermentation. He pointed out that all sugary solutions which fermented spontaneously gave a precipitate which in external appearance resembled the sediment found during the fermentation of beer. He regarded the yeast as a ferment and believed its action was similar to that of a chemical substance.

Erxleben, 1818, was possibly one of the first to express a definite view regarding yeast as the initiator of fermentation. He concluded that yeast was a vegetative organism.

Kircher, 1601–80, stated that animalculae are the cause of putrefaction and decay, but whether scientific observations were relied on to support his theories is not known.

Willis, 1623–75, Stahl, 1660–1734, and Liebig, 1803–73, adopted the view that fermentation was caused by a substance which, owing to its molecular instability, communicated its vibration to other substances present in the medium.

In 1822 a chemist named Tiele pointed out the value of malt, which he attributed to the presence of sugar. He did not recognize enzyme

activity, but Kirchoff in 1811 found that starch is converted to glucose by mineral acids, and in 1816 it was demonstrated by Kirchhoff that boiled starch could be converted to sugar by cereal grains. Payen and Persoz in 1830 named the active principle which converts starch to glucose "diastase."

Ludersdorff about 1801–86, developed methods for souring wort, improved methods of developing stock yeast methods of control such as the use of the saccharimeter, the microscope, and the thermometer, and the determination of acidity in the yeast mash. He stated in modern terms the nature of malt and the function of acids in controlling contamination. He observed that the addition of hops would prevent contamination of the fermenting wort.

About 1800, compressed yeast began to be known in England, Holland, and Germany. The first compressed yeast is stated to have been prepared in 1792 by an Englishman named Mason. Von Dunndorf stated that compressed yeast production began in Holland in 1781.

1. Recognition of Various Types of Fermentation

About the year 1800 various types of fermentation began to be definitely recognized. About 1809 Appert showed that he could preserve foods from putrefaction and decay by sealing them in glass vessels during cooking so that when sealed the air was reduced to nearly a vacuum. You may recall that air or oxygen was believed to support putrefaction, and Schwann had shown that cooked foods decayed when exposed to air. However, he found heated air or sulphur dioxide treated air remained sterile. He finally stated that it is not the O_2 or the air that causes decay, but some factor present in air, probably an organism. Kützing stated that vinegar production was associated with a small organism. He also associated the yeast cell with fermentation. Cagniord de la Tour definitely related the yeast cell to the action of fermentation.

2. Fundamental Concepts[1]

From our vantage point we can understand how the course of thought was influenced by the following: the use of the microscope by Kützing and Schwann; the theories and observations of Sylvius deBoe who, in 1659, distinguished between putrefaction and fermentation and other reactions in which gas is given off; the statement of Becher, 1635–82, that only sweet solutions fermented; Van Helmont, 1577–1644, and Lavoisier, about 1780, who stated a specific agent was present in fermentation and that a gas was released during fermentation of wine; and the observations and studies of Thenard, 1777–1857, Gay-Lussac, 1778–1850, Black, 1728, McBride, and Cavendish, 1731–1810.

Black, 1728–99, had shown how to distinguish gases and showed that carbon dioxide is the only gas formed during fermentation. McBride found that all fermentation gases consisted of carbon dioxide, and

[1] Delbrück and Schrohe *(12)*; LaFar *(32)*.

Cavendish found that 57 percent of the gases given off during fermentation of sugar consisted of CO_2 (fixed air).

Spallanzani, 1765, called attention to the fact that boiling water for three quarters of an hour destroyed the infusoria present.

3. Chemical Theories of Fermentation

Lavoisier, 1743–94, applied himself to the problem of fermentation. In 1789 he stated that during vinous fermentation, alcohol and carbon dioxide are formed. He undertook the first modern quantitative study of the products of alcoholic fermentation. His results were not very accurate from our point of view, but he correctly pointed out the important fact that alcohol, CO_2, and acid are produced; 95.9 parts of sugar break down into alcohol, 57.7 percent, plus CO_2, 35.3 percent, and what he believed to be acetic acid, 2.5 percent; a total of 95.5 parts, without an addition of matter from extraneous sources. The agent causing fermentation does not contribute any material.

Gay-Lussac, about 1815, formulated the equation of fermentation as follows: cane sugar $C_{12}H_{24}O_{12} = 4\ C_2H_6O + 4\ CO_2$, or 45 parts glucose yield 23 parts of alcohol and 22 parts of CO_2.

Dumas, 1800–1884, and Boullay corrected Gay-Lussac's formula for cane sugar and showed that his formula applied to glucose. Gay-Lussac analyzed Appert's sealed foods and found no oxygen, so he concluded O_2 was necessary for fermentation.

The correct formula for the fermentation of sugar by yeast was developed by Pasteur, (*51*). He showed that 100 grams of cane sugar yielded 105.4 parts invert sugar. This sugar is decomposed into the following: 51.1 grams alcohol and 49.4 grams CO_2. In addition 0.7 grams succinic acid, 3.2 grams glycerol, and other substances, possibly yeast tissue, 1 gram, were produced; total 105.4 parts.

When sugar solution is fermented without aeration, more alcohol is obtained than in the aerated solution. The rate of CO_2 production is higher in a fermenting solution than in an aerated solution where respiration is taking place. In respiration practically the only waste product is CO_2. Consider the equation:

180 grams glucose $\rightarrow 2\ C_2H_5OH$ (92 grams) $+ 2\ CO_2$ (88 grams)
92 grams will be transformed to yeast substance, protein, carbohydrates, fats, et cetera; that is, 48 parts of sugar carbon. The average carbon content of yeast is about 47 percent, so, theoretically, 48 parts carbon from 180 grams glucose will form 102.1 parts of yeast dry matter. This is a yield of 189 grams fresh yeast of 30 percent dry matter. This yield is achieved in practice today.

4. The Vitalistic Theory of Fermentation

Blondeau in 1846 stated that every type of fermentation has its inception in a specific organism. However, this idea as well as that of Schwann, 1837, was not readily accepted. Schwann observed that the fermentation rate increased as the number of globules in the suspension

increased. Quevenne in 1838 stated that yeast is not analogous to a chemical compound but is an organized body of a new character.

In 1854 Schroeder and van Dusch performed a critical and important experiment. Following the procedure of Schulze they were successful in preserving malt wort by boiling, but not eggs, milk, and meat. Filtered air was drawn into the flasks. They concluded that an organic substance is broken down by two processes: one, decomposition requires oxygen for its activation, whereas the other does not. They found that milk and eggs could be preserved if heated above 100°C. Pasteur explained this difficulty during the course of his experimental work in disproving the theory of spontaneous generation advanced by Needham, 1745, and later supported by Bastian, and by Joly and Pouchet about 1865. Van Broek in 1859 stated that fermentation of grape juice is caused by a vegetative cell, the yeast cell, and during fermentation yeast cells are developed.

Mitscherlich, 1794–1863, made an exact analysis of yeast. He discovered the inverting enzyme activity of yeast and distinguished between top and bottom yeast. Fermentation is produced by a vegetable organism which he explained on the basis of the catalysis theory of Berzelius, 1779–1848. He repeated the experiments of Schroeder and von Dusch, and that of Helmholtz, and found that fermentation did take place on the side of the membrane where yeast is present, but not on the other side.

Pasteur (*50, 51*), 1850, showed that yeast could be grown on a synthetic medium to which the ash of yeast was added. He was careful to point out that in the presence of organic matter, growth is accelerated. His studies on aerobic and anaerobic fermentation indicated that aeration might be useful in growing yeast. The studies on the diseases of beer had a great influence in promoting the study of pure cultures of microorganisms.

The work of DeBary, about 1850–60, Brefeld, 1860–70, Hansen, 1881, and finally the researches of Koch, 1885, brought about modern techniques in the development of pure cultures. However, these discoveries were long in being put into practice. Aeration was practiced by Marquardt in 1879. The Garunge Institute, Berlin, about 1900, instituted the plan of providing pure cultures for the use of the yeast and spirit industries of Germany. The study of yeast nutrition was carried on by a number of prominent researchers in France, England, Holland, Germany, and the Scandinavian countries.

Liebig stated that he was unable to grow yeast of the type he employed in a synthetic solution. Wildier (*62*) and Ide, 1901, reported that it was necessary to add some organic material, such as yeast extract, to promote yeast growth in synthetic solutions.

This is not the time to discuss the long and bitter struggle that took place concerning the relation of the yeast cell to alcoholic fermentation. Pasteur boldly declared, "No fermentation without life," and this set

the stage for a dramatic discussion. Liebig and Wohler, who had synthesized urea in 1828 and discovered the enzyme emulsin, had scornfully criticized the biological concepts of Schwann and maintained that the yeast cell contained a ferment which by its activity breaks down the sugar.

Later Traube, 1858, in trying to reconcile the views of Pasteur and Liebig enunciated his enzyme theory. He held that enzymes (ferments) are responsible for the changes produced during fermentation. The enzymes are produced by living matter; in this case, the yeast cell. Naegeli and Claude Bernard tried to reconcile the theories of Liebig and Pasteur. They did not fully accept the vitalistic theory.

Many years later, 1896, Buchner and Meisenheimer demonstrated that enzymes could be separated from the yeast cells, and in the absence of living cells, they were able to ferment sugar to alcohol and CO_2.

5. Carbohydrate Metabolism

Carbohydrates serve as sources of energy and supply tissue-building material to the cell for growth and reproduction. To understand the steps that take place during these metabolic processes we should know the chemical nature of the energy sources and the transformation that takes place when the cells utilize carbohydrates.

The work on carbohydrate metabolism of yeast began with early observations on the character of the gas formed during fermentation, and later, on the relation of the alcohol produced to the amount of gas liberated during the reaction. In the first section of this paper some of the significant contributions to the knowledge of the nutrition of yeast were pointed out, and thus it will not be necessary to consider the older studies at this time. Lavoisier and Gay-Lussac made the first serious studies of the chemical reactions taking place during fermentation. The observation of Schwann, Cagniard de la Tour, Kützing, and Pasteur stimulated the intensified attacks on the problem of fermentation. The discoveries of Buchner indicating that the fermentation enzymes could be separated from the yeast cells opened a new era in the field of fermentation and biochemistry.

This work was followed by the important contribution of Harden and of Harden and Young (*23*, *24*), 1906, who showed that fermentation of sugar involves phosphate and sugar reactions in which sugar combines with phosphates (phosphorylation), enzymes react with the phosphorylated sugars, and the final reaction products are alcohol and CO_2.

Emil Fischer had opened up vast areas of protein and carbohydrate chemistry, thus preparing the field for the biochemical investigations that enriched science during the last 55 years. The basic sciences of chemistry, physics, and physiology were rapidly developing, and the tools for sound and critical studies were being made available.

Among those who advanced the knowledge of carbohydrate utilization by microorganisms following the early work of Pasteur are: Lebe-

dev. Nägeli, Loew, Bokorny, Boseken, Waterman, E. Laurent, O'Sullivan, Dumas (*14*), Buchner, Harden, (*23*), Harden and Young (*23, 24*), Stern, Neuberg (*45*), A. J. Brown, Iwanowski (*28*), G. Harker, Meyerhoff, Nathan, Fuchs, Von Euler (*16*), Nauman (*44*), Emden, Warburg, Christian, Gunsalus (*22*), Umbreit, Wehmer, Lipman (*37, 38*), Cori and Cori, Lohman, Krebs (*31*), Wood, Snell, Green, du Vigneaud, Elvehjem, Roger Williams (*65*), Kluyver, E. B. Fred, Peterson, Blanchard, Ochoa (*47*), Doudoroff, Barton-Wright (*4*), Keilin, Horecker, Van Niel, Lampen, H. K. Ling, M. Gipps, Werkman, Nord, Negalein, Wieland, Raistrick, F. Bachman, Lash Miller, Shive, Eakin, Fernbach, Langlykke, Foster (*17*), Herrick, May Karow, Lundsgaard, and M. Laufer. The contributions of Carl Neuberg, Warburg, and Otto Meyerhoff should be specifically mentioned because of their fundamental nature.

The use of tagged atoms has been an important tool in advancing the knowledge of carbohydrate metabolism. Lipman's (*37, 38*) work on coenzyme A has greatly extended our understanding of the interrelationships of the intermediate products of carbohydrate metabolism and fatty acid metabolism. The relationship between the intermediates of carbohydrate metabolism and the citric acid cycle as advanced by Krebs (*30, 31*) has given new insight into yeast metabolism and unified our knowledge of this field.

Cane and beet molasses, which consist of cane and invert sugar, and maltose and dextrose, produced by conversion of starch by enzymes, constitute the main sources of carbohydrates for growing yeast.

6. Nitrogen Metabolism[2]

The study of nitrogen metabolism occupied a prominent role in the investigations on yeast. Pasteur (*51*) in 1874 concerned himself with the question of the growth of the yeast cell in mineral solutions. He pointed out that ammonium salts could be used as sources of nitrogen for yeast growth. Duclaux's work confirmed the investigations of Pasteur. Pasteur showed that inorganic nitrogen was not as good as organic nitrogen for yeast growth. The investigations of Adolph Mayer (*41*) in 1871, of Naegeli (*43*) in 1879, and Kossowicz substantiated the work of Pasteur on the utilization of ammonium salts by yeast. Kossowicz also showed that yeast would not grow normally in synthetic solutions if small seedings were employed.

In 1901 Wildiers (*62*) endeavored to grow yeast in mineral solutions with sugar as a source of carbohydrate. He was unsuccessful unless he used large seedings of yeast or added organic matter such as peptones or organic extracts such as malt extract. Several amino acids and salts such as asparagine failed to produce normal growth. He postulated that a factor which he called "bios" was necessary to promote growth. This factor was not a protein or an amino acid.

Pringsheim (*53*) did not accept the theory of Wildiers. He stated

[2] Kirby (*29*).

that normal growth was obtained if one employed nitrogenous compounds having the RNHCHCO-structure. He also believed the yeast required time to adjust itself to ammonium salts. Pringsheim's work delayed the development of the bios problem because he attempted to eliminate bios as a specific factor in yeast growth.

Nitrogen metabolism was extensively studied by a large number of investigators. Some of the important ones are: Pasteur (*50, 51, 52*), M. Hayduck, (*25*), Raulin, Thomas (*57*), H. T. Brown, Stern, G. Leuchs (*35*), Bioloblocki, Hopkins, Boeseken, Ide, Waterman (*61*), Nauman (*44*), Pringsheim (*53*), Bokorny (*7*), Bertrand, Adolph Mayer (*41*), Ehrlich (*15*), Henneberg (*26*), Elion, Naegeli (*43*), Schulze, F. Hayduck (*25*), Nessler, Deslov, Stickland, (*56*), Muller-Thurgau, Lange (*33*), Lindner, Slater, Laurent, Rasler, Kossowicz, A. J. Brown, Rubner, Delbrück (*12*), Duclaux (*13a*), Iwanowski (*28*), Von Euler (*16*), R. S. W. Thorne (*58, 59*), Barton-Wright (*5, 6*), Hartelius, Northrup, Linderstrom-Lang (*36*), Fruton (*20*), and Christensen (*9*). The work of these investigators has given us considerable insight into biosynthesis and the nitrogen metabolism of the yeast cell. A number of important questions arose during the development of these studies.

The importance of aeration was not fully appreciated. Consequently, some of the early experimental work cannot be accepted today. Another problem involved the metabolism of amino acids. Are amino acids taken up as such during growth? Are they decarboxylated and deaminated according to the theory of Ehrlich? Is organic nitrogen necessary for growth, or can the organism use ammonia nitrogen as a sole source of nitrogen? Nitrate nitrogen was found to be unavailable.

The influence of ammonium salts on the rate of fermentation was not fully appreciated until the work of Kohman and Hoffman appeared about 1914. The complex physiology of growth and fermentation was a challenge to generations of investigators. Years of research were required to develop comprehensive and satisfactory generalizations on the data covering the early observations.

Duclaux and O'Sullivan pointed out that more rapid fermentation occurred with the addition of Pasteur's mineral nutrients. A lack of clear appreciation of the difference between the metabolic activity of fermentation and that of growth and respiration may have been responsible for the delay in the application of these observations to industrial processes. Some application of the use of ammonia was made in the fermentation of wine. The addition of mineral salts including ammonia seemed to improve the fermentation rate and gave better attenuation. The situation was further complicated by the presence or absence of bios factors. However, the effects of B_1, inositol, biotin, pantothenic acid, and B_6 on fermentation and growth were not clearly understood before 1935. Until the essential factors had been isolated and their functions and interrelationships established, progress was slow, and conclusions and generalizations were of uncertain validity.

The use of isotopic nitrogen and enzymic digested as well as acid hydrolyzed protein has greatly advanced our knowledge of protein formation and metabolism. Progress has been rapid since the new procedures involving isotopes became available.

The greater part of the yeast produced commercially in the United States and Canada at present is grown on molasses. Ammonia and phosphoric acid or salts of these compounds are employed. Very little of the ordinary nitrogenous material present in molasses is utilized by the yeast in the production of commercial bakers' yeast.

7. The Role of Bios Factors in Yeast Nutrition

In the preceding discussion on nitrogen compounds it was pointed out that Wildier (*62*), 1901, had found that yeast did not grow readily in a sugar, yeast ash medium using ammonium tartrate as a source of nitrogen. Some growth was obtained if a large inoculum was employed, but with a small inoculum no growth was observed. Growth was obtained when a sterile water extract of yeast was added. As mentioned earlier, he postulated that a bios factor found in yeast extract and malt extracts is necessary for growth.

Amand stated that failure by Wildiers to obtain growth in synthetic media by a small inoculum was not due to the medium which is nontoxic.

Freda Bachman, 1919, and Roger Williams (*64*), 1919, employed synthetic media in experimental work on yeast. Bachman noticed that an increased rate of fermentation was obtained when substances known to be rich in water-soluble B were added to a synthetic medium. Williams observed that yeast would not grow in the synthetic media he employed when seedings of 1 to 3 cells were used. Substances rich in water-soluble B produced an increased rate of growth.

In 1924 Euler and Swartz (*16a*) stated that yeast contained a fermentation factor. About 1920, Lash Miller and his students began working on the nature of the bios factors. They isolated inositol, and concentrates of crude biotin, II A and II B were made. These proved to be growth factors for the yeast they employed. A factor which functions as II A was identified by Roger Williams, 1929, as pantothenic acid, and II B was found by Kögl and Tönnis to be the substance known today as biotin. In 1942 du Vigneaud determined its structure. Roger Williams, in 1931, also isolated a growth factor, beta-alanine, which functions as factor II A for some strains of yeast.

In 1939, Schultz, Atkin, and Frey (*19*), and also Roger Williams, found pyridoxine to be a growth factor for yeast. It has a fairly definite influence on the fermentation rate of certain strains of yeast. Schultz, Atkin, and Frey (2–3–19), also found that nicotinic acid has a slight effect on the fermentation rate.

Roger Williams (*65*), 1930, showed that crystalline vitamin B_1 was a growth factor for strains of yeast he employed.

Schultz, Atkin, and Frey (*54*) pointed out that vitamin B_1 is a fermentation factor for a number of strains of yeast. The pyrimidine, component of vitamin B_1, was also found to serve as a fermentation factor and growth factor for yeast. Under proper conditions yeast can synthesize thiamin from thizole and pyrimidin.

It is evident from this brief discussion that the bios-factor which Wildiers postulated is not a single substance. A number of factors have been identified. Yeast strains vary in respect to the bios factors required for growth. Some yeasts do not require B_1 for growth; others do not require inositol. Consequently, each strain must be grown on synthetic media in order to determine its nutritive requirements.

Yeast grown under good nutritive conditions and with proper aeration and pH will have a maximum fermentation rate, and the yield of yeast, based on the nutrients employed, will be economic. Full utilization of tthe nutrients will be obtained. Our present concern is to obtain more knowledge in regard to the relation of vitamins to the enzyme systems of the cell and their intermediates, means of increasing enzyme content and stability, the nature of the enzyme systems, and the types of nitrogen and mineral compounds most suitable for yeast growth and fermentation activity.

Gunsalus (*22*) has recently shown that lipoic acid and diphosphothiamine function in the oxidation of pyruvic acid. Lipmann (*37*) found pantothenic acid is a constituent of coenzyme A. Snell showed that thioethanolamine is combined with pantothenic acid in coenzyme A. Lynen found that the acetyl group combines with the sulfhydryl to form the thioester, the active group. Nicotinic acid is a constituent of coenzymes I and II. Vitamin B_1 is a constituent of the phosphothiamine. These are examples of the important functions vitamin compounds have in the metabolism of the yeast cell.

The literature covering these studies is vast and impressive, but unfortunately, discussion of this work in this paper will have to be limited.

In the preparation of active dry yeast a number of problems are still unsolved. More information on the vitamin requirements of yeast is needed. Perhaps if we had more knowledge concerning the enzyme systems of yeast, we would be able to produce an active dry yeast of greater stability, at lower cost, and with a higher fermentation rate than that of any yeast we are producing at present.

Better procedures and better equipment for dehydration of yeast to avoid enzyme destruction are subjects for further investigation. The geneticist and the student of metabolism will also find the enzyme field a fertile one for further exploration in respect to producing better commercial strains of yeast. The study of nitrogen compounds suitable for yeast growth, the nitrogen requirements of the cell at various stages of its activity, and the composition of the cell, that is, the glycogen, trehalose, protein, nucleric acid content, et cetera, when the cell

is to be dehydrated and following dehydration, are areas that need intensive study. The bios factors are essential in mineral, carbohydrate, and nitrogen metabolism, and they must be present in suitable amounts to develop maximum enzyme activity in order that the dehydrated product will have activity equivalent to that of the original yeast.

8. Nucleic Acid

Nucleic acid is one of the important components of the yeast cell. It comprises approximately 4 to 8 percent of the weight of the active dry yeast, the percentage varying with the strain and the mode of growth. There are two different types of nucleic acid present in the yeast cell, known as deoxyribonucleic acid and ribonucleic acid. They exist in the cell combined with a protein. The studies on the structure, formation, function, and molecular weight of these constituents, Brown (*8*), Christman (*10*), Allen (*1*), are very definitely expanding, and great advances have been made in recent years. Unfortunately, the study of the chemistry of nucleic acid has been, until recently, somewhat neglected.

Nucleoproteins are present in the nucleus and in the cytoplasm of the cell. The nucleus, or nuclear material, in yeast contains large amounts of nucleoprotein. The chromosomes, which carry the hereditary factors, are composed largely of chromatin, a protamin combined with nucleic acid. The reactions and characteristics of this material are not fully understood, but it is one of the important constituents of the cell.

Adenine had appeared in a number of roles not previously identified. Adeninethiomethylriboside has been found in yeast as a reservoir of sulfur and methyl groups.

Many important enzymes which function in the growth and reproductive processes of the yeast cell contain components found in nucleic acid. A number may be mentioned to indicate the character and important function these components play in the metabolic processes of the cell.

Adenosine triphosphate ATF
Adenosine diphosphate ADP
Adenylic acid
Coenzyme II triphosphopyridine nucleotide TPN
Coenzyme I diphosphopyridine nucleotide DPN
Coenzyme A (contains pantothenic acid and adenine)
Cytrochrome C reductase

Adenosine combined with three molecules of phosporic acid is known as ATP. It is a phosphorylating agent. The decomposition of ATP yields energy. Coenzyme I DPN, a hydrogen acceptor, consists of the following: nicotinic acid amide, ribose, phosphoric acid, phosphoric acid, ribose, adenine. Coenzyme II TPN has three phosphoric acid groups. Both coenzymes are the prosthetic groups of specific conjugated proteins and when combined, perform their functions in carbohydrate metabolism.

Coenzyme A as S-acetyl CoA is involved in fatty acid metabolism in the active yeast cell. Coenzyme A contains adenine, pantothenic acid, phosphoric acid, ribose, and a sulfur compound, mercaptoethylamine.

The flavo proteins are hydrogen acceptors. The dinucleotide (prosthetic group) consists of riboflavin, phosphoric acid, ribose, and adenine. It is combined with a specific protein, an albumin sensitive to heat. Riboflavin phosphate (alloxazine-ribityl-phosphate) is the prosthetic group of cytochrome C-reductase.

In the yeast cell during dehydration the nucleic acid metabolic system may be disturbed by unfavorable pH or by temperatures that cause autolytic changes. A short period of autolysis is sufficient to destroy all the nucleic acid; at least it can no longer be removed as the tetranucleotide.

It is not possible to find data that give information on the amount of nucleoprotein that should be present to give maximum fermentation rate and stability to active dry yeast. The degree of autolysis which is sufficient to affect the fermentative activity and stability of the cell is not known.

The geneticist has a vast field for investigation. It may be possible to alter the structure of the nucleic acid in chromatin or to induce variations by irradiation, introduction of virus, nucleic acid or nucleotides, or by use of isotopes. This field should be diligently explored.

9. Enzyme Studies

Enzyme studies on yeast really began with the work of Mitscherlich, 1794–1863, who discovered the enzyme, invertase. The most startling discovery was that of Buchner, 1896, who separated yeast zymase from the yeast cell. In the absence of living cells he was able to ferment sugar to alcohol and CO_2. This work was succeeded by that of Harden and Young (*23, 24*).

They found that it is necessary for sugar and phosphate to combine (phosphorylation) in order that fermentation may take place. The researches of Euler (*16*), Meyerhoff, Warburg, Keilin, Quastel, Lohmann, Neuberg (*45, 46*) Lipmann (*37, 38*), F. Ehrlich (*15*), Thunberg, Wieland, Butkewitsch, Elvehjem, du Vigneaud, Roger Williams, Green, Ochoa (*47*), Gunsalus, Snell, Shive, Umbreit, Fred, Peterson Robison, Krebs (*30, 31*), Parnas, Lindegren, Novelli, Cheldelin, Horecker, Spiegelman, and others have extended the researches on metabolic enzymes. Their work led to outstanding progress in this field.

The use of isotopes, or tagged atoms, has hastened progress on enzyme studies and is largely responsible for the great progress that has been achieved during the last decade. One is amazed by the accomplishments and by the accuracy obtained by investigators who worked without these important techniques which the newer knowledge has given us.

10. Sterols

Sterols occupy a poorly defined role in the metabolism of the yeast

cell, but they have some importance in the economy of the yeast industry. Ergosterol is the precursor of vitamin D. During growth the amount of sterols present in the cell is limited. However, under certain cultural conditions the amount of total sterols can be increased many fold Whether the increase in sterol content is related to respiratory activity is not clear. Coenzyme A activity may be involved.

Progress is being made in our understanding of the biosynthesis of the sterol ring. The role of acetate as a building unit for sterol production in which coenzyme A is involved is being clarified.

The relation of sterol content to the activity and stability of active dry yeast has not been definitely developed, and there is apparently no published literature covering this subject.

11. Lipides

The role of lipides in the dehydration of active dry yeast and their function in the stability and activity of the dehydrated yeast are not well defined. Phospholipides, such as lecithin, are present in yeast cells during all stages of growth, and are also present in the dehydrated yeast. Studies on the functions of lipides in various stages of production, storage, and industrial use of active dry yeast are indicated.

12. Fats[3]

Recent studies support the view that acetic acid is one of the fundamental units for fat and cholesterol synthesis. Little is known of the metabolism of fat in the fermenting yeast cell. At maturity the cell contains a relatively small amount of fat. Cells that have aged or autolyzed contain large amounts of fat. Degradation of fats may proceed by the removal of two carbons from the chain (F. Lynen. Lipide metabolism. *Ann. Rev. Biochem. 24,* 653, 1955), but whether synthesis of long chain fatty acids proceeds by reversal of the oxidative pathway is a matter still under investigation. DPN, ATP, and acetyl CoA collaborate in the metabolic reactions. The relation of fat content and fat metabolism to the stability and fermentative activity of active dry yeast has not been adequately investigated.

13. Genetics

It may be said that Hansen had a significant place in laying the basis for our knowledge of yeast strains by his single-cell pure culture technique. As the science of genetics developed, a number of investigators, Harper, Strasburger, Dangeard, Guilliermond (*21*), and others attempted to study the sexual stages in the reproduction of fungi.

Winge maintains that the genetic behavior of yeast is controlled by normal Mendelism. Lindegren and Lindegren have reported formation of diploid ascospores, and tetraploid strains have also been reported. Subromaninam stressed the necessity of applying orthodox criteria in characterizing cell inclusions.

[3] Stephenson (*55*).

Fowell, British Distillers, Ltd., points out that genetical studies enable yeast manufacturers to maintain strains of uniform yeast having constant quality and uniformity of action. He favors an improved Lindegren technique using mass mating followed by single-cell isolation for breeding strains for industrial use.

Wickerham points out the difficulties inherent in yeast taxonomy. For classification of Hansenula, reliance is placed on the ability of different species to assimilate carbohydrates, on vitamin requirements, and on sporulation characteristics. Many of the older techniques and criteria have been discarded.

Shultz *et al.* have used vitamin requirements as a means of identifying strains. Response to sulfur compounds has also been investigated. Lodder (*39*) and Kreger-van Rij in their detailed monograph have utilized metabolic studies as a basis for characterizing species and strains. These studies should prove helpful to the geneticist.

14. Mineral Metabolism

Pasteur (*51*), M. Hayduck, F. Hayduck (*25*), Raulin, Bertrand, Adolph Mayer (*41*), Ehrlich, Clerfeyt (*11*), Henneberg, Elion, G. Leuchs (*35*), Pringsheim, Iwanoswki (*28*), Laurent, Harden and Paine, Czapek, Naegeli (*43*), Schulze, Kossowicz, A. J. Brown, Rubner, Delbrück (*12*), Maercker, H. T. Brown, Duclaux (*13a*), Nauman (*44*), and others, and more recently, Stadtman, Barker, Schultz *et al.*, and Elvehjem studied the mineral nutrition of yeast. Synthetic solutions were employed. The use of vitamins or bios factors was not well known until 1930, nor was adequate aeration given in the early experiments. Consequently, we cannot accept as final the results obtained by workers prior to 1930 as accurately reflecting the actual requirements of yeast cells growing at maximum rate. The following elements appear to be essential: phosphorous, magnesium, potassium, sodium, iron, sulphur, copper, manganese, zinc, and calcium.

15. Classification of Yeast

Schwann, 1837, published a treatise on beer yeast. He concluded that yeast was a vegetable organism, and communicated with Meyen, a mycologist, concerning his theory of fermentation. Meyen believed the yeast organism to be a fungus. Because of its capacity to ferment sugar, he named it "Saccharomyces," the sugar fungus. The fact that the organism was found in beer resulted in the name of "cerevisiae." Under the species, cerevisiae, are classified many strains of beer, ale, and bakers' yeasts. The recent work of Lodder and Kreger-van Rij (*39*), "The Yeasts. A Taxonomic Study," should be consulted for information concerning the present status of yeast classification. Mrak and Phaff (*42*) and Wickerham have made important contributions. Schultz *et al.*, as mentioned above, have used vitamin requirements as a means of typing yeast.

16. The Development at Mellon Institute

Prior to 1920 no dry yeast of suitable stability and with a fermentation rate comparable to that of fresh yeast on the solids basis had been prepared. True, there were dry yeasts on the market, but they had very little activity and required a long sponge period of at least 15 to 20 hours to bring their activity to the level where good bread and yeast-raised goods could be produced.

There was interest in an active dry yeast. The baking industry was producing at least 50 percent of all bread consumed in the United States, and demands for yeast were good, inasmuch as mechanized equipment and newer methods of breadmaking involved larger percentages of yeast. However, to service a baker and to supply fresh yeast to all parts of the country, the yeast manufacturer had found it necessary to build plants in various parts of the country. Refrigeration had to be provided in industrial centers, and transportation for the yeast to the refrigerator and from the refrigerator to the consumer was expensive.

Mr. Corby of the Corby Yeast Co., later purchased by the Fleischmann Co., believed a dry yeast might provide the answer to many of these problems. The active dry yeast should remain active at ordinary room temperature for 6 months to a year and retain its fermentation rate at practically 90 to 100 percent of the original activity in the fresh state. It should go readily into suspension, have a good odor and color, and it should be possible to market the product in an ordinary nongas-proof container; otherwise, a sealed container should be developed. This was an extensive assignment.

Prior to 1920 a large number of patents for the production of dry yeast had been issued in Europe and America. However, none of the ideas or any combination of the ideas embodied in the patents we tried gave a yeast with a good fermentation rate combined with stability over a period of 6 to 8 months.

A German patent, 257176, issued to P. D. H. Ohlhaver (*48*), in 1913, contained information that was helpful. Ohlhaver found that if he conditioned bakers' yeast in a sugar solution and then dried the yeast in a hot air dryer at 50°F., he obtained a yeast with a longer survival time than when the yeast was dried directly after the usual production. It should be noted that the yeasts produced before World War I were generally grown on grain-malt extracts, or in some cases, grain-malt extracts and beet molasses.

A patent issued to J. Pritzker, 1926, a Swiss, for dry yeast,Florylin, of 8 percent H_2O and 6.3 percent ash, having one-third the fermenting strength of fresh yeast, was also helpful.

When we tried the Ohlhaver method on yeast grown in grain extracts or in molasses solutions with nitrogen, as $(NH_4)_2SO_4$, and acid phosphate as source of phosphorus, our results were very erratic. However, it was of interest that at times fairly good results were obtained; that is, the yeast might retain 40 to 50 percent of its activity for several

months. Consequently, it was necessary to investigate the reason for this behavior.

It will not be possible to describe the vast number of experiments that were tried before more insight was obtained in respect to the factors involved, and the proper perspective as to the balancing and activating of the various factors was achieved. An unlimited number of combinations can be visualized, for the nitrogen nutrients alone offer a tremendous number of complex combinations with the other known and established nutrients, to say nothing of the unknown factors, such as bios factors, trace elements, et cetera.

We were convinced that it was necessary to investigate the following:

a. To systematically study yeast nutrition, preferably in synthetic media. This was difficult, as we learned later, because of our limited knowledge of bios factors (vitamins).

b. To find the proper strain of yeast or to develop one.

c. To find whether or not respiratory activity and growth should be followed by a period of fermentation. What temperature and time should be given to each stage.

d. To determine whether or not fermentation should be omitted.

e. To determine what degree of aeration should be given.

f. Should there be a ripening period of the yeast and gradual withdrawing of nutrients followed by a fermentation period?

g. What nitrogen content should yeast have during growth? during the final stage? during dehydration? What source of nitrogen is most satisfactory?

h. What carbohydrate content should the yeast have during growth and during final growth and dehydration?

i. What sources of carbon are best suited to produce growth and a good dry yeast?

j. What minerals are necessary and at what levels should they be added? What levels should exist in the finished yeast?

k. What pH should the yeast have at all stages? What acids should be present?

l. What glycogen, fat, gum, trehalose, and cellulosic content should the yeast have at various stages, especially after dehydration?

m. What vitamins or bios factors are essential in each stage of development?

n. What degree of maturity should the cells have to dehydrate well and to resist aging?

o. Should the yeast be frozen and dehydrated, or cooled to 35 or 40 degrees and then dehydrated? or dehydrated at the temperature it leaves the fermenter? What time period should be given for dehydration? At what humidity should the yeast be dried? Many other factors might be mentioned, but these are sufficient to illustrate the complexity of the problem. Needless to say, not all of these factors and their proper combination are known today.

The dehydration of yeast offered many difficult problems, many of which are still subjects for research. A number may be mentioned.

a. What is the proper temperature and humidity of the atmosphere for dehydration?

b. What is the minimum time for dehydration?

c. Should a filler be employed? an antioxidant? or a coating, such as a hard fat?

d. Should the yeast be granulated or in pellet form? What size granules and pellets should be made? How rapidly should they disperse in water?

e. What is the optimum moisture content for the dehydrated yeast?

f. What is the proper storage temperature? how low can the temperature fall? how long an exposure can one have at low temperature and how high can the temperature rise? How long an exposure can the yeast endure at each temperature level where activity is still normal?

g. Should the yeast be packed in carbon dioxide, nitrogen, vacuum, or air? What moisture content should be present in each case?

h. What tests can be employed to establish the activity of yeast in various doughs?

Many other factors could be mentioned, but these factors were of pressing and compelling importance and required investigation. A number of laboratories were engaged in this work: the University of Wisconsin, Anheuser Busch, Red Star, Northwestern Yeast Co., National Yeast, Federal Yeast, DCL in England, Ward Baking Co., and Standard Brands, Inc.

The first successful yeast was prepared at the Mellon Institute in 1920. It had been found that the bakers' yeast usually prepared for the market had 50 to 53 percent protein. It had poor keeping quality and autolyzed readily. This yeast, Corby yeast, could be conditioned in a 5 percent sugar solution to which limited amounts of NH_4 tartrate and secondary calcium phosphates, $MgSO_4$ and K_2SO_4, were added. Aeration was about 30 to 70 percent of that required for maximum yeast growth. To 5 liters solution, 300 grams of yeast were added. The pH was maintained at about 4.5 to 6.0. Neutralization was accomplished by means of $NaHCO_3$.

The yeast was removed at the end of 6 to 8 hours when the sugar had been used and the cells fully matured. The protein content was 38 to 44 percent, and the glycogen content, 5 to 6 percent. The yeast was centrifuged, pressed, and frozen at a temperature of about 0° to 10° F. After freezing it was broken up and mixed with two-thirds of its weight of corn flour and dried at a temperature of 40° to 70°F. (yeast temperature) over a period of 18 to 22 hours. The air temperature was not over 80°F. The moisture of the drying chamber was determined by taking relative humidity readings.

The yeast was dried to 7 to 8 percent H_2O and sometimes as high as 9 to 10 percent. Lower levels were also tried. It was tray-dried and

granulated by passing it between rolls or through a coffee grinder. The granules were placed in glass jars and sealed. Vacuum packing was also tried. The yeast in sealed jars retained its full original activity for 8 to 9 months at room temperature.

17. Development of Baking Tests

Storage of yeast at 86°F. and in the ice box at 25° to 30°F. in sealed Mason Ball jars was the procedure usually practiced. Baking tests, gas tests, and gas tests in doughs were made at intervals of one month.

The following baking test was employed:

Flour	300.0 grams	Volume of dough	1300 cc
Yeast	5.0 grams on fresh basis	Dough rise	3′ 45″
Water	200.0 cc	Proof to top of pan	
Salt	5.1 grams	Volume of loaf	2100 cc
Sugar	12.0 grams	Range	1900 to 2200 cc
Shortening	5.0 grams	Pan size,	
		top inside	7⅝ x 4½ in.
		bottom inside	6⅝ x 3⅝ in.
		depth	5½ in.

The use of adjuvants to aid in stabilization of the dehydrating yeast was tried experimentally for many years. Salts such as $CaSO_4$, $ZnSO_4$, $MgSO_4$, NH_4Cl, NH_4 tartrate, mineral oil, $CaHPO_4$, K_2SO_4, NaCl, $CaCl_2$, $Ca_3(PO_4)_2$, wax, and hardened fat did not always prove effective.

18. Conditioning Yeast in Grain Extracts and Molasses Solutions Using Organic Nitrogen and Organic Phosphates

We began our experimental studies by growing and conditioning yeast in glucose or cane sugar with salts. Usually an extract of malt or sprouts or rice polish was added in order to promote growth and maturing of cells. A study on bios requirements was initiated which was carried on for 25 years. During this period six bios factors were found to be of interest in connection with growth and fermentation of bakers' yeast. The bios requirements of different species and strains vary considerably.

When we had achieved the production of a satisfactory yeast, the problem was to find more economical nutrients. Grain extracts in combination with $(NH_4)_2SO_4$, urea, or NH_4 tartrate were found to offer possibilities. Mr. Shaver, Dr. Schultz, Mr. Kirby, and I prepared some excellent yeast using these nutrients. We were able to control protein, gum, glycogen, phosphates, and sulphur content, thereby producing a stable yeast which dehydrated well, retained activity 80 to 100 percent of original value for many months, had good color, and a good pH range. This type of yeast was high priced for it required more expensive materials than yeast grown on molasses and ammonium salts. The molasses yeast usually had a higher initial activity. It seemed desirable to study the utilization of molasses.

Molasses must be made filterable and it must be clarified. We found

that it had mineral and bios deficiencies. Some molasses could not be filtered or clarified readily. Sulfur dioxide, zinc salts, and toxic products produced by overheating molasses were often present. Yeast in the presence of certain types of molasses is not able to utilize urea.

Several methods of clarification were employed. Phosphate clarification, using $NaHCO_3$ and $CaH_4(PO_4)_2$, was successful. $(NH_4)H_2PO_4$ + $NaHCO_3$ was used widely. The use of silica gel and sodium bicarbonate was also a successful combination and produced a well clarified wort. Centrifuging was also satisfactory when combined with chemical treatment.

The use of inorganic nitrogen such as $(NH_4)_2SO_4$ presented a serious problem. Fermenters were made of copper and as the acidity rose, the copper went into solution and hindered yeast growth and discolored the yeast. Mr. Shaver, Dr. Schultz, Mr. Kirby, and I were interested in the problem of using molasses and mineral salts. After years of experimental work, methods of controlling growth and pH by use of NH_3 were achieved. Control of nitrogen content of yeast as well as its mineral and glycogen content were established. The maturity of the cells was regulated. The yeast was fully as stable as yeast grown on grain extracts, its initial activity excellent, and its color and odor were very good and nearly equal to that of yeast grown and conditioned on synthetic media or grain extracts.

Research by Schultz and Light has been devoted to reducing the conditioning period where growth is restricted and the ammonia reduced. The cells must be fully matured before dehydrating. Efforts were made to grow the yeast fully conditioned when its growth cycle is completed.

19. Dehydration

Two types of yeast were produced, the granular and the pellet type. In the former, the yeast may be extruded and dried on a continuous belt in a chamber where air flow, humidity, and temperature are controlled. Every succeeding stage of dehydration varies in temperature and humidity from the preceding stage. The drying time may be approximately 4 hours, but in some cases it may extend to 8 to 10 hours.

In the pellet type the yeast may be extruded into a revolving tunnel dryer. Heated air is passed through the tunnel. The humidity of the air is controlled solely by the temperature of the incoming air and the rate of evaporation. The moisture content is usually about 7 to 8 percent in the finished yeast. The time of drying may vary considerably, but it is seldom less than 12 hours. The temperature and humidity of the incoming air are uncontrolled factors, and thus uniformity of operation is impossible. The only control of humidity is to increase or decrease the temperature of the dryer. With the belt type dehydrator good dehydration can be obtained in a much shorter period and with better control. Spray drying was used experimentally, but uniform stability was not attained. Drum drying at a vacumm of 1 to 3 mm. was also

used experimentally. Freeze drying did not prove to be superior to belt drying and was often erratic.

20. Packaging

The dehydrated active dry yeast may be sifted to remove fines. Controlled production should be able to limit fines to a small percentage. The yeast is placed in metal cans, tinned. Each can holds 2 pounds. The can may be sealed in air, or it may be vacuum packed, or the air may be removed and replaced by nitrogen.

Composition of active dry yeast, pellet, and continuous belt-dried yeast.

Protein	40 to 43 percent	Glycogen	15 to 18 percent
P_2O_5	1.8 to 2.15	Fat	5 to 7 percent
Ash	4 to 5 percent	Water	7.0 to 8.5

Commercial use of active dry yeast

The standardization of the yeast was accomplished by means of punch tests using the following formula:

300 grams flour high protein—mixed in Fleischmann mixer
5 grams salt
15 grams sugar
10 grams shortening
Water—62 percent or as required
Volume of dough—1180 cc
Temperature—30°C.
R. H. 90 to 100 percent
Three punches—95", 55", 56", approximately
Battery jar for sponge rise

21. Baking Tests

A similar formula was used for the baking test. The dough is allowed to rise to 1180 cc. It is then rounded and given a rise of 10", molded, and put in the pan and proofed at 40°C. and 100 percent R. H., to top of pan (size given previously), placed in oven at 225°C. and baked for 30 minutes.

Tests were made at definite periods of 1 month to determine the stability of the yeast at room temperature. 70°F. and 40°F. Cyclic tests were also conducted in which temperature and humidity were controlled for 12-hour periods daily at 95°F. and at R. H. of 85 to 90 percent, and 12 hours daily at 75°F. and R. H. 95 to 100 percent.

To determine the stability of the yeast and to maintain a constant type so far as activity and stability are concerned, samples were set aside at 86°F., 40°F., and 0°F. The older samples were compared with the recent ones and after the new samples were tested, they became the new standards by making a composite of a given number of batches. In this way continuity of type was maintained.

22. Commercial Use—Marketing

The dry yeast was marketed in the early nineteen thirties in South

America, and in the forties it began to replace the fresh foil yeast for household use in the United States. The Armed Forces became interested in active dry yeast about 1940–41. Colonel Paul P. Logan took up the problem of studying and introducing the new product, and later Colonel Rohland A. Isker carried on extensive investigations at the Quartermaster Laboratory at Chicago.

23. Military Specifications

Requirements. Yeast must be pure culture of *Saccharomyces cerevisiae* grown on a molasses mash with suitable nutrients; all yeast must conform to the provisions of the Federal Food, Drug, and Cosmetic Act and regulations promulgated thereunder.

Type II. Active dry yeast shall be a granular particulate product. The moisture content shall not exceed 8 percent. The rope spore count shall not exceed 100 per gram.

3.5.1. The active dry yeast shall be of such quality that after it has been held for 18 days at 100°F., the dough made therefrom shall require not more than 8 percent additional time to reach the 1400 cc. mark (on each of the 2 rises) as required by the test procedures specified in 4.3.1 for standard active dry yeast which has been stored for the same time at the same temperature.

3.5.3. The active dry yeast shall be of such quality that after it has been held for 18 days at 100°F., it shall yield a loaf volume which is not less than 92.0 percent of the loaf volume produced by the test procedure specified in 4.3.2 for standard dry yeast which has been stored for the same length of time at the same temperature. In addition, after 18 days at 100°F., the active dry yeast shall yield a loaf of bread of approximately the same quality with respect to grain, texture, crumb color, flavor, and taste as that produced by the standard active dry yeast which has been stored for the same time at the same temperature.

3.7. *Workmanship.* The product shall be prepared, processed, and packaged under modern sanitary conditions and by such methods as will reflect good standards of workmanship and quality in the finished product.

4.3.1.1 gives the preparation of samples for punch and baking tests. Formula:

Ingredients	*Pretreatment*	*Dough*
Active dry yeast	3.30 g.	
Water	50.00 ml.	168.0 ml. (variables)
Sugar	0.75 g.	16.5 g.
Salt	—	6.6 g.
Nonfat dry milk solids	—	6.6 g.
Shortening	—	9.9 g.
Flour	—	330 0 g.

Flour shall be straight grade (or better), milled from hard wheat. It shall have a diastatic activity of not less than 275 nor more than 375 milligrams of maltose.

4.3.1.3. *Pretreatment.* Add active dry yeast to the sugar-water solution at 105° to 110°F. without stirring. Let stand for 5 minutes, then stir. After standing for an additional 10 minutes, stir again until the yeast is dispersed.

4.3.1.4. *Dough mixing.* Combine the dough ingredients and add the pretreated yeast last to avoid the possibility of chilling the yeast. Mix the dough until properly developed. The temperature of the dough after mixing shall be not less than 84° nor more than 86°F. Any test dough shall not differ by more than 0.5°F. from that containing the standard dry yeast.

4.3.1.5. *Fermentation.* Place the dough in a greased glass jar having an inside diameter of about 5 inches and a height of 7 inches. The jar shall be marked with a line indicating a volume of 1400 cc. Place the dough in a cabinet maintained at 86°F. (30°C.) with the humidity adjusted so as to prevent crusting of the dough. Allow it to rise until the edge of the dough in contact with the glass jar reaches the 1400 cc mark. (The cone of the dough is above this mark.) Record the time required. Remove the dough from the jar and expel the gas by placing it in a mixer for a definite time (from 10 to 15 seconds) or by hand manipulation. Replace the dough in the jar and press down to eliminate air pockets and obtain a reasonably level surface. Allow the dough to rise until it reaches the 1400 cc. mark. Record the time required.

4.3.2. *Baking test.* Using the formula specified in 4.3.1.2, preheat the yeast (4.3.1.3) and mix the dough (4.3.1.4) as described in the punch test. Transfer the dough prepared with the standard active dry yeast to the glass jar and allow it to rise until it reaches the 1400 cc. mark. Note the time required. Punch, round up, and allow it to rest 15 minutes at 86°F. Mold, place in pan, and proof at not less than 96° nor more than 104°F. until the dough attains an average height of three-eighths inch above the top of the pan. Note the time required. Bake at not less than 415° nor more than 440°F. Allow the bread to cool for 1 hour and determine the loaf volume in cubic centimeters. Cut and examine the bread the following day for grain, texture, crumb color, flavor, and taste in comparison to the loaf made from the standard active dry yeast.

Pan dimensions	*Inches*
Top inside	9¾ by 4¼
Bottom outside	9 by 3½
Depth	2¾

The dough containing the active dry yeast under test shall then be handled on exactly the same time schedule throughout, disregarding the volume attained by the dough during fermentation and the height attained during the pan-proofing period.

4.3.3. *Rope spore count.* The rope spore count shall be made according to the method of Hoffman, Schweitzer, and Dalby, "Control of Rope in Bread" *Ind. Eng. Chem., 29*, No. 4 (April 1947), p. 464 (6.3).

4.3.4. *Maltose value.* The maltose value of the flour used in the

punch test and baking procedure for active dry yeast shall be determined according to the American Association of Cereal Chemists' method for measuring the diastatic activity of flour (Cereal Laboratory Methods, 5th Edition (1947), p. 100). Pressuremeter value shall be determined according to the American Association of Cereal Chemists' method for measuring gas production in doughs (Cereal Laboratory Methods, 5th Edition (1947) p. 101 (6.2).

4.3.5. *Moisture determination.*

4.3.5.1. *Compressed yeast.* Place approximately 5 grams of compressed yeast in a tared metal dish provided with a cover, and weigh. Add 8 milliliters of 95 percent ethyl alcohol and mix thoroughly with a gentle rotary motion. Evaporate the alcohol on a steam bath. Dry overnight in an air oven at 100° C. Replace the cover, cool in a desiccator, and weigh.

4.3.5.2. *Active dry yeast.* Proceed as with compressed yeast, omitting the addition of alcohol.

4.3.6. Unless otherwise specified, other chemical analyses, when required, shall be made in accordance with the methods of the Association of Official Agricultural Chemists (6.4).

4.3.7. Oxygen shall be determined in accordance with the Orsat method as described in "Standard Methods of Chemical Analysis" (6.5). Mercury shall be used in the measuring burette and leveling bottle. Results shall be reported on a basis of gas being at atmospheric pressure in the container. The pressure may be determined by use of a manometer inserted in the gas analyzer.

24. Packaging

5.1.2.1. Two pounds of active dry yeast shall be packaged in hermetically sealed and open-top style round, metal cans, approximately size 502 by 309, 502 by 310, 502 by 312, or 404 by 700 (depending upon the density of the product). The cans shall have soldered side seams and compound-lined double-seamed ends and shall be made throughout from commercial 0.50 electrolytic tin plate. The cans shall be coated outside in accordance with 5.1.2.3 or 5.1.2.4, as specified (6.1).

25. Summary

The development of the modern concept of fermentation is discussed, and some of the first attempts to manufacture dry yeast are mentioned. The influence of the vitalistic theory of fermentation in stimulating research and the effect of cogent thinking by many investigators concerning the metabolic activities of yeast and the mechanism by which the cell accomplishes the transformation of sugar and other nutrients into cell substance and carbon dioxide are discussed briefly.

Drying yeast in order to retain its quality and fermentation rate has been a long and arduous research. Many scientific and industrial researchers have given years of activity to this study and thus contributed

greatly to the successful fulfillment of this endeavor. Certain strains of yeast can adapt themselves to dehydration more readily than others.

The physiological state of the cell has been found to play a role in determining the stability of the cell during and following dehydration. Some of the factors that have been found to be involved are the type of cell—genetically, its maturity, the nitrogen content of the cell, its glycogen and trehalose content, and the nature of the carbohydrates of the cell and reserve tissue. Mineral nutrition also plays an important part in the life and stability of the cell. The optimum moisture content of dehydrated yeast has not been definitely determined.

The dehydration of yeast must be conducted in such manner that very little impairment of the normal physiological state of the cell takes place. The delicate balance of the complicated enzyme systems must remain essentially unaltered. The cell must function normally in the dough; that is, be free from reducing action and the production of abnormal byproducts. Its ability to reproduce, and to ferment sugar in the dough should be essentially like that of the undehydrated cell from which it is derived.

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Genetic Principles as Tools for the Taxonomic Study of Yeasts

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1. The Genus *Hansenula*

YEASTS, UNLIKE ALL OTHER FUNGI, show chromosomal replication from the haploid number found in primitive species, to diploid and even triploid and tetraploid numbers. As with many of the higher chlorophyllous plants, the degree of ploidy in yeasts is a measure of their evolutionary development. The most primitive species in the genus *Hansenula* are haploid, and the consecutively more recently evolved species produce successively higher ratios of diploid to haploid cells.

In this genus a comprehensive study has been made of the change in morphological characteristics and the increase in intensity of biochemical activity which parallels an increase in ploidy. Let us consider these relationships first in a group of homothallic species, the most primitive of which are exclusively haploid and are found only in association with coniferous trees and bark beetles, while the most highly evolved species of this group are diploid and free-living in soil and streams.

The colonies of the completely haploid species are mucoid and glistening, tending to flow when the plate is placed upright on its edge. The cells are very small. Fermentation is limited to glucose and galactose of those sugars commonly used for identification of yeasts, and the intensity of fermentation is weak. Vitamins are required for growth; neither pellicles nor esters are produced. Sporulation is almost always preceded by fusion of nuclei produced by a cell and by its bud. Neither germinating ascospores nor the vegetative cells they produce conjugate so long as their environment is favorable for vegetative growth.

The homothallic species of intermediate development are found in association with trees or in soil. The colonies are butyrous and glistening, the cells are larger, fermentation intensity is much stronger, pellicles, esters, and all required vitamins are produced by the species of higher development in this intermediate group. Some of the asci they produce consist of two conjugated cells, indicating the vegetative cells producing such asci were haploid, and some asci consist of a single cell, indicating they were produced from diploid vegetative cells. It is believed that the greater the ratio of asci consisting of single cells to those

consisting of two conjugated cells, the more recent is the evolution of the species and the greater its physiological activities. The reason both haploid and diploid cells are found in a single strain is the gradually increasing tendency of each successive species to diploidize by conjugation of vegetative cells even when growing on freshly inoculated, highly nutritious media. Some of the cells conjugate to yield diploid buds, others do not conjugate and thus remain in the haploid state.

This increasing tendency to diploidize is so strong that 98 to 100 percent of the cells of the most recently evolved homothallic species are diploid. They produce mat, usually rugose colonies, the cells are large, they ferment strongly, they require no addition of vitamins to the medium, and they produce thick pellicles and esters.

The comparable heterothallic line of species show exactly the same changes in characteristics as they progressed from primitive haploid species to completely diploid species, but the primitive heterothallic species, consisting of two sexes, could not conjugate simply by the passage of a nucleus from a bud through an already existing opening into a mother cell and there fuse. The nucleus had to pass from one cell into another independent cell of opposite sex. The process of getting a nucleus through two cell walls into a different cell would seem to have many obstacles in yeasts with basically very little impulse to diploidize even long enough to form ascospores. In fact, only in the following exceptional cases has it been accomplished in the laboratory, though perhaps in nature it may occur more frequently.

The most primitive heterothallic haploid species of the genus *Hansenula* is *H. holstii*, which is found in association with coniferous trees and in the gums of some deciduous trees, such as the wild cherry. It produces mucoid colonies, and slowly ferments glucose and rarely galactose. Its characteristics regarding pellicles, esters, and vitamin requirements are likewise those of primitive species. Fifty-five strains were isolated before 2 were found which would conjugate and form spores when mixed on a sporulation medium. Three weeks were required to produce only 2 or 3 percent of ascospores. However, when the ascospores were isolated and cultivated, then mated back to their parents, 1 of the 10 matings produced 30 percent spores. Ascospores of this one fruitful mating were similarly isolated and mated back to their parents and to each other. This time the majority showed abundant mating and many produced 30 to 50 percent ascospores in 12 days of incubation. However, these inbred mating types showed little, if any more, ability to mate with natural isolates of the species than did the original natural isolates from which they were derived.

The next species up the phylogenetic line of heterothallic *Hansenula* species is one we have not yet named. It is found in association with conifers. It produces butyrous colonies, all isolates ferment slowly both glucose and galactose, and about 75 percent of the isolates conjugate

when the opposite sexes are mixed. All of these properties indicate a position on the phylogenetic line somewhat above *H. holstii*.

The manner in which this yeast conjugates is novel indeed. Instead of conjugation occurring exclusively between 2 cells, many cells may be involved. On close examination of small cell groups that occur in naturally spreadout formations, conjugation tubes may be observed connecting several cells, but none of the cells has more than 2 tubes entering it, so far as known. The tubes are so fine that it is doubtful whether nuclei could pass through them. Spores are produced so sparingly that an estimation of 1 spore per 100 billion cells is believed to be conservative for matings of natural isolates. Backcrossing of ascospore isolates to their parents increases the number of cells which conjugate, but probably does not increase the ratio of the number of spores per number of conjugated cells. It does increase the number of spores to vegetative cells to about 1 spore to 14,000 cells in the most fruitful inbred lines.

Fortunately, this species is high enough up the phylogenetic line to produce an infinitesimally small number of diploid cells. Such cells have not been isolated from nature, though it is assumed they occur there to a minute degree, but are quickly returned to the haploid state by sporulation. One diploid was isolated during the course of the experiments on backcrossing. It produces spores abundantly, with about 80 percent of the cells being ascospores when sporulated. Its physiology is identical with that of its parents, and ascospore isolates obtained from it remain exclusively haploid, so far as tested. When mated, these haploid isolates produce many of the multiple conjugations so characteristic for this species, but no more than the inbred isolates of this yeast. The diploid form is readily maintained in the laboratory by lyophilization.

Farther up the phylogenetic line is another heterothallic species, which will be named *Hensenula fabianii* when it is described. It ferments 3 sugars strongly and 1 weakly, produces glistening colonies, makes esters, but requires added vitamins. It exists in nature in the haploid state so far as we know, although we have only five strains of the species. Four strains produce spores when mixed with strains of the opposite sex. The mating types conjugate when placed on sporulation medium. Some of the conjugated cells form ascospores while others produce diploid buds. These diploid cells bud in turn and may be isolated from the culture by streaking it and picking the largest of the resulting colonies. The diploid cultures sporulate both on sporulation and vegetation medium but the ascospores and the haploid cells that result from them have little tendency to conjugate on vegetation medium although they have a strong tendency to conjugate on sporulation medium. Thus, a diploid culture when serially transferred at approximately 10-day intervals on vegetation medium returns to the

haploid state, though by similar serial transfer on sporulation medium it remains largely in the diploid state.

Above *H. fabianii* the tendency to diploidize is so strong on all media that the remaining species in this line are isolated from nature almost exclusively as diploid yeasts. After killing the vegetative cells in a sporulated culture of any one of these species, the haploid ascospore isolates may be cultured. The opposite sexes conjugate and sporulate readily on either sporulation or vegetation media. The three species thus characterized ferment strongly 3 or more sugars, the cells are large and almost completely diploid, and all produce esters. *Hansenula subpelliculosa* requires vitamins, but *H. anomala* and *H. ciferrii* do not. The first produces glistening colonies, the latter 2 produce both mat and glistening colonies. The strains which produce mat colonies form heavy pellicles to assimilate the abundant fermentation products of the cells growing at the bottom of the liquid cultures. The tendency to diploidize is so strong in *H. anomala* that when haploid cultures of a single sex are aged on sporulation medium, very few cells may self-diploidize to form a unisexual diploid. In fact, some of the most strongly sporulating stock strains of this species which we have in our collection have proved to be unisexual diploids.

In conclusion, it may be stated that the most basic trend in the evolution of the genus *Hansenula* is to exist in the vegetative state as diploid cells. Each more recently evolved species in the evolutionary chain possesses this tendency to a greater extent than its progenitor. In the two branches of the genus we have just discussed, this increasing tendency to diploidize is correlated with increasing size of cells; with a change from mucoid, to glistening butyrous, and finally to mat colonies; to ferment more sugars and to ferment them more rapidly; to become independent of the environment as a source of vitamins; to produce pellicles; to synthesize esters and to become free-living rather than remaining dependent upon an association with trees and bark beetles.

It should be noted that in another group of species within the genus *Hansenula* the tendency to become diploid in the vegetative state was coupled with a tendency to lose physiological activities as they became more dependent upon coniferous trees and their associated bark beetles.

2. The Proposed New Genus *Dekkeromyces*

We have noted that both heterothallism and diploidization came in at an early period in the evolution of the genus *Hansenula*. Though it was not discussed, no two species in this genus have been induced to hybridize. Let us consider another genus which as yet has not been described. It will be removed from *Saccharomyces* and will be proposed under the name of *Dekkeromyces*. It is related to *Hansenula*, but shows

interesting differences when its evolutionary accomplishments are compared with those of *Hensenula*.

Only one species of *Dekkeromyces*, now known as *Saccharomyces fragilis*, is entirely diploid. It is believed to be a naturally occurring hybrid, for it can be duplicated by crossing *Zygosaccharomyces ashbyii* with *Saccharomyces lactis*. *Saccharomyces fragilis* is the most recently evolved species in a line of crescent-spored yeasts. Only one species, *Saccharomyces lactis*, is heterothallic, and it too shows the results of hybridization by its wide range of variable characteristics. It is the last species to have been evolved in a line of haploid, round-spored species.

Hybridization of most species is readily effected. Strains representing two species are mixed together on a sporulation slant and the mixture is serially transferred four times at 10-day intervals at 25 C. The parents are so selected that the hybrids may be isolated by physiological properties. One of the characters that is shared to varying degrees by strains of most species of *Dekkeromyces* is the ability to produce a pink pigment closely related to pulcherrimin. When strains of different species are hybridized, the hybrids produce more pigment than the two parents, and on this basis the hybrids may be separated from the parents; or parents may be selected which differ in their ability to assimilate two carbon sources, A and B. The hybrids are produced by serial transfer of the mixed cultures, then they are serially transferred in flasks of media containing carbon source A until the parent incapable of assimilating A has disappeared, but the parent which assimilates A and the hybrids which assimilate A and B are present in large numbers. Then cells from the last flask of medium A are transferred to a flask containing only compound B as a source of carbon. In this series of flask cultures the parent which cannot assimilate carbon source B is eliminated and only the hybrids are left. To use actual species and carbon sources to illustrate this biochemical method of isolating hybrids, one may hybridize *S. fragilis* and *Z. dobzhanskii*. The former assimilates and ferments lactose but has no action on maltose, and the latter assimilates and ferments maltose but has no action on lactose. The hybrids ferment both maltose and lactose. The lactose- and maltose-fermenting hybrids produced by crosses of various appropriate species within this genus are the only ascosporogenous species known to be exceptions to Kluyver's third rule of yeast fermentation. This rule states that a yeast which ferments lactose does not ferment maltose.

It is always of interest to determine how the properties inherited from the two parents will segregate when the hybrid sporulates. The sporulated hybrid is heated just enough to kill the vegetative cells, then the ascospores are isolated by streaking on plates. Some of the resulting colonies are restreaked, and a colony from each plate is selected for study. It is of interest to note in this connection that all hybrids studied thus far, where one parent attacks alpha-glucosides,

specifically maltose, melezitose, alpha-methylglucoside and trehalose, and one attacks lactose, the ascospore isolates show a marked tendency to gradually lose the ability to metabolize alpha-glucosides but retain their ability to metabolize lactose. It should be pointed out, however, that most of the hybrids are stable and are readily maintained in an unaltered state in the laboratory.

3. Conclusion

In conclusion, it may be stated that yeasts of the genera *Hansenula* and of the anticipated new genus *Dekkeromyces*, as well as other genera of sporogenous yeasts, are like some genera of the most highly evolved chlorophyllous plants in that the greater the degree of ploidy of a species, the more recent is its origin. In some genera, as in *Hansenula*, no two species will hybridize, and this is a very important fact for determining the limits of species. Other genera, such as *Dekkeromyces*, hybridize readily, and this is an important criterion for the determination of the limits of such genera.

Conditioning Yeasts for the Production of Enzymes[1]

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1. Introduction

AS MY CONTRIBUTION to the present conference, I was asked to discuss the topic "Conditioning of Yeasts for the Production of Enzymes." In point of fact the best I can do is to provide a preliminary discussion to this rather important practical problem. We cannot at the present time say that our knowledge of the enzyme-forming mechanism is so extensive and detailed that complete control can be exercised so as to yield yeast preparations of prescribed enzyme patterns and levels of activity. Nevertheless, rapid progress has been made in the past decade in delineating factors controlling enzyme synthesis. I shall here attempt to summarize the present status of our understanding of the problem.

Those of us who are interested in yeast as an organism can well be proud of the historically important role that it has played in serving as ideal experimental material in some of the most significant advances of modern biochemistry. To cite but one example, we might note that it was the study of yeast cells that led to the ultimate elucidation of the energy metabolism of muscle. By possessing one peculiar ability, the yeasts also contributed immeasurably in opening up the field I shall be describing today. You are all aware that growth of microorganisms in different cultural media results in populations possessing varied properties and enzymatic activities. This is essentially the phenomenon of "enzymatic adaptation." There were always two alternative biological mechanisms which could be invoked to explain such observations. They may be summarized as follows:

a. Selection of a preexistent mutant type possessing the physiological properties characteristic of the final population.

b. An induced change in the enzyme pattern of all (or nearly all) of the cells initially present due to the presence in the medium of a substance we will refer to as an inducer.

Until one could decide which one of these two mechanisms was operating, it was obvious that the phenomenon of enzymatic adaptation could not be employed with any certainty as a tool for probing

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into the properties of the enzyme-forming mechanism. The fact that yeast cells can adapt under conditions where growth is virtually eliminated provided the experimental situation permitting a resolution of this dilemma. Yeast cells, for example, can be made to adapt to the fermentation of maltose when suspended in a phosphate buffer solution of the substrate and in the absence of an external nitrogen source. This type of experiment precludes immediately the possibility that mechanism 1 is functioning. A change in the physiological properties of the population, which is to be ascribed to the selection of a pre-existent mutant type, necessarily involves extensive multiplication. If the latter is not possible, mechanism 1 is eliminated. It was further possible to show that the change in enzyme activity of the whole cell was indeed referable to the formation of new enzyme molecules. I shall not attempt to detail these researches since they have been thoroughly summarized and discussed in recent reviews (*37*, *51*, *58*) and need not be detailed again here. Certain consequences may perhaps be noted (*48*, *49*). The most important of them was the emergent experimental potentiality. In successfully establishing that enzymatic adaptation did indeed involve the induced synthesis of new enzyme molecules, a demonstration was provided that the synthesis of specific enzymes could be attained under relatively simple and controllable conditions against a constant genetic background. It was evident that a system had been found and defined which converted into experimental reality the possibility of inquiring into the mechanism of enzyme synthesis.

The very nature of the phenomenon virtually dictates the kinds of questions which are initially asked of it. The presence of certain agents called inducers can, in the presence of a suitable energy supply, stimulate the formation of specific enzymes. The use of inducible systems to elucidate the mechanism of enzyme formation resolves itself quite naturally into attempts to provide adequate answers to the following questions.

(1) What is the nature of the precursor material which is transformed into active enzyme molecules?

(2) What is the nature of the enzyme-forming mechanism which converts the precursor material into active enzyme?

(3) What is the role of the inducer, the presence of which specifically stimulates the appearance of the corresponding enzyme?

In the remaining time at our disposal we will turn our attention to an estimation of the extent to which answers can be provided to some of the questions which have been posed.

2. The Nature of the Precursor

The problem of the precursor is perhaps most dramatically exhibited by considering inductions carried out under the simplest circumstances. It has been shown by a number of workers with a variety of enzyme systems (*37*, *51*, *58*) that enzyme synthesis can be induced, in the absence

of a nitrogen source, in cells suspended in a buffer solution of the inducer. That the appearance of enzyme activity actually involves the formation of enzyme has been established in these cases by exhibiting the homologous enzyme in extracts prepared from the induced cells. In such inductions, the nitrogen employed by the cell in fabricating the new enzyme molecule must come from some preexisting nitrogenous components, and one is immediately faced with the obvious necessity of identifying the components so employed.

Before considering the most recent experiments which have led to a satisfactory resolution of this problem, it is of interest to note briefly some of the earlier work which, although inconclusive, nevertheless exerted a strong influence on the subsequent development of this aspect of the problem. Monod (*35*), in his classic investigation into the growth of bacteria, discovered the existence of a severe interaction between enzyme-forming systems which was expressed by the fact that simultaneous synthesis of 2 metabolically unrelated enzymes did not occur on exposing cells to a mixture of the 2 relevant inducers. In general, only one of the enzymes was formed at a time. A similar situation was uncovered in the yeasts by Spiegelman and Dunn (*53*). With yeast the presence of an external nitrogen supply greatly suppressed the severity and the extent of this interaction, and indeed under certain circumstances, simultaneous formation of two otherwise interfering enzymes was made possible.

Although these interactions were discovered relatively early in the renewed investigation of the phenomenon of enzymatic adaptation, their detailed significance remains to be delineated. Nevertheless, at the time, they were interpreted to suggest a competition for some commonly required nitrogenous material and, therefore, implied the existence of the precursor not as yet specified as to its ultimate enzymatic function. To these findings may be added those which established that the induction of enzyme requires the participation of a functional and utilizable energy-generating mechanism. Agents such as 2,4-dinitrophenol (*34*), sodium azide (*50*), and arsenate (*59*) which prevent the utilization of energy generated by metabolism also inhibit the induction of enzyme activity.

The importance of these early observations was derived from the fact that one obvious and attractive hypothesis of explaining the phenomenon of induced enzyme formation was made unlikely. The hypothesis which was eliminated assumed that the mechanism involved is one akin to the activation of trypsinogen to trypsin. Of course, the fact that energy is required for the induction precludes at once any simple application of this concept. In addition, such a model would suppose the pre-existence in a cell of inactive forms of enzymes already fully determined as to their specificity and their function. The inhibitory interactions observed would be difficult to explain under these circumstances. While observations of this nature could not definitely decide

the issue, they clearly encouraged the search for a nitrogenous precursor not as yet restricted in its specificity and potentially convertible into more than one kind of enzyme molecule.

In designing experiments which seek to reveal the nature of this precursor, one can be guided by the fact that, in principle, three mechanisms of enzyme synthesis can be written down, as follows:

1. complex precursor → active enzyme
2. complex precursor + free amino acids → active enzyme
3. free amino acids → enzyme

Reaction 1 assumes the preexistence in the noninduced cell of a complex precursor which can be converted into active enzyme without the involvement of the free amino acids. This property distinguishes it from mechanisms 2 and 3 and permits an experimental decision.

Evidently, what we are asking is whether it is possible for the precursor to become active enzyme without the participation of the free amino acid pool. Putting the question this way suggests immediately the necessity of examining the effect on the synthesis of enzyme of any experimental condition which decreases the availability of the free amino acids. Several methods are available and have been employed for achieving a restriction of this nature and they may be listed as follows:

1. The use of amino acid analogs as specific agents to prevent the incorporation of the free amino acids into protein.
2. In the case of those cells which possess an internal amino acid pool, to examine the effect of depletion and replenishment of this pool under conditions which would minimally disturb other components of the cell.
3. The use of amino acid-deficient mutants which would make unavailable specific components.

Experiments along all of these lines have been realized with yeast and the bacteria. The following paragraphs summarize briefly the evidence obtained.

A. *The Effect of Amino Acid Analogs on Enzyme Synthesis*

Halvorson and Spiegelman (*19*) carried out a study with a series of more than 40 analogs of amino acids for their effects on induced formation of a-glucosidase in *S. cerevisae*. A parallelism was established between the capacity of an analog to inhibit net protein synthesis, as measured by growth, and its ability to suppress enzyme synthesis. In the case of the effective analogs, complete and specific reversal of the inhibition was achieved by the addition of the homologous amino acids. The generality of these findings was extended by the independently performed experiments of Lee and Williams, (*31*), who demonstrated that the administration of ethionine to the intact rat prevented the formation of tryptophan peroxidase. In the experiments with the yeast (*19*, *21*, *22*, *55*), it was possible to demonstrate by direct analysis that the presence of an effective amino acid analog inhibits incorporation from the free amino acid pool into the protein fraction. One interest-

ing feature which emerged from these experiments is that the presence of any one of the active amino acid analogs prevents the incorporation not only of its homolog but virtually of all the other amino acids as well. Further, no peptide fragments, unique to pools derived from cells incubated with an amino acid analog, could be found.

In these studies, no evidence for an amino acid-independent transformation of a complex precursor into active enzyme was obtained. The data rather led to the conclusion that the primary pathway of the induced formation of enzyme in nondividing cells of yeast involves the compulsory utilization of the internal free amino acids. The fact that the utilization of nonhomologous amino acids was blocked concurrently suggested further that the first stable intermediate formed in the synthesis of an enzyme molecule is of such complexity as to demand the simultaneous availability of a large portion of the component amino acids.

B. *The Effect of the Availability of Free Amino Acids on Enzyme Synthesis*

If the conclusions derived from the experiments with amino acid analogs are correct, it would be expected that the ability of cells to form enzyme should parallel the availability of free amino acids for protein synthesis.

One striking difference between the enzyme-forming capacity of yeasts as distinguished from that of many gram-negative bacteria receives simple explanation in these terms. Thus, yeasts are able to form enzymes when suspended in nitrogen-free solutions of inducer, whereas the gram-negative bacteria in general require an exogenous supply of nitrogen as a necessary concomitant of enzyme synthesis. The work of Taylor (*62*) suggests a reasonable explanation for this apparent independence of the yeast enzyme-synthesizing mechanism. This investigator surveyed a variety of yeast and bacteria for the presence of free amino acids in their internal environment. Of the three yeast types examined, all possessed detectable quantities of the five amino acids looked for. Amongst the bacteria, the gram-negatives possess primarily glutamic acid and lysine. None of the gram-negatives included in the survey contained detectable free amino acids by the procedures employed.

The possession of an internal pool was subsequently found to be a universal attribute of a wide variety of yeasts (*56*). It would appear that the ability of yeast to get along without an external source of nitrogen for enzyme synthesis is due to the fact that they have internal supply. To examine the question, then, of the effect of free amino acid availability on enzyme formation in the yeast, it was necessary to devise and employ procedures capable of modifying these pool levels both quantitatively and qualitatively. Using such procedures, Halvorson and Spiegelman (*20*) demonstrated a strong correlation between enzyme-forming capacity and pool level in both depletion and replenish-

ment cycles. The results obtained in these studies supported the conclusion that free amino acids constitute the quantitatively predominant sources of nitrogen in the formation of new enzyme molecules. Again, no evidence was uncovered which suggested the existence of a detectable amino acid-independent transformation of a pre-existing complex precursor into active enzyme molecules.

C. *Enzyme Formation by Amino Acid Auxotrophic Mutants*

The third approach mentioned which could provide relevant information involves the use of the auxotrophic mutants deficient in the ability to synthesize one or another of the amino acids. It was obvious that organisms such as yeasts, which accumulate an internal free pool, would be difficult to employ in such studies and the not unexpected difficulties arose when they were attempted with the yeasts. Fortunately, however, this approach was successfully applied almost simultaneously in two laboratories and it is interesting to note that in both instances the organisms employed, *E. coli* and *Aerobacter aerogenes*, possess a vanishingly small internal supply of free amino acids.

One of these investigations stems from the illuminating studies of Monod and Cohn (*37*) and their collaborators into the formation of B-galactosidase by the ML strain of *E. coli*. It is interesting to note that, in the course of these studies, Cohn and Torriani (*11, 12*) had discovered the existence of an enzymatically inactive protein, Pz, which was serologically related to the B-galactosidase. In addition to this obvious structural relationship, they established a suggestive correlation between the distribution of the Pz protein and the capacity of the cells to synthesize the B-galactosidase. Finally, they also showed that a significant decrease in Pz occurred in cells during the induced synthesis of B-galactosidase. Although not the only possible hypothesis entertained by the authors, it is clear that all of these observations would receive ready explanation if Pz were indeed the precursor of the B-galactosidase. In any event, taken together the observations noted offered the most impressive evidence existent in the literature to support the suggestion that a preexistent complex specific precursor is involved in the synthesis of a known enzyme.

There was, however, one fact that militated against the acceptance of this view. Nitrogen-starved cells, though possessing normal amounts of Pz, showed no ability to synthesize the B-galactosidase unless an external source of nitrogen was provided. It was nevertheless still possible to imagine that the starvation procedure interfered in some way with the metabolic step required for transformation of Pz into active enzyme.

Monod, Pappenheimer, and Cohen-Bazire (*38*) undertook to investigate this question further by employing a series of mutants, each of which was deficient in the ability to synthesize a single amino acid. These mutants were subjected to a "specific starvation" by being grown in a medium in which the required amino acid was present in limiting quantities, whereas all other compounds were in excess. Immediately

upon the cessation of growth which attended the exhaustion of the amino acid, an inducer of the B-galactosidase was introduced. It was found that little or no enzyme was synthesized by the cells so treated, despite the fact that they contained normal amounts of Pz. Such cells do, however, form enzyme immediately upon the addition of the amino acid they are unable to synthesize. These results made it necessary to abandon any interpretation of the relation between Pz and the B-galactosidase which involves a direct, amino-acid-independent conversion of Pz into active enzyme. The large number of amino acid auxotrophs employed in this study would suggest further that if Pz is indeed the precursor a considerable number and variety of amino acids must be added to it before it is converted into active enzyme.

Ushiba and Magasanik (*63*) employed essentially the same approach in their study of the adaptive utilization of myoinositol by mutants of *A. aerogenes*. The results obtained led these authors also to the conclusion that the induced formation of the enzymes they were studying involved extensive synthesis from the amino acids. In a subsequent study, Rickenberg, *et. al.*, (*44*) reported similar experiments and results with *E. coli* strain K12.

D. *Is There a Preexistent Complex Precursor?*

The experiments cited thus far make unlikely any mechanism of synthesis which presumes the conversion of a preexistent precursor into enzyme by a process which is independent of the free amino acids. The only alternatives left are, either that there is no preexistent complex precursor, or that one does exist but becomes active enzyme only after the incorporation of amino acids. The most obvious experimental approach aimed at a choice between these alternatives would appear to be the use of isotopic labels. Thus, the induction of enzyme synthesis in uniformly labeled cells suspended in unlabeled medium should provide the necessary data providing the enzyme synthesized can be isolated in a pure state and its isotopic content determined. Rotman and Spiegelman (*46*) and Hogness, Cohen, and Monod (*24*), using the B-galactosidase system in *E. coli*, independently undertook to provide data relevant to this issue.

Rotman and Spiegelman (*46*) secured uniformly labeled cells by growth in C^{14} lactate. Enzyme was induced for short periods while the cells were suspended in nonradioactive medium. The B-galactosidase synthesized was isolated and purified by means of zone ionophoresis through starch columns. Further purification was achieved with the aid of specific precipitation with purified antiserum. The results obtained revealed that less than 1 percent carbon of the newly formed enzyme molecules could have been derived from any cellular components existing prior to the moment of the addition of the inducer. In the experiments of Cohn, Hogness, and Monod, S^{35} was employed as the isotopic label and similar methods for the isolation of the enzyme. Identical results and conclusions were obtained.

These findings virtually eliminate any hypothesis which assumes the preexistence of a complex precursor material which is convertible into enzyme. It is evident that a mechanism suggesting the *de novo* formation of enzyme from amino acids is at present the only one which has received experimental support.

A consequence of considerable importance issuing from this last conclusion is that induced enzyme synthesis is thereby *equated* to the process of protein synthesis. It follows that data derived from the study of enzyme induction are pertinent and relevant to the more general problem of protein formation. Further, the use of inducible enzymes as model systems of protein formation can, in principle and in fact, confer two significant operative advantages. In the first place, one is assured that the synthesis of a protein is being examined, a certainty not available to experiments dependent solely on incorporation studies. Secondly, the formation of as little as 0.01 μg of new protein can be detected with ease and precision.

3. On the Role of the Inducer

One of the most dramatic features of the phenomenon of enzyme induction is the requirement for the presence of the inducer and the apparent precision with which it specifically stimulates the formation of a particular enzyme.

Monod and Cohn (*37*) have recently reviewed the bulk of the published information relevant to inducer function. Much of this knowledge we owe primarily to the efforts of these two men and their collaborators. Certain experiments are particularly pregnant with interesting implications for the nature of EFS, and it is these which will occupy our attention.

A. *Specificity and the Relation of an Inducer to the Homologous Enzyme*

There are two possible mechanisms of inducer action which can be formulated with sufficient exactitude to permit their experimental elimination. These have been labeled by Monod and Cohn (*37*).

1. *The Functional Hypothesis.* The synthesise of an enzyme is presumed to be linked to its activity, from which effective inducers would necessarily be substrates.

2. *The Equilibrium Hypothesis.* It is assumed that inducers must form complexes with enzyme synthesized as a *sine qua non* of enzyme formation.

A number of observations eliminated the first hypothesis. Thus, Spiegelman, Reiner, and Sussman (*57*) demonstrated that maltose can induce α-glucosidase at pH values that preclude detectable metabolism of the maltose even by fully induced cells. Analogs of natural substrates have been employed for similar purposes. Thus, a-methylglucoside can be shown (*52, 54*) to be an inducer of maltase under conditions in which utilization cannot be detected. Further, Monod,

Cohen-Bazire, and Cohn (*36*) have demonstrated similarly that thiomethyl-B-D-galactoside and melibiose, neither of which are detectably metabolized by strain ML of *E. coli*, are nevertheless inducers of B-galactosidase.

The second hypothesis was proposed in its simplest form first by Yudkin (*66*) who did so in terms of a mass-action hypothesis in which combination between inducer and enzyme is assumed to drive the reaction in the direction of precursor conversion into enzyme. An uncomplicated application of this complexing concept leads to several predictions, the experimental violation of any one of which would require either its reversion or complete abandonment. We may list some of these as follows:

1. Substances which can complex with enzymes should be effective inducers.

2. Substances shown to be incapable of complex formation with a given enzyme likewise should be unable to induce this enzyme.

3. The dissociation constant of the substance measured in terms of its inductive effect on enzyme synthesis should be comparable in magnitude to the value obtained in experiments in which the constant is derived from complexant properties of the inducer with enzyme.

It should be noted that the term "complex formation" employed here is meant to subsume both the *specific* type, characterized by combination between an enzyme and its substrate or competitive inhibitor, and the *nonspecific* type, represented by a complex between an enzyme and a noncompetitive inhibitor. Thus, proof that an inducer does not form a specific complex with enzyme does not eliminate it as an enzyme complexant, since it leaves open the possibility of nonspecific complex formation. There is no *a priori* reason for believing that nonspecific combinations cannot function in the process of enzyme synthesis.

Involving as it does only negative properties, convincing exceptions to the second deduction mentioned are difficult to provide. However, violations of both 1 and 3 have been found. Thus, Lederberg (*30*) exhibited a mutant of *E. coli* which fails to respond to lactose as an inducer of B-galactosidase, despite the fact that lactose is a substrate and therefore a specific complexant.

Monod, Cohen-Bazire, and Cohn (*36*) subjected these considerations to the first systematic and thorough analysis. They revealed that thiophenyl-B-D-galactoside, although a potent competitive inhibitor of the B-galactosidase, was nevertheless incapable of inducing the formation of this enzyme. Other specific complexants, e.g., neolactose and phenyl-B-D-galactoside, were similarly found devoid of inductive capacity.

An instance of an apparent violation of the third deduction was provided by Spiegelman and Halvorson (*54*). The dissociation constant of methyl-a-D-glucoside, an inducer of a-glucosidase synthesis in *S. cerevisiae*, was determined and compared with the constant derived

from its complexant properties with enzyme. The two values were found to differ by a factor of about 200.

It must be emphasized that the types of hypotheses made unlikely by the experiments cited are highly restricted in nature. All of these experiments had in common an examination of the effect of the inducers as enzyme complexants in terms of measurable effects on enzymatic function. By their very nature, they could not preclude the possibility of combination at some site which possessed no consequences in terms of a detectable modification of enzyme activity.

It must further be noted that such experiments do not eliminate the possibility that inducer serves as a coupling agent which leads to the formation of a stable complex amongst enzyme, inducer, and a third component. If the inducer could combine with enzyme effectively only in the presence of a third component, violation of all three predictions could obtain.

B. *The Question of Stoichiometry of Inducer Action*

Until the advent of the resourceful exploitation by Pollock of the penicillinase-forming system of *B. cereus*, the question of stoichiometry was one which could be formulated but hardly resolved. Pollock (*40*, *41*, *42*) discovered that fleeting exposures of cells to minute concentrations of penicillin at 0° C. can lead to the specific adsorption of enough inducer molecules to permit penicillinase formation even subsequent to the destruction of all unbound penicillin. Using S^{35} labeled penicillin, it was found that specific fixation is saturated at about 80 atoms per cell. Having purified the penicillinase and determined the minimum turnover number, Pollock and Torriani (cited in *43*) were able to demonstrate that each molecule of penicillin thus fixed can cause the formation of at least ten molecules of penicillinase. These observations then establish that an inducer can act catalytically.

The unique feature of virtually irreversible adsorption of inducer has thus far not been exhibited in other enzyme-forming systems and hence this type of experiment is at present only feasible in the penicillinase producing system.

Specific fixation of inducer molecules, although not as irreversible as in the case of penicillin, has however been exhibited in B-galactosidase synthesis in *E. coli*. Cohen and Rickenberg (*8*) used S^{35} labeled thio-methyl-B-D-galactoside and showed that it was concentrated in the cells. Further, the addition of thio-phenyl-B-D-galactoside, an inhibitor (*10*) of B-galactosidase synthesis, led to the elimination of the labeled inducer from the cells. These experiments show, therefore, that the inducer is concentrated in the cells by specific adsorption at sites from which they can be displaced by an inhibitory analog.

4. Precursor and the Nature of the Enzyme-Forming System

In the present discussion, the term "enzyme-forming system," hereinafter referred to as EFS, will be used to designate that structure in the

cell which is directly and personally involved in the process of fabricating the enzyme molecule. This verbal device is employed to isolate the EFS conceptually from all the other cellular components which can and probably do intervene more or less directly in the synthetic process. It will, of course, be noted that there is an assumption made here. By so stating the problem, we do presume the existence of such a unique structure and, at least implicitly, ignore the possibility that proteins and enzymes are formed by a multitude of cooperating and sequential reactions.

In thinking about the possible nature of the EFS and in designing experiments to clarify the mechanism of its functioning, it is difficult to avoid being influenced by the results of the investigations into the precursor question. In a sense, these findings force the institution of an active search for EFS. The data we have reviewed relevant to the precursor problem are satisfyingly clear-cut, almost distressingly so. They lead compellingly to the conclusion that, in fabricating a new enzyme molecule, the cell prefers to weave it rather than to stamp it into existence. In this process the simplest components are employed. Further, we find no evidence for any stable intermediates smaller than those requiring the presence and utilization of all the amino acids. A stepwise formation beginning with simple peptides and proceeding through polypeptides of intermediate lengths would appear to be eliminated. From one point of view, this is, of course, a pessimistic conclusion. It suggests that a successive approximation to an understanding of how proteins are synthesized will not be achieved in terms of a gradually better insight gleaned from the study of intermediate pieces of increasing complexity as they approach the final stage of synthesis. A door is thus slammed upon an extremely attractive approach for tackling the question of protein formation. Those who accept these conclusions obviously are faced with the necessity of finding a new approach to the solution of the problem of protein synthesis. One is forced to turn one's attention to the possible nature and mode of functioning of the template.

5. The Chemical Nature of the Template

A template which is to serve as a device for protein synthesis must be at least as complicated and as large as the molecule which it is forming. Other than the protein molecule itself, there are relatively few candidates one can propose which can satisfy the two criteria of size and informational complexity. With these restrictions in mind, the two known possibilities are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

In the following paragraphs, we will briefly examine the available evidence for and against the possibility that one or the other of these substances is associated with the enzyme-forming mechanism.

A. *DNA as a Component of EFS*

Evidence from work with the transformation principles (*25, 68*) offers convincing evidence that genetic information can be stored in and transmitted through DNA. The potentiality, therefore, of forming any specific kind of protein molecule must ultimately be referable to the DNA of the cell. The question, however, which we would like to entertain at present is whether DNA is directly and *personally* involved in the synthesis of protein or whether it effects its influence via an intermediary. Definitive evidence one way or the other is, at present, not available. Presumably, an unequivocal demonstration will ultimately emerge from experiments analogous to those being performed in the laboratories of Brachet (*6*) and Mazia (*33*) with enucleated fragments of amoeba. At present, the best that can be offered is a series of experiments inquiring whether a correlation can be established between the metabolic activity or state of DNA and the act of protein synthesis.

There exists a variety of experiments in which it is possible to demonstrate a complete dissociation of DNA metabolism from protein synthesis. DNA formation is known (*1*) to be far more sensitive to inhibition by radiation with X-rays than is protein formation. Baron, Spiegelman, and Quastler (*3*) have shown that X-ray dosages, far exceeding those expected to stop the formation of DNA completely, permit normal enzyme formation in yeast. Similar dissociations have been achieved with other systems and by different means (*17, 39*).

Kelner's (*28*) studies on photoreactivation of *E. coli* following exposure to ultraviolet have provided an elegant method for a virtually complete separation of RNA and protein formation from net DNA synthesis. Halvorson and Jackson (*18*), employing yeast, have recently repeated and confirmed these results. The results obtained suggest again that protein and RNA continue to be synthesized subsequent to UV doses which completely inhibit the DNA formation.

Cohen and Barner (*9*) have reported the ability of a thymineless mutant of *E. coli* which can synthesize xylose isomerase in the absence of an added supply of thymine. This important finding was confirmed in our laboratory (*56*) using the same mutant and examining B-galactosidase forming capacity. It was found that cells of this strain synthesized considerable amounts of enzyme when suspended in a synthetic medium lacking thymine. This behavior is in striking contrast to that observed with mutants possessing other metabolic deficiencies. Thus, in our own experience and in that of others (*38, 39, 56*) adenineless, uracilless, or amino acid-deficient mutants form little or no enzyme in the absence of the required metabolite.

An interesting apparent exception to the information cited is that of Allfrey's (*2*) observation with isolated nuclei. He found that treatment with DNase suppressed the ability of his preparations to incorporate labeled amino acids, whereas RNase had little or no effect. The situation observed here may, however, be a reflection of the low nuclear

RNA content. If, as seems likely, RNA is derived from DNA, destruction of the latter would eliminate protein synthesis in such systems.

The data cited above demonstrate that drastic interference with DNA synthesis is often not accompanied by very striking effects on the formation of protein. While such findings cannot eliminate DNA as an active component of the EFS, they hardly lend support to the supposition that it is. The credence assignable to such negative conclusions with respect to DNA gains further weight from similar experiments examining RNA metabolism which yielded strikingly different results.

B. *RNA as a Component of EFS*

Many have postulated RNA as a key substance in protein synthesis. Chantrenne (*7*) has succinctly summarized such speculations and the supporting evidence. Here we would like to confine our attention to the information derived from the study of enzyme synthesis. Again, as in the case of DNA, correlative experiments have been performed with intact cells examining the effects on enzyme formation of agents or conditions which influence RNA metabolism.

1. *Experiments with Ultraviolet Light.* Swenson and Giese (*60, 61*) demonstrated that exposure to ultraviolet dosages, far exceeding those required to stop DNA formation, results in the inhibition of induced enzyme synthesis in yeast. Examination of the action spectrum of the inhibition revealed that it coincided with the absorption spectrum of nucleic acid. Halvorson and Jackson (*18*) extended these interesting observations. They examined the effects of various dosages on the synthesis of a-glucosidase, the ability to use free amino acid pool components, and incorporation of P^{32} into the nucleotides of RNA. Their results established an excellent parallelism between the loss in capacity to utilize the free amino acids and the ability to synthesize enzyme. It was further found (*56*) that even slight damage of RNA metabolism, as measured by ability to incorporate P^{32}, had profound effects on enzyme-forming ability. Thus, at a dose which achieved a 25 percent inhibition of RNA metabolism, enzyme formation was suppressed to the extent of 95 percent.

2. *The Effect of a Uridine Analog on Enzyme Synthesis.* One obvious approach which could in principle yield information pertinent to the role of RNA is to examine the effects of various analogs of uracil and its derivatives on enzyme formation. Ben-Ishai and Spiegelman (*5*) undertook such a study. One of the most effective compounds found was 5-OH-uridine, which the experiments of Roberts and Visser (*45*) suggest is able to prevent the utilization of uracil for the synthesis of RNA. The presence of as little as 5μg/ml of this compound results in virtual cessation of B-galactosidase formation by *E. Coli.* Further, this inhibition can be achieved even if the OH-uridine is introduced subsequent to the addition of inducer, at a time when maximal rate of enzyme formation had been attained.

Several illuminating facts emerged from those experiments. One was

that the OH-uridine could effect a complete inhibition of B-galactosidase formation at concentrations which had no effect on over-all protein synthesis. The apparent greater sensitivity of the B-galactosidase forming system suggests that it requires a larger effective supply of RNA precursors than other protein synthesizing systems. A second fact of interest is the ability of the OH-uridine to prevent enzyme formation even after its onset. This would suggest that continued synthesis of RNA is required for the uninterrupted production of enzyme. The same conclusion is derivable from the observation (*38, 39*) that, unlike the previously noted experience with thymineless mutants, uracilless mutants cease making enzyme immediately upon the exhaustion of externally supplied uracil.

C. *Competitive Interactions Amongst Protein Synthesis Systems for RNA Precursors*

The marked response of the B-galactosidase forming systems to 5-OH-uridine and its interpretation in terms of an elevated requirement for RNA precursors suggests other types of experiments for exhibiting this kind of interaction. *E. Coli* cells growing logarithmically in a synthetic medium with ammonia as the sole source of nitrogen do not accumulate a detectable internal pool of amino acids. The rate of protein formation is apparently limited by the synthesis of amino acids since an immediate increase in growth rate follows the addition of an external supply of amino acids. It would be expected that the sudden stimulation of protein synthesis caused by the amino acid addition would exert an exhaustive demand on the metabolic devices which supply the derivatives needed for ribonucleic acid synthesis. In view of the suggested sensitivity of the B-galactosidase-forming system to the supply level of the RNA precursors, the addition of amino acids might be expected to result in an inhibition of B-galactosidase formation. This prediction is experimentally realized (*17*). Thus, the presence of inducer fails to stimulate enzyme formation if amino acids are added simultaneously. The suppression is virtually complete for a period of a half-hour following which some recovery of enzyme-forming capacity occurs. That the inhibition is related to RNA precursor supply is supported by the ability of purine and pyrimidine bases to reverse it.

This dependence of enzyme formation on an adequate supply of nucleic acid precursors has also been exhibited (*5*) in the case of glucosidase formation in *S. cerevisiae*. In addition to their free amino acid pool, yeasts also possess a considerable internal supply of nucleotides and their polyphosphate derivatives (*47*). It was found possible (*5*) to specifically deplete the nucleotide pool by incubation in the presence of an external supply of amino acids and energy. This treatment leads to a loss of enzyme-forming capacity while leaving the free amino acid pool intact. If cells are first partially induced and their nucleotide pool then depleted, they fail to form enzyme on being again exposed to inducer. If their nucleotide pool is, however, replenished,

enzyme synthesis proceeds normally. These experiments illustrate in a different manner and with another system the apparent requirement that RNA synthesis be possible if enzyme formation is to continue.

D. *Experiments with Subcellular Fractions*

The experiments thus far described strongly implicate RNA as the template in the process of enzyme synthesis. They cannot, however, be taken as conclusive. It is painfully obvious that, while interesting and perhaps even ingenious experiments can be performed with intact cells, the distance between the data and the conclusions derived from them is too great for certainty. Definitive identification of the chemical nature and the mode of action of the template is not likely until the latter has been physically isolated in a functional state. *In vitro* performance of its function by the isolated enzyme-forming system may be suggesting the impossible, since it demands even more than that which has already been accomplished in the case of transformation in the bacteria. In the latter, genetically competent material has been separated from other cell components. However, the transforming principles have been asked to function only after reinsertion into an intact living organism.

Nevertheless, that the ideal *in vitro* situation may be attainable in the not too distant future is prophetically foreshadowed by the striking successes which have recently been recorded with subcellular fractions. Many of these deal primarily with incorporation studies. To this extent it is uncertain they necessarily represent model systems which will permit the further dissection of the protein synthesizing mechanism. While the data must therefore be interpreted with caution, their uniqueness and potential value command consideration.

Zamecnik and Keller (*67*) succeeded in preparing a microsome fraction which actively incorporates amino acids when supplemented with some component of the supernatant and an ATP-generating system. Subsequent work on the supernatant fraction by Keller and Zamecnik (*27*) indicated the presence of an enzyme which generated guanosinetriphosphate, a derivative of which functions in the insertion of the amino acids into peptide linkage. The work of Hoagland (*23*) and DeMoss and Novelli (*13*) strongly suggests that polyphosphate derivatives of nucleotides activate amino acids prior to their incorporation.

Lester (*32*) and Beljanski (*4*) examined the ability of lysozyme-treated preparations of *B. megaterium* to incorporate amino acids and labeled amino acids. Both authors found that treatment with RNase abolished this ability whereas exposure to DNase was stimulatory.

The most extensive investigation on the properties of subcellular fractions has come from Gale's (*14, 15*) laboratory. In these studies, cells of *S. aureus* are disrupted by sonic disintegration and a fraction obtained by differential centrifugation which is relatively low in viable cells and, therefore, presumably in intact cells. Although it is unlikely that this preparation is homogeneous, it nevertheless is of the greatest

interest since it is amenable to enzymatic and extractive resolution. Removal of the nucleic acid from such disrupted cell preparations leads to a marked lowering in their ability to incorporate amino acids. This loss can be restored by the addition of nucleic acids from the same species, DNA being more active than RNA on a dry weight basis. This latter finding may be merely a consequence of the greater stability of DNA to isolation procedures. The data are consistent with the concept that the RNA made from the DNA supplied is the active agent.

A most interesting recent development has been the discovery by Gale and Folkes (*16*) that the presence of specific di- and tri-nucleotides are extremely active in promoting the incorporation of specific amino acids. Thus, for example, di-nucleotides containing adenine and cytosine can completely replace the intact RNA in promoting the incorporation of aspartic acid. Indeed, on an equivalent weight basis the di-nucleotide is more than a hundred times as active as the intact RNA. Interpretation of these findings is yet uncertain. It may indeed be, as suggested by Gale and Folkes (*16*), that these small fragments represent that part of the RNA template which is concerned with the insertion of the corresponding amino acid into peptide linkage. An argument which can be raised against this assertion stems precisely from the observed high activity of the di-nucleotide. It seems unlikely that nucleotide pairs are sufficient to specify the relevant amino acids, since only 16 possibilities are uniquely determined. At least three bases of the RNA template would have to be involved in the specification of a given amino acid if 20 or more choices have to be made. This reasoning assumes, of course, that the four bases are the only components of the code.

An alternative explanation of these findings can be offered. It may be that the active fragments of Gale and Folkes may, by transfer reactions, generate the nucleotide components functioning in the activating mechanism suggested by the work of Hoagland (*23*) and DeMoss and Novelli (*13*). Whatever the interpretation, it nevertheless remains true that these results are pregnant with many possibilities.

As distinguished from incorporation studies, the attainment of protein synthesis has been reported in only two sorts of subcellular fractions. One is the system of Gale and Folkes (*15*) in which the development of "glucozymase," catalase, and inductive formation of B-galactosidase have been demonstrated. When the preparations are sufficiently resolved either by removal of RNA or DNA, it is found that both DNA and RNA stimulate the formation of both catalase and B-galactosidase.

Again, the relative high activity of the DNA may be a consequence of great stability to extractive procedures. No limits to the potentialities of this system are apparent. It is difficult to believe that its future study can fail to provide definitive answers to the basic problems of template function and specificity.

Another system which gives great promise of future fruitfulness is the so-called protoplasts of *B. megaterium*. Weibull (*64*) showed that

these could be prepared by treatment of cells with lysozyme in hypertonic medium. Wiame *et al.*, (*65*) showed that these preparations were able to synthesize arabinokinase as demonstrated by an increased QO_2 during incubation. Simultaneously, Landman and Spiegelman (*29*) isolated a lactose positive mutant of *B. megaterium* and devised a stabilizing medium for protoplasts which permits synthesis of B-galactosidase. Virtually all the enzyme-forming capacity is recovered in the protoplasts. When supplemented with amino acids, hexosediphosphate, and inducer, they synthesize enzyme at rates comparable to intact cells. The B-galactosidase formed has been isolated in solube form and purified. These preparations are amenable to enzymatic resolution, their enzyme-forming activity being suppressed by RNase. This treatment does not destroy them physically but selectively removes 80 percent of the RNA.

It is evident that the search for a system which would permit the further experimental probing of protein synthesizing systems is at present in an exciting but preliminary stage. There seems little doubt, however, that a new era is being opened which will ultimately permit a description in chemically defined terms of the nature of the protein-synthesizing machinery.

6. Summary and Concluding Remarks

We have here surveyed the data which have accumulated on the phenomenon of "enzymatic adaptation" with particular emphases on the efforts of the past decade. In view of the complexity of the problem initially posed and the difficulties which could easily have hindered understanding or led to irrelevant confusion, the progress which can be recorded is satisfying.

The problem has been brought to the point where further questions must be posed in terms of the chemical structures and reactive interrelation of the components identified. From the experiments reviewed in the last section, it would appear that the systems needed for the experimental resolution of precisely such questions are now on the way to development.

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III. FUNCTION OF YEAST IN BAKED PRODUCTS

Effects of Yeast on Bread Flavor

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THE FLAVOR OF BREAD is largely a matter of opinion. One may like that which another does not favor. Some of the statements made here may be partly the result of personal likes and dislikes, and may conflict with the opinions of others. Fortunately, we can rely on the public demand as a measure of flavor approval. This is indicated by types of bread that enjoy the widest sale. The baking industry offers nearly every conceivable kind of bread to the trade. They naturally concentrate on that which sells. The popular types of bread should then be our yardstick and study be made of their flavor and appeal.

The flavor of bread arises from its ingredients, as altered by fermentation, baking, and handling.

The very mild flavor originating from wheat flour can be observed in cooked flour-water paste or in noodles. When such pastes are salted one observes the basic flavor of all wheat products. This flavor is very mild and possesses very little appetite appeal unless amplified or accented by other means. In breadmaking, desirable taste qualities are obtained by using the ancient arts of fermentation and baking. The combined action of these processes added to the basic flavors of wheat produces one of the most appealing flavors known. Who cannot recall joy upon finding fresh bread out of mother's oven or remember the pangs of hunger stirred by its fragrance?

This paper is limited to a discussion of the effects of yeast on bread flavor, and hence will not attempt to cover the entire scope of the subject.

1. Yeastless and Yeasted Doughs Compared With Regard to Flavor Production

To study the relation of yeast and fermentation to bread flavor, comparisons were first made between yeastless and yeasted doughs.

Doughs were made from flour, water, sugar, and salt without yeast and small pieces dropped into heated water. Higher temperatures were required to cause stiffening of dough than in cooking wheat-starch paste which thickens at 136° F. The dough pieces stiffened between 140° and 150° F. but continued to become firmer up to 175° F. Further heating to 212° F. gave the dough a springy property probably due to gluten coagulation. The flavor changes during these heatings were very

small and mild. The raw flour taste disappeared above 175° F. and the dough became more chewy at higher temperatures.

It is apparent from the properties of these cooked pieces of dough that bread will acquire increased structural strength as the interior becomes more cooked and affects eating quality.

When a similar dough containing yeast was likewise rapidly heated before fermentation had taken place, little difference from the yeastless dough was noted. When this latter dough was allowed to ferment before heating, marked differences were found. The dough became gassy and acquired a pronounced yeasty flavor. When heated to 150° F. much expansion occurred and the dough started to firm. It now looked and tasted somewhat like bread crumb having a strong fermentation flavor, but lacking the crust characteristics. When heated above 175° F. a slightly firmer product was obtained. Further heating increased firmness but reduced the fermentated flavor.

Figure 1 compares the temperature rise in 2 doughs when heated as a resistor in an electric current of constant input. This manner of heating produces a uniform temperature rise throughout the entire dough mass. By cutting off the current the heating can be stopped at any desired temperature for examination of the dough.

Yeastless dough rose in temperature at a uniform rate until the boiling point of water was reached.

Fermented dough deviated from a uniform temperature rise, though the heat input rate was constant. The deviation from linear began at about 150° F. and became steadily greater as heating continued.

The increased absorption of heat by fermented dough was due to the products of fermentation. Carbonic acid was decomposed and carbon dioxide driven from solution. Other highly volatile compounds such as diacetyl, acetaldehyde, and esters were gasified Above 175° F. alcohol began to evaporate causing the temperature rise to be retarded. This effect became steadily greater as alcohol was removed until at 212° F. only water remained to evaporate. It is thus apparent that fermentation has increased the time required for baking.

One can readily appreciate from these curves that the fermented flavor remaining in bread is due not only to the amount of fermentation it has received, but also to the temperature attained in the loaf interior.

It is obvious that fermentation retards the temperature rise in baking bread. The more the dough is fermented, the slower will the interior of a loaf become heated; thus more of the fermentation products are retained and the loaf will be less cooked and softer.

Yeast produces many different products in dough that alter its flavor. The most noticeable change is loss of sweetness due to fermentation of sugar. This reaction produces numerous by-products and intermediates in addition to alcohol and carbon dioxide. During baking, fermentation is at first accelerated and then abruptly terminated as

yeast is killed by the rising temperature. Thus, any of the substances from the arrested fermentation reaction may be present. Compounds remaining in dough may be carbon dioxide and alcohol; lactic, succinic, and pyruvic acids; acetaldehyde, ketone, acetylmethyl carbinol, diacetyl, isoalcohols, butylene glycol, and dihdroxy acetone. Those compounds that boil below temperatures attained in the dough during baking may distill off and their effects on flavor be lost. Among such volatiles are acetaldehyde, acetone, diacetyl, alcohol and ethyl acetate. The compounds of higher boiling points that may remain in the finished bread are pyruvic, lactic, and succinic acids, acetylmethyl carbinol, butylene glycol, iso-alcohols, dihydroxy-acetone, ethyl lactate, and ethyl succinate.

These less volatile compounds present in dough are not very flavorful in themselves. Alcohol, if not all distilled off, can be perceived. Acetylmethyl carbinol, though not appreciable itself, reacts later with oxygen to yield the very pleasant odor of diacetyl. The remaining fermentive compounds from sugar are of less importance, due to lack of flavor or small concentration.

The appetite of yeast for amino and ammonium compounds causes it to deaminize amino acids and leave residues of iso and other alcohols which flavor the bread. Yeast also releases acids bound by the ammonia it metabolizes, thereby lowering the pH of bread.

Nitrogen appetite of yeast is so great that when all other supplies are exhausted, yeast may attack the free amino groups of proteins thus denaturing them to that extent and reducing their availability for forming melanoids and their hydrogen bonding capacity. This statement is to some extent theoretical but is supported by the observation that yeast can reduce the Van Slyke amino nitrogen of milk during fermentation in the presence of added sugar.

No way of applying the Van Slyke method to flour proteins has been found, but it would seem probable that yeast will attack such proteins as well as milk. This is also suggested by the lesser ability of overfermented doughs to develop brown crusts, though they still contain ample sugar, and also by their lowered crumb quality.

Figure 2 shows the oven spring of doughs cooked as resistor. By this method of heating all reactions occur simultaneously without formation of a crust. The relation between temperature, oven spring, and the reactions in the dough may thus be more readily demonstrated.

The chart shows the spring of 2 yeasted doughs, 1 of which was proofed to height without previous fermentation; the other dough with adjusted yeast, sugar and yeast food was fermented 4 hours before panning. The adjustments were such that proof times were about the same.

The unfermented dough expanded during heating until starch swelling began after which upward motion soon ceased.

The fermented dough expanded to about 175° F. at which temperatures soluble proteins should be denatured. Motion then ceased

notwithstanding that large additional supplies of gas for expansion were available from evaporating alcohol.

It is apparent that fermentation improved the baking quality of the dough. The cells retained gas after starch swelling and expanded over twice as much as those in unfermented dough.

Bread from fermented dough had a fine grained, thin walled cell structure, whereas bread from unfermented dough had coarse grain and thick cell walls. Bread of fine grain requires a larger supply of volatile, flavory substances than does that of coarse grain, to yield a fermented flavor. This is due to the thicker walls retaining the volatiles longer than do the thinner walls of well developed bread.

The addition of suitable oxidizing agents to an unfermented dough can yield oven spring and cell structure equal to or greater than obtained from fermentation. This indicates that yeast has metabolized or in some manner acted on the substances that oxidation similarly affects. These are possible sulphur compounds in dough such as glutathione.

2. Relation of Crust Formation to Flavor

The preceding experiments have described the flavor of bread cooked without crust formation.

When dough is baked in a conventional oven the penetration of heat is from the exterior progressively toward the middle. Thus, during bread baking, all the reactions described at different temperatures are starting at the exterior from the beginning and progressing inward toward the loaf center.

If one will open loaves of bread at different stages of baking, the outer layers will be found expanded, cooked, and volatiles largely gone. Inward will be found a cooked layer containing volatiles which advances to a sharp boundary beyond which is found only uncooked, gassy dough.

Dough placed in the oven soon forms a dried and browned outer layer or crust that confines the dough within its boundaries. The pressure from within must be relieved by breaking the crust or by forcing gas out of the outermost layers of cells and packing them against the inner crust. A fermented dough is improved and develops strength so the cells retain their integrity and force the crust to yield. This results in smooth side breaks, thin crusts, finer grain, and larger volume, with resulting effects on flavor.

Crust color is influenced by fermentation. The melanoidin reaction in browning of crust occurs between sugar and nitrogen compounds such as ammonia or amines. Yeast also utilizes these same substances. Thus excessive fermentation results in light crusts and reduced flavor that comes both from loss of sugar and nitrogen with less browning reaction. This difficulty may be overcome by adding sugar and ammonium compounds to doughs. It is also aided by oxidation which reduces the requirement for fermentive development.

In the absence of simple forms of reactive nitrogen, both the browning reaction and fermentation are of such strength that with sugar present the amino nitrogen from proteins can be utilized. The browning reaction can occur with both milk and flour proteins. Mixing renders the flour proteins more available for the reaction.

The principal products from the browning reaction that are important flavorwise are pyruvic aldehyde from breakdown of sugar; iso-aldehydes from amino acids; furfural from pentosans and the melanoidin itself. The aldehydes and furfural are volatile and can be lost, but the brown melanoid is not and carries its slight bitter taste to provide part of the delightful flavor of many browned baked goods.

The baker must balance the supply of sugars and nitrogen compounds utilized by browning so that there will be the right amount remaining unfermented in the dough when it enters the oven. The materials ordinarily supplied for this purpose are either dextrose or sucrose and ammonium salts. The nitrogen supply should also be balanced against the amount of fermentation since an oversupply will yield too dark a color and may alter the flavor to a less appealing character.

Milk furnishes a noncompetitive source of sugar (lactose) for browning, which yeast does not ferment. Its use protects the browning reaction from the exhaustion of sugar by fermentation.

A byproduct of the action of yeast on sugar is acetylmethyl carbinol. This compound, though without appreciable odor itself, is readily oxidized by atmospheric oxygen to diacetyl which possesses a potent, pleasant odor often found in butter and in bread. The amount of acetylmethyl carbinol formed in bread depends on the method of manufacture. Little is found in straight dough. Sponge and broth methods give the higher yields, when proper amounts of sugar, oxidation, and fermentation are used.

While bread is baking, most of the volatiles are largely distilled and lost. They carry in their combination one of the most appealing aromas known, which as far as possible should be retained in bread. The compounds responsible for this pleasant odor in the order of their probable importance are diacetyl, ethyl alcohol, pyruvic aldehyde, acetaldehyde, furfural, iso-aldehydes, iso-alcohols, acetic acid, and traces of esters. Efforts to capture or imitate the odor of baking bread have not been too successful.

In breadmaking, best results for flavor may be obtained from a hot oven so that a desirable brown crust is formed before the interior volatiles are all distilled. Also a quick cooling by chilling is desirable so that the maximum of volatiles will be retained.

The amount of gas escaping from a baking loaf, due to the evaporation of alcohol, is much greater than that due to carbon dioxide in the earlier stages of baking.

The two compounds are generated by fermentation in approximately equivalent amounts, hence yield equal volumes of gas when evaporated. Carbon dioxide is largely lost during the operations of making and forming the dough. Alcohol is not lost, so upon baking yields a relatively large volume of gas which passes out of the baking bread through the crust and holds its temperature down much below that of the oven.

The more a dough is fermented, the greater is this ventilation. Ordinarily, this holds the crust below 150° C. under conventional baking conditions. Reduced fermentation will result in higher crust temperature.

Sugar will not brown at 150° C. but when mixed with ammonium-type compounds will brown readily, yielding flavory pyruvic aldehyde and the bitter melanoid complex formed by reaction between the two. When ammonium-type compounds are in excess, less pyruvic aldehyde escapes to flavor the bread. When sugar is in excess, less pyruvic aldehyde escapes to flavor the bread. When sugar is in excess and ammonium in balance then both compounds are formed. The crust browns and the aldehydes escape to the air and into the crumb.

In the latter case aldehydes are condensed in the crumb and held to flavor the crumb and to be released on reheating to yield a fresh odor and flavor to such reheated product. This adsorption occurs with all aldehydes formed in the crust, such as furfural from the pentosans and

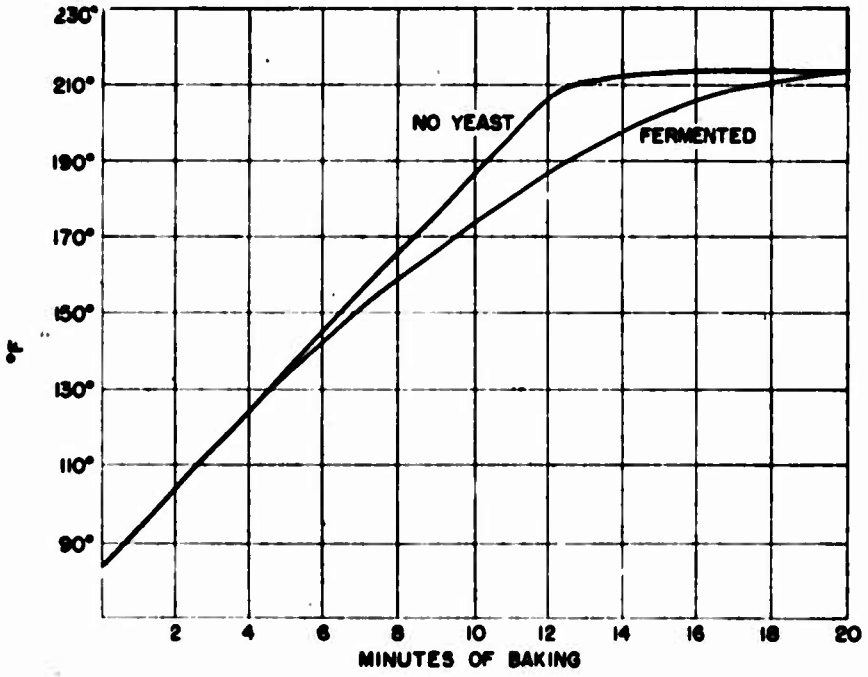

Figure 1. Temperature rise in dough baked as resistor with constant input.

iso-aldehydes from amino acids. They are drawn into the interior, especially during cooling, and held by the crumb to yield their potent flavors.

The well known Italian type of bread is made by straight dough methods from the four essential ingredients: flour, water, salt, and yeast. Because of the slow formation of sugar by enzymes, it requires a low concentration of yeast and a long fermentation time. When properly made it yields a light brown crust and some pyruvic and other aldehydes to flavor the bread. It has little sweet taste and no acetylmethyl carbinol to oxidize and yield the pleasant odor of diacetyl. Its grain is coarse and cell walls thick. It yields a chewy bread which retains enough flavor in its coarse structure to be very pleasant eating.

If sugar and a large amount of yeast are used in dough and fermentation carried to exhaustion of all available ammonium-type compounds, the dough upon baking does not brown. The colorless crust formed yields no flavor to the bread. Such white-crusted bread tastes little different than the electrically baked doughs above described. If a hotter oven is used, crust flavor will develop.

A dough made up by a conventional formula for bakers bread containing the usual liberal supply of sugar, fat, milk, and ammonium salts, but oxidized with larger amounts of iodate and well mixed, can be immediately panned after mixing without any fermentation, proofed, and baked to yield a good-looking loaf of bread of fine grain, large volume and well-browned crust. Such bread is very poorly flavored. It contains low levels of fermentation products. A similar dough, made by the sponge process with proper adjustment of oxidation, has a rich fermentation flavor and odor of diacetyl. When browned properly the bread possesses appealing qualities that cause it to be the largest selling type of bread in the United States.

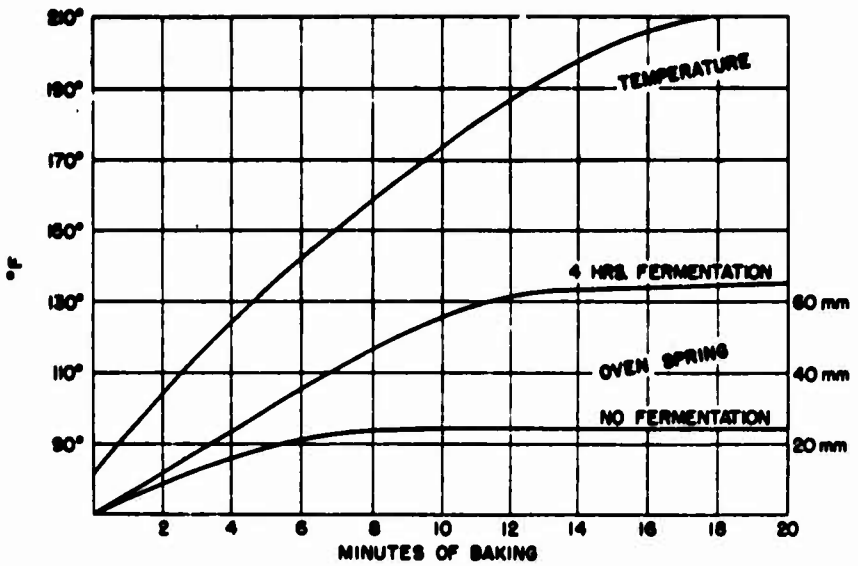

Figure 2. Oven spring of dough baked as resistor with constant input—no oxidation, 60-minute proof.

Evaluation of the Bread Baking Properties of Various Genera and Species of Yeasts[1]

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DURING THE FALL OF 1953 many discussions were held relative to setting up the work on stability of active dry yeast. Those taking part in these discussions were Dr. Larsen, Mr. Cryns, Dr. Goresline, Captain Yare, Miss Heiberg (Mrs. Wolkoff), Dr. Wickerham of the Northern Utilization Research Branch, USDA, and Dr. Mrak, University of California, who was serving as a consultant on the problem. It was decided that the first part of the program would be a screening of a number of yeast cultures to determine which of them could be used successfully in making bread. The second part of the program was to consist of stability evaluations of dry yeast prepared from the most promising of these cultures.

Dr. Wickerham supplied 75 cultures for evaluation and 4 were isolated from a can of active dry yeast which had been held in storage for 1 year at 90° F. at the QMFCI.

In order to obtain enough yeast for a baking test, the cultures were grown in 2-liter Fernbach flasks each containing 1 liter of malt extract-yeast extract broth (3 grams malt extract, 3 grams yeast extract, 5 grams peptone, 10 grams dextrose, 1 liter water) for 24 hours on a shaking machine. Each flask was equipped with a wooden paddle to facilitate aeration of the culture. The temperature was held at 88° F. The inoculum was a heavy cell suspension of the yeast culture in 10 cc. of broth incubated at 88° F. for 24 hours. Certain yeasts grew at such a slow rate that 20 cc. of inoculum instead of 10 cc. were used per liter of malt extract-yeast extract medium in order to obtain 8 grams of yeast necessary to bake a loaf of bread. The straight dough method was used in the baking tests. No modifications were made in fermentation time or formulation. In most instances only one loaf of bread was made due to the small amount of yeast available; however, in those cases where loaf volume was very poor repeat runs were made.

Of the 79 cultures, 17 did not leaven the dough as determined by lack of an expansion during an incubation period of 2 hours at a temperature of approximately 90° F. The 62 other cultures produced

[1] The author wishes to emphasize that he is reporting the results of the work of Captain Robert S. Yare and Mrs. Barbara Wolkoff, who were closely connected with growing the cultures used in the bread-baking tests and the evaluation of the tests. Not having been associated with this work at the time it was accomplished, he can present only those data contained in the progress reports of the two investigators mentioned.

bread ranging from very acceptable to non-acceptable. Criteria used to judge quality of the loaf were volume, flavor, odor, texture, and "eating quality," a term used to describe the feel of the bread in the mouth. Evaluations were made by an expert panel of six people.

When all of the yeasts had been tested a conference was held to set up a priority list of cultures (Table 1) for detailed propagation and storage studies. Group I shows those having highest priority, followed in order of priority by groups II, III, and IV. The organisms included in these groups total 49. Seventeen additional yeasts did not cause an expansion of the dough in baking tests, while 13 others did not produce acceptable bread.

In comparison with the known growth rates of *S. cerevisiae*, the cultures in group I may be divided into subgroups (Table 2). Subgroup A consists of 4 yeasts having growth rates similar to that of *S. cerevisiae;* subgroup AA, 1 yeast, having a growth rate 10 percent higher; subgroup B, 7 yeasts, having growth rates 20 percent lower; subgroup C, 3 yeasts, having growth rates 30 percent lower; subgroup F, 1 yeast, having a growth rate 60 percent lower; and subgroup I, 1 yeast having a growth rate 90 percent lower.

Stability evaluations of the promising yeasts discovered in this screening operation have not yet been made. It is anticipated that this work will go forward within the near future.

Table 1. Priority List of Yeast Cultures

Group I	Group II	Group III	Group IV
H–37 (a hybrid)	*S. carlsbergensis var. polymorphus*	*S. fragilis* 665	*Z. fermentati*
H–44 (a hybrid)	Colony #10 (a hybrid)	*S. intermedius*	*S. fragilis* #1109 (a hybrid)
Distillery yeast 1941	*S. cerevisiae* 684	*S. chodsti*	H–87d
QM top	*S. cerevisiae* 132	*S. cerevisiae* B–1779	*S. pombe*
QM 6b	*S. cerevisiae* 978	*S. cerevisiae* 898	*T. colliculosa*
Z. lactis	*S. cerevisiae* 1188	*S. tubeformis*	*T. dattila*
S. carlsbergensis mandshuricus	*S. carlsbergensis*	*S. fragilis* 876	*H. subpelliculosa*
S. chevalier	Hawaiian Food 254	*S. ellipoidius thermophilus*	*C. arborae*
Z. drosophilae	*S. cerevisiae* 643	*S. oviformis*	
S. cerevisiae thermophilus	*S. logos*	*S. tropicalis* 1767	
S. cerevisiae 567		*S. osmophilus*	
S. cerevisiae 1386		*S. diastaticus*	
C. pseudotropicalis			
S. italicus			
Undetermined B–932a			
S. fragilis #610			
S. marxianus #1052			
C. tropicalis 1410			

Table 2. Subgroups Based on Comparative Growth Rates

Subgroup A	Subgroup AA	Subgroup B	Subgroup C	Subgroup F	Subgroup I
QM–top QM–6b *S. cerevisiae* 567 *S. cerevisiae* 1586	*Z. drosophilae.*	*C. pseudotropicalis* *S. marxianus* 1052 *C. tropicalis* 1410 *S. carlsbergensis mandshuricus* *S. chevalier* *S. cerevisiae thermophilus* *S. fragilis* 610	H–37 (1140 x 610) H–44 (1140 x 1052) Distillery 1341	Unidentified B–932a	S. italicus 1573

Theoretical and Practical Aspects of Brew Fermentation

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1. Introduction

THERE ARE SEVERAL METHODS of making bread, among which, at present, the most common are the sponge-dough and straight-dough methods. Table 1 summarizes the basic features of these methods and offers a comparison with the brew method. The sponge-dough technique usually involves the mixing of part of the ingredients including about 60 to 70 percent of the flour, all of the yeast, yeast food, malt, and part of the water. After a controlled fermentation period of about 3 to 5 hours the sponge is returned to the mixer and the remaining ingredients are mixed in. These include the remainder of the flour, water, sugar, shortening, salt, milk solids, and so on. The complete dough is then given a floor time of about 30 minutes.

In the straight-dough method all of these ingredients are incorporated into the dough in one mixing, followed by a fermentation period of 2 to 3 hours. After the complete doughs have been mixed and fermented, the two processes then involve quite similar steps of dividing, overhead proofing, makeup, pan-proofing, and baking.

Table 1. Bread-Making Processes[1]

	Sponge dough	Straight dough	Brew dough
Mix	Part of ingredients	All ingredients	Brew ingredients
Ferment	3 to 5 hrs.	2 to 3 hrs.	3 to 4 hrs.
Remix	Remaining ingredients	.....	Brew and dough ingredients
Floor time	½ hr.	.....	½ hr.

[1] Dividing, overhead-proofing, makeup, pan-proofing and baking procedures are much the same in all three methods.

The basic ingredients of a *brew fermentation method* are yeast, sugar, and water. These basic ingredients are mixed together, as will be brought out later, usually with some other ingredients and allowed

to ferment for specified times. After brew fermentation, the other usual dough ingredients are mixed with the brew to form a dough which is then carried on through the basic steps of bread manufacture without, of course, a dough fermentation period but with the usual floor time given a sponge dough. One might look upon the brew fermentation method as a combination of both the sponge-dough and straight-dough processes, where the brew replaces the sponge and all of the flour goes into the single mixing stage. The analogy can be extended even further by saying that the brew is a replacement for a "master sponge," that is, one large enough for several doughs, even for several doughs of different varieties of yeast-leavened goods. Of course what makes this possible is the absence of large quantities of flour from brews and the stability of brews.

2. History of Brews

In the book *"The Technology of Bread Making,"* written in 1911 by Jago and Jago there are listed several methods of breakmaking, among which is a process called "Ferment and Dough." The origin of this method is not given, but the authors stated that at the time of writing it was being used very largely in London and the South of England in the manufacture of crusty bread and was also well adapted for other types of bread. This process involved a fermentation of about 6 hours using yeast and a suspension of boiled potatoes in water. A straight dough was then made with the ferment, which dough was then allowed to ferment from 2 to 5 hours as compared to contemporary conventional straight-dough fermentation times of 10 hours. The authors claimed that bread with good volume, flavor, texture, etc., could be obtained by this method.

This technique undoubtedly has had many variations since the early 1900's. In more recent years we have all heard of the continuous bread-making process conceived by Dr. Baker and his associates of the Wallace & Tiernan Company. This method makes use of a brew with a fermentation time of about 4 hours. The dough that is subsequently mixed from the brew is given its development not by a long dough fermentation but by mechanical mixing in a specially contrived mixer or "developer" as it is called. Shortly after this development came the buffered brews. These brews are buffered at about a pH of 5 either with inorganic compounds or proteins. The doughs, which are allowed to ferment for only short times, are made up in conventional bakery equipment.

3. Bread Flavor and Brews

One of the functions of a brew is to supply the flavor to the finished loaf of bread. Dr. Baker gave an excellent talk this morning on bread flavor so there is no need for me to repeat what was said except to summarize the sources of bread flavor. The origin of flavor in bread is

firstly from the ingredients. It is also from the effects of dough conditioning on eliminating the rawness or "greenness" from the dough. This dough conditioning takes place not only during mixing, but also during fermentation where the sponge dough is in a continuous state of agitation because of the vigorous fermentation. If sponge or straight dough fermentation is eliminated, as it is in the brew process, the dough conditioning can be done mechanically. A third source of bread flavor is from the fermentation products resulting from the action of yeast enzymes on sugars. Since brews contain yeast and sugar they supply this aspect of flavor. And then we have flavor resulting from the baking process including the browning reaction and any chemical reactions that might take place within the interior of the loaf.

Bread made by the brew process can achieve its flavor, as by other methods, from the ingredients, the yeast enzymes acting on sugars, and the baking reactions. The development of bread flavor in the brew process then can only be hindered by the absence of dough conditioning as ordinarily occurs during sponge or straight dough fermentation. This can be overcome by increasing the dough mixing time or possibly by chemical means. This point will be further discussed later.

4. Functional Ingredients of Brews

The ingredients of brews have to include yeast, some form of fermentable carbohydrate, and water. These are the basic necessities, and under certain conditions good bread can be made from them.

A. *Yeast*

The yeast can be either compressed or active dry. When compressed yeast is used, usually from 1 to 3 percent is added to the brew and the remainder, if any, to a total of 3 percent, is added to the dough.

In experiments in which we divided a total of 3 percent compressed yeast between the brew and dough stages, thus using as little as 1 percent in the brew with 2 percent in the dough or as much as 3 percent in the brew and none in the dough; and comparing the results with similar combinations using 2 percent yeast, the bread with the best overall score was made with 3 percent total yeast. This was so regardless of how the yeast was proportioned between the brew and dough stage.

This is a good point at which to mention that these experiments on the combinations of 2 percent and 3 percent yeast in the brew and dough revealed no differences in proof times of the doughs with the different proportions of each percentage. For example, 1 percent yeast in the brew and 2 percent yeast in the dough gave a proof time of 49 minutes; 3 percent in the brew and none in the dough gave a proof time of 48 minutes. These results indicate that yeast activity is not enhanced as a result of brewing.

In addition to our proof-time results with different levels of yeast proportioned between the brew and the dough, we have run gas meas-

urements in the fermentometer on brews buffered with either milk or calcium carbonate and stored at 40°, 75°, 86° and 100° F. These data show no increases in yeast activity of the brews after the initial fermentation period by fermentometer tests.

When using active dry yeast in brews, the conditions for obtaining optimum results are slightly different than with compressed yeast. Compressed yeast is merely crumbled and added to the brew. Active dry yeast must be rehydrated at a temperature of 110° F. and then added to the brew. A brew with active dry yeast is preferably maintained at 86° F. We have found that the use of brew temperatures higher than 86° F. with active dry yeast result in slower proof times and poorer bread quality. The direct addition of active dry yeast to a brew at 86° F. does not give the best results. Apparently the active dry yeast has to be rehydrated in water rather than in the brew with its other dissolved ingredients.

When using active dry yeast in the brew process, conditions can be simplified with no effect on bread quality by adding all of the yeast to the brew stage. Thus, the yeast pretreatment step for the dough stage can be eliminated.

B. *Sugar*

The sugar levels in the brew that we have found satisfactory for full flavor development range from about 1 to 3 percent based on the flour. There does not seem to be any advantage to using more sugar than this.

C. *Salt*

We have also been adding about half of the total salt to the brew, i.e., about 1¼ percent based on flour. By measuring gas production rates in the fermentometer, we found that in the absence of salt, gas production in the brew is very slightly faster in the first hour of fermentation. However, proof times of dough made with brews with and without salt were practically identical, so adding 1¼ percent of salt to the brew has no adverse effect on the dough proofing. We do not know whether this amount of salt affects brew stability.

D. *Malt*

Malt, either syrup or dry, can be put into either the brew or the dough. If added to the brew, its fermentable fraction, which amounts to about 50 percent, can be used to advantage.

E. *Yeast Nutrients*

As far as yeast nutrients are concerned, we have found that the only ingredients which have an influence in the brew stage are ammonium salts. Other ingredients of a yeast food such as Arkady, that is, potassium bromate and calcium sulfate, have no effect on brew fermentation rates. The ammonium salt, however, was found to speed up yeast fermentation in the brew.

Some fermentometer and pH data illustrating these results are shown

in figure 1. In this figure are plotted the gas production rates of the various brews *versus* time of fermentation. As can be seen, the brews with yeast food and those with calcium sulfate plus ammonium chloride produce gas at a faster rate than brews with no yeast food or with only calcium sulfate plus the oxidant, potassium bromate. Therefore, it is evident that the ammonium salt is responsible for the faster gas production rate. In addition, the ammonium chloride has a slight acidifying effect on the brews, resulting in lowered pH's. These particular brews were made using milk solids as the buffer.

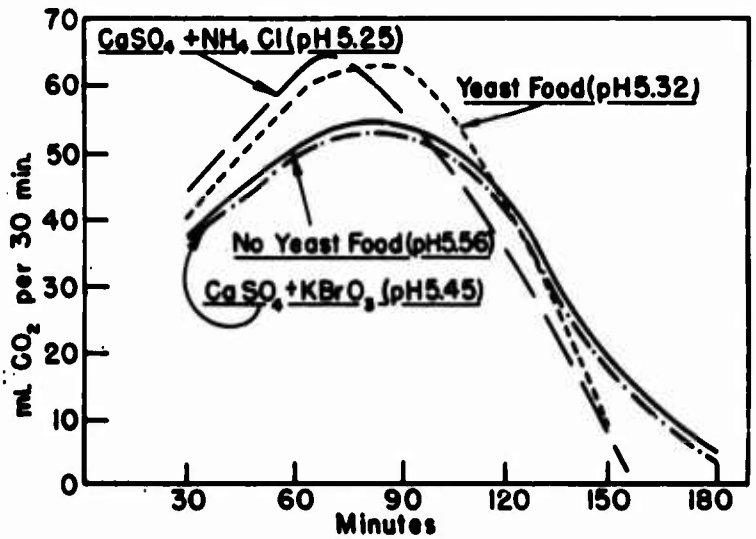

Figure 1. Effect of yeast food components on gas production and brew pH.

F. *Enrichment Wafers*

In addition to the brew ingredients already mentioned, enrichment wafers may be added to the brew or to the dough.

G. *Buffer*

Finally, in order to stabilize the brew, a buffer is necessary. The buffer, which can be either a protein-containing material such as milk or an inorganic compound such as calcium carbonate, should maintain the pH between 4.5 to 5.5. The reason for the necessity of a buffer will be discussed in detail later. I realize that, technically, calcium carbonate is not a buffer, but since it can function as such, for the sake of convenience, we will group it with protein buffers.

H. *Other Ingredients*

There is an advantage, in formulating a brew, in adding as many ingredients as possible to the brew. By doing this, one weighing of an ingredient can suffice for several doughs. Large quantities of ingredients,

enough for several doughs, can be weighed out and added to the brew, and in subsequent dough mixing the correct quantity of each ingredient for single doughs is obtained merely by measuring an aliquot of the brew and mixing it with the dough ingredients. Many small individual weighings can then be eliminated.

I. *Ingredients to be Omitted*

Needless to say, there are some ingredients that have to be omitted from the brew. They include the final dough sugar. This sugar is necessary for doughproofing and for sweetness. Shortening should be omitted from the brew since it would not disperse homogeneously unless emulsified. Also, if the brews are cooled, the shortening might solidify. When preparing brews with milk as the buffer, proteinases should be excluded, or at least only predetermined satisfactory levels should be used, since they cause milk to clot under certain conditions. And finally, some of the salt should be left out of the brew since amounts over 1¼ percent tend to retard the fermentative activity of yeast in the brew and would lengthen the brew time somewhat.

J. *Summary of Brew Ingredients*

Based on what was said about brew ingredients, we have prepared a summary of brew ingredients using a calcium carbonate buffer. This is shown in Table 2. The brew improver, mentioned in the table, contains ammonium chloride, a yeast nutrient, and calcium carbonate, a buffer.

5. Importance of pH Control

As mentioned before, one of the necessary conditions for a stable brew is maintaining the pH around 5.0 with a buffer.

In figure 2 is shown one of the reasons for buffering a brew. The buffer used in this case was calcium carbonate (in the brew improver) in sufficient quantity to keep the pH around 5.0. The total gas evolution, as measured in the fermentometer, is compared with a brew with no added buffer. It can easily be seen that in the presence of the buffer the rate of gas production is much faster indicating, of course, more yeast activity. The total amount of gas evolved in both cases is the same since sugar levels were identical.

Table 2. Brew Formula (100 lbs. flour dough)

Water	45 lbs.
Yeast—Compressed	1.0 lb.
Active Dry	1.2 lbs.*
Sugar	2 lbs.
Salt	1.25 lbs.
Malt	0.25–0.5 lbs.
Brew Improver	3¼ oz.
Enrichment	As required

* Total yeast.

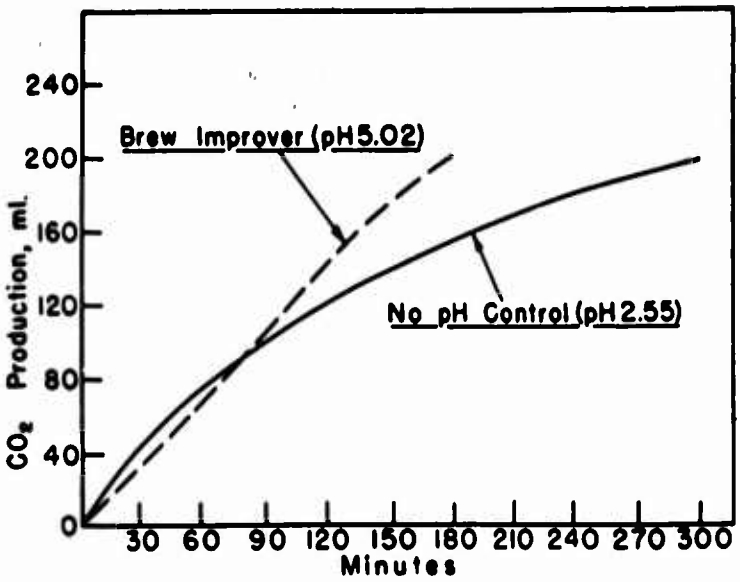

Figure 2. Effect of pH control on gas production.

A further illustration of this same point, but with data of a quantitative nature, is shown in figure 3. These bar graphs illustrate that with different levels of brew improver the time necessary for conversion of practically all the sugar can vary from 4 hours to 5½ hours with the fastest rates of fermentation occurring with the intermediate levels shown. These intermediate levels give brew pH's from 4.01 to 5.42; pH's above and below these levels result in slower rates of evolution.

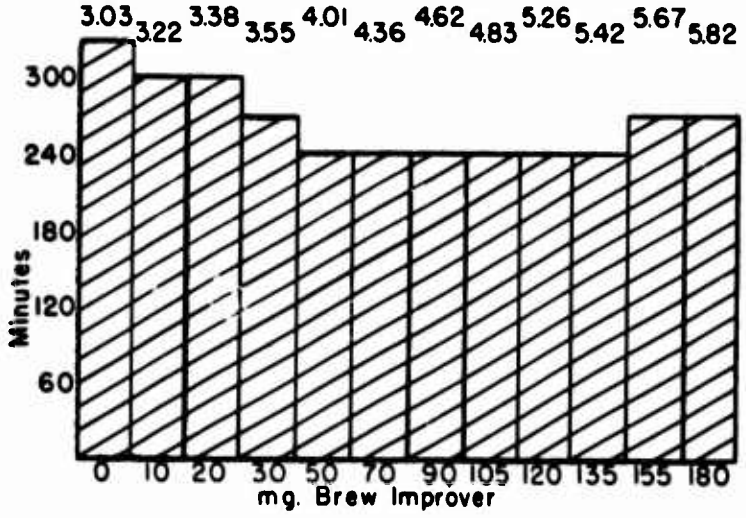

Figure 3. Time necessary for 95 percent sugar conversion final brew pH.

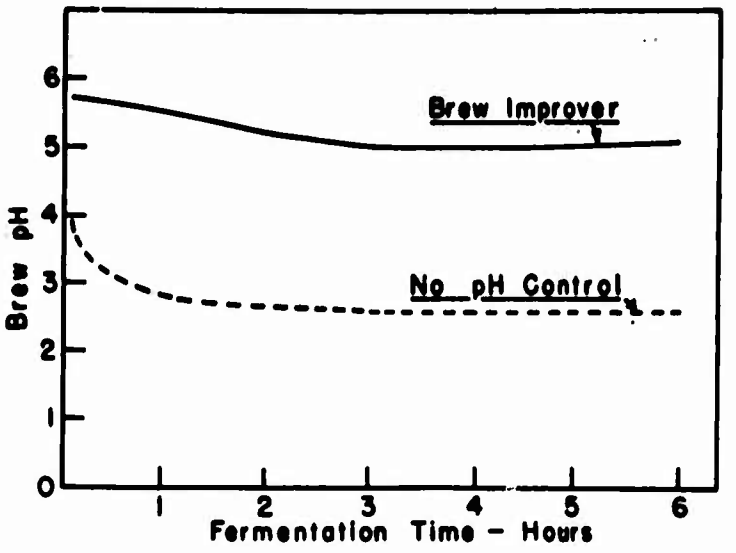

Figure 4. Changes in brew pH.

Figure 4 has curves showing pH changes during fermentation in the presence of a calcium carbonate buffer and in the absence of a buffer. The formation of fermentation acids occurs quite rapidly as can be seen from the pH changes in the unbuffered brew. The presence of these acids seems to be responsible for the lowered yeast activity. Our brew-storage tests indicated that this inhibition is nonreversible to a certain extent and becomes more severe with time. Therefore, in order for a brew to show stability on storage, a buffer must be used.

When bread is made from freshly prepared buffered and unbuffered brews, the baking data as shown in table 3 are typical of the results. With a buffered brew, the proof time is faster, the loaf volume is larger, and the pH is higher. In addition, bread from a buffered brew always

Table 3. Baking Data: Using Brews With and Without pH Control

	Buffered Brew (pH 5.08)	Unbuffered Brew (pH 2.66)
Proof time (min.)	40	50
Loaf volume (cc.)	2140	2000
Bread pH	5.32	4.97

Bread score:
(comparative basis)
Grain, crumb color, odor and taste—Buffered brew bread preferred.

has a better grain score, lighter crumb color, and better flavor. These differences are more outstanding if the brews are stored before use.

6. Stability of Brews

This is illustrated by figure 5 which shows the gas evolution data of brews made with and without pH adjustment after storage for 24 hours and 48 hours at 75° F. These data were obtained by adding sucrose and phosphate buffer to both brews after the storage periods in order to ensure that they were being tested under the same conditions. It is quite evident that the brew made without pH adjustment and allowed to stand at a pH of about 2.5 lost considerable yeast activity and was not satisfactory for use in making bread; whereas the brew which was kept at a pH of 5.0 during fermentation still had satisfactory yeast activity after 48 hours at 75° F.

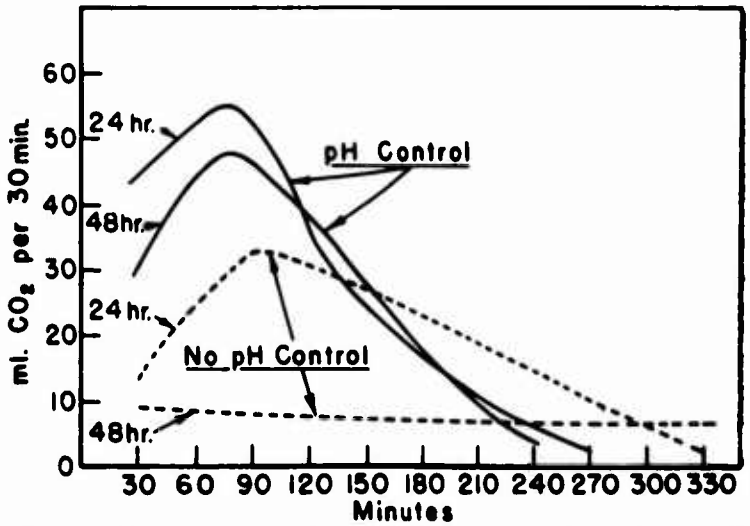

Figure 5. Gas production; brews made with and without pH control and stored at 75°F.

7. Protein Buffers

The discussion of pH control so far has centered around non-protein buffers, such as calcium carbonate. In addition, as mentioned before, protein sources can be used as buffers. The most widely used protein for this purpose, of course, is milk, and the recommended level is 6 percent with 2 to 3½ percent sugar. In addition, we have tried different types of soy flour at different levels and have made satisfactory bread. The final pH of a brew made with 3 percent sugar and 1 percent soy is about 3.6; with the same level of sugar and 3 percent soy it is 4.4. We have not done very much work with nonmilk protein buffers, but with the proper levels of protein and sugar we see no reason why brew bread of high quality cannot be made. A disadvantage common to all protein buffers is their tendency to foam, but this can be largely overcome by the addition of anti-foaming agents.

8. Concentrated Brews

The data we have been using so far to illustrate our results were obtained with brews containing either 100 percent or 65 percent of the total water as used in the dough. We have also had success with brews containing as little as 30 percent of the total water. In the tests we have done so far with brews containing 30 percent or 50 percent of the total water, the final bread was either equal or superior to that made with more dilute brews. The only adjustment that seems to be necessary with more concentrated brews is in salt levels. They should be reduced accordingly. We are not prepared to say much at this time about the stability of more concentrated brews. We are presently working on this aspect.

The American Dry Milk Institute has recently released a new concentrate brew formula which contains approximately 50 percent of the total water. We have done some laboratory tests on this new formula in comparison with their previous one which contained all of the water in the brew and found that the use of the concentrated brew results in better bread.

9. Advantages

There are definite advantages in using more concentrated brews. In making up the dough, cooling water can be used for adjustment of the dough temperature. Also, absorptions and yeast levels for different products can more easily be adjusted; and finally, tank sizes can be smaller or higher production can be maintained with tanks already on hand.

10. Brew Conditions

There is a lot of flexibility in the brew method, particularly in the brew conditions. The American Dry Milk Institute has defined these

Table 4. Brew Formula and Conditions
Brew Formula (100 lbs. flour dough):

Water	45 lbs.
Yeast—Compressed	1.0 lb.
or active dry	1.2 lbs.
Sugar	2 lbs.
Salt	1.25 lbs.
Malt	0.25–0.5 lbs.
Brew improver	3¼ oz.
Enrichment	As required

Brew Fermentation Time: 3 to 4 hours.

Brew Temperature: Set at 85°F. Temperature not critical. From 75° to 95°F. satisfactory.

Stability: At least 24 hours when held from 75° to 80° F. If held longer it should be refrigerated.

conditions and published them in various bulletins, and hence we will only consider calcium carbonate buffered brews in a discussion of brew conditions. Table 4 summarizes the brew formula and conditions.

11. Temperature

Our brew recommendations are as you have seen. We have standardized on a brew temperature of about 85°F. after finding that fermentation temperatures from 75° to 95°F. gave equally good bread.

12. Time

The recommended time for brew fermentation is from 3 to 4 hours. We have used Ca CO_3 buffered brews held at 85° F. for anywhere from 2 to 24 hours and have obtained consistently good results. If the brew is to be held for longer than 24 hours we suggest that it be refrigerated to be on the safe side. A typical brew, as shown in this table, will have no fermentable sugar left after 3½ hours fermentation and will have a final pH of about 4.8.

13. pH Control

As you would expect, the final brew pH can be adjusted very conveniently by changing the level of brew improver. This is illustrated in figure 6 which shows the relationship between the level of brew improver and final brew pH. Incidentally, the level of sugar used also affects the final brew pH, since when more sugar is fermented more acidic products are formed. For that reason, our brew improver has been formulated so that its usage level should be about 10 percent of the sugar level.

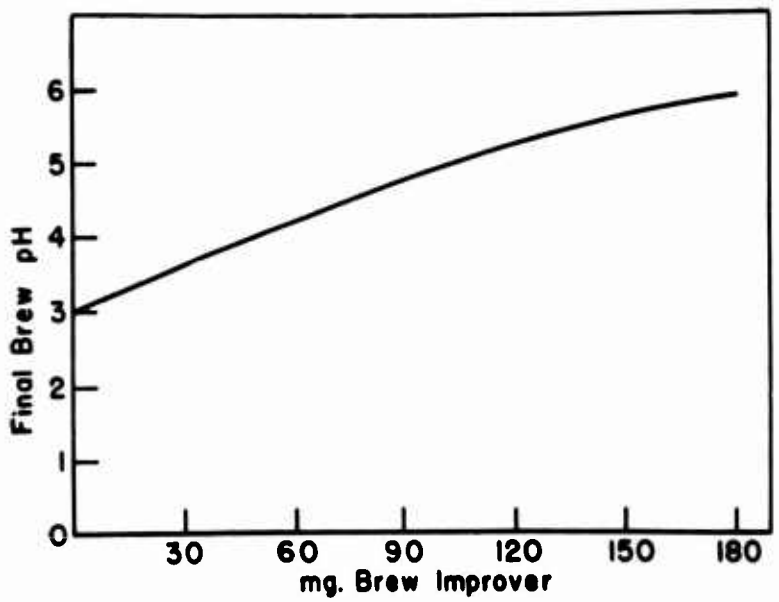

Figure 6. Relationship between level of brew improver and final brew pH.

14. Dough Conditions

The dough conditions for the brew process are summarized in table 5. The ingredients used at this stage are the usual ones that are put into the dough side of sponge-doughs. The one notable exception to this statement is that additional yeast is added to the brew-dough stage when compressed yeast is used. The amounts of any of these ingredients may, of course, be considered as variable depending on conditions and preferences. Any effect of oxidizing salts will be noticed in the dough. The effects of other ingredients of dough improvers, such as monocalcium phosphate or ammonium salts, might also be noticed in the dough stage.

15. Enzymes

Proteinases may have a function in brew doughs. We do not believe they can have much effect on the mixing time requirements of brew doughs since there is not sufficient time for them to exert their action.

Table 5. Dough Formula (100 lbs. flour dough)

Brew	47 lbs.[1]
Water	24 lbs. (variable)
Flour	100 lbs.
Sugar	6 to 8 lbs.
Yeast—Compressed	2 lbs.
or active dry	All added to brew
Yeast food	¼ to ½ lb.
Salt	¾ lb.
Shortening	3 to 4 lbs.
NFDMS	Variable.

[1] Approximate brew weight when 65 percent of the total water is used in making the brew.

However, when used at appropriate levels, proteinases should function to help machining properties and prevent dough buckiness. Another baking enzyme which we found to be effective in brew doughs is lipoxidase. Lipoxidase, as you probably know, can be used to advantage as a dough bleaching material. This enzyme is present in soy beans in high concentration. In tests in which ¼ percent of an enzyme-active soy flour was used a noticeable improvement in crumb color was obtained, even with bleached bread flour.[7]

16. Mixing Times

It is fairly evident that bread quality, including loaf volume, improves with longer mixing-time periods. Our experience has indicated that the mixing time may be from 30 to 50 percent longer than for sponge doughs. This might be expected since some mechanical action does occur in sponge fermentation, which, of course, cannot occur in a

brew dough. From a theoretical point of view, the extra mixing requirements of brew doughs are really less than might appear, if one considers that the sponge-mixing stage is eliminated. If the time required to mix a sponge is added to that required for the remix or dough stage, the total is usually only a minute or 2 less than required for brew doughs. From a practical point of view, however, the longer brew dough mixing times might necessitate a revision of production schedules in a large shop. Table 6 summarizes the brew conditions.

17. Dough Temperature

It has been our experience that brew doughs yield better bread if they are set at warmer temperatures than sponge doughs. Temperatures from 84° to 88° F. have been found to give good results.

18. Floor Time, etc.

Floor times of 30 minutes or even a little longer have been found satisfactory. The final steps in bread production, including makeup, pan-proofing, and baking can be as usual for conventional bread.

Table 6. Brew Dough Conditions

Mixing: Mix dough 3 to 4 minutes after dry up. The total mixing time may be 30 to 50 percent longer than for sponge doughs made with the same flour.
Dough Temperature: Set dough at from 84° to 88°F.
Floor Time: 30 minutes (approximately).
Makeup, Proof, Baking: As usual.

19. Comparison with Conventional Bread

A. *Keeping Qualities*

One of the important comparisons between brew bread and conventional bread that should be considered is keeping quality. We have compared sponge-dough bread with brew bread to see if any differences in keeping quality or staling rate exist. The dough formulas were essentially the same insofar as they could be considering the differences between the two types of bread. Naturally, no bread softeners were used in these tests. Bread softness was measured with the Bloom gelometer. For some of you who might not be familiar with this instrument, it measures the weight necessary to force a plunger a definite distance into a slice of bread. For tests of this type to be successful, many factors have to be controlled. These include baking conditions, the temperature at which the bread is wrapped, the temperature of storage, the use of enough samples to give accurate results, and so on. We generally test bread for softness after 24 hours and after 72 hours at 78° F. Some results we have obtained are as shown in table 7. These data show very slight differences in the softness of brew bread and sponge-dough bread after 24 hours and even less difference after 72 hours. Also included for your information are bread pH's showing practically no

Table 7. Compressibility Data

Age of bread	Brew bread	Sponge-dough bread
At 78°F.	*Gm.*	*Gm.*
24 hours.	106.00	99.00
72 hours	157.00	150.00
pH of bread	5.25	5.29

differences between the two types of bread. So, in keeping quality, it seems safe to conclude that there is practically no difference between brew bread and conventional bread.

B. *Brew Bread Quality*

In order to discuss other aspects of bread quality, such as grain, texture, flavor, and so on, I have taken advantage of the excellent talk given by Mr. L. F. Marnett at the annual meeting of the American Society of Bakery Engineers here in Chicago last March. Mr. Marnett spoke on the comparison of brew bread *versus* conventional bread in keeping qualities, flavor, and general characteristics. In preparation for this report, he evaluated only those ferment breads which were made in bakeries employing brew processes as regular production procedures. These brew breads were compared to conventional bread from the same area. In all, bread from 6 areas was scored by a system in which 86 is about average and below 80 definitely substandard. The findings were that the keeping qualities of the brew breads and conventional breads were about equal. The average bread quality of the brew bread was 82.3 and of the conventional bread was 86.3. However, there are two bakeries producing brew bread with average quality scores very close to or better than the competitive conventional bread. Thus, one bakery is producing brew bread with a score of 86.4 as compared to conventional bread from the same area with a score of 86.6. Another bakery is making brew bread with a score of 83.9 as compared to conventional competitive bread with a score of 83.7. What lowered the overall brew bread average score to 82.3 were the other 4 markets with scores ranging from 79.2 to 82.8. Apparently, the bakers in these markets do not have their process under as good control as the two bakers who are making brew bread comparable in quality to conventional bread. Mr. Marnett stated, and I agree with him, that if some bakers can make brew bread of comparable quality to conventional bread, the process should be sound.

20. Conclusion

A. *Variety Goods*

This report has centered mainly around the production of white pan bread by the brew process. The reason for this is fairly simple.

Most of the work done in our laboratories has been on white pan bread. However, a limited amount of work in our laboratories and in the field indicates that most yeast-leavened products can be made by the brew process.

Mr. Anton Bosch, at a meeting of the New York Metropolitan Bakery Production Club last February, presented formulas for the production of several yeast-leavened items by the brew process. These included rye, sweet rye, white, Vienna, whole wheat, and raisin bread; rolls, coffee cakes, buns, danish pastry, and donuts. If anyone is interested in the procedures for making these items by a brew method, the information is available in the May 23, 1955 issue of Baker's Weekly. I might mention here that I have seen these items produced in Mr. Bosch's bakery and was pleasantly surprised at the excellent quality of this large variety of products, all made from one brew formula.

B. *Economics*

In respect to the economics of the brew process, there is very little detailed information as yet. All we can venture to say is that, on paper, it appears that the brew process might be more economical than present production methods.

C. *Future*

As far as the future of the process is concerned, it would be unwise to make a prediction. The American Dry Milk Institute, which as you probably know, has been extremely active in the brew process, recently released some interesting figures at the annual meeting of the American Dry Milk Institute in April of this year. Since the first announcement of its ferment process in April, 1954, it has received 696 written inquiries on the process, 432 coming from bakery operations. At present the institute knows of 34 bakers using a brew process for at least part of their production. If some of the bakers using the brew process can prove to themselves and the rest of us that they can make baked products at least as good as by conventional methods and make them more easily and economically, then we'll probably be witnesses to a major change in our centuries-old traditional methods of making bread. Of course, as with all new developments in any industry, only time and the ingenuity of curious men will give us an answer.

Relationship of Yeast Fermentation, Ingredients, and Bread Processing Methods Upon Finished Bread Quality

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WHITE BREAD IS A BASIC ELEMENT in the American dietary. It is economical, possesses wide acceptability, and is nutritious. It is produced by combining with flour significant proportions of fat-free milk solids, sugar, fat, and the elements of enrichment—thiamin, niacin, riboflavin, and iron. Under the skilful hands of the baker, these materials are converted into well piled, golden crusted, even textured, loaves of tasty bread, full of ready energy.

1. Ingredients

Bread quality begins with the use of quality ingredients. The right type of flour, maintained functionally uniform, is basically important. Having in mind the imposed processing conditions and the desired character of the finished bread, the baker must carefully specify his requirements to the miller in order to obtain flour with the baking characteristics that lend themselves to his purposes.

He must indicate the general nature of the wheat from which the flour is to be ground and supplement this information by the range within which the quantity of protein must fall, together with the mixing characteristics the flour should possess. This will enable the miller to understand the baker's needs intelligently. As a basis for trading, the ash content will be specified to indicate the grade of flour wanted. The diastatic activity level must be prescribed, too, in order to build into the flour the factor of fermentation tolerance. Some bakers try to impart to the miller the degree of maturing that is to be applied. Bright crumb color has appeal to the consumer of white bread and, since flour has an effect on it, the color of flour is specified. Absorption is included in flour specifications. It is of interest to the baker because, within certain limits, it is associated with bread yield.

Formula-wise, white bread is a rather standardized product throughout the baking industry. The enriching ingredients, fat-free milk solids, sugar, and fat are used over a rather limited range of proportions. These proportions are more generally established by the baker on the basis of economic and acceptance considerations rather than for functional reasons. Fat-free milk solids, for example, are used over the range from 0 to 8 percent, with the industry average at approxi-

mately 4 percent. Care must be exercised to use fat-free milk solids which have been superheated adequately to render them compatible to the breadmaking process. They should therefore be pretested by test baking or some other method to cull out any lots of poor baking milk. Generally speaking, as the proportion of milk solids in the formula is increased, absorption must be increased, the oxidation level of the dough can be increased with advantage, and the dough mixing time is extended. In the finished loaf, fat-free milk solids contribute to crust color, toasting properties, and richness of taste. They bring to bread the nutritional properties inherent to fat-free milk solids and supplement the wheat proteins, thereby enhancing their nutritional index.

Sugar is used in the form of cane, beet, or corn sugar up to about 10 percent, with the industry average in the neighborhood of 6 to 8 percent. It contributes to an environment of sufficiency and tolerance for yeast fermentation. As increasing proportions of sugar are used, fermentation in the early stage is slowed up, but this effect does not assume major proportions when ordinary practice is followed. Only when proportions of sugar in excess of 10 percent are used does this effect have to be taken into consideration. Sugar exercises a softening effect upon wheat gluten, so that as sugar is increased it may need to be compensated for by a reduction in absorption. Crust color, toasting properties, and sweetness of taste are the chief contributions of sugar to the quality and characteristics of the resultant bread.

A wide variety of edible oils and fats of animal or vegetable origin may be used in making bread. One study of J. C. Baker (*1*) and his co-workers suggests that there is some advantage in the use of firmer fats containing a relatively high-melting fraction. When fat is added to the bread formula, an improvement in volume and texture is realized up to about 3 to 4 percent. Beyond this point, no further advantage is realized and when a level beyond 6 percent is used, volume begins to diminish and texture becomes heavy and greasy. Fat appears to have a lubricating effect on dough consistency and for this reason a significant increase in the amount of fat used is generally accomplished by a downward compensation on absorption. Along with the effects of fat on loaf volume and crumb texture, it tenders the crust, shortens the crumb, and contributes a note of richness to taste.

In discussing bread ingredients one must not neglect to mention salt. It is seldom used in excess of 2 percent, otherwise the taste of the bread would be too salty. When used at the level of 2 percent or fractionally less, it rounds out the taste of bread by supplying body to it. Salt has a governing or retarding effect upon yeast fermentation. A practical and common application of this fact is to add the salt in the sponge stage in seasons of warm weather in instances where control over fermentation room temperature is lacking.

Two other bread ingredients deserve mention. One is the so-called dough conditioner which embodies elements to minimize the difference

in hardness of water and to supply additional nutrients for yeast. It also contains an oxidizer in the form of a bromate or iodate which enables the baker to make the specific adjustments called for in his shop on this factor. The other is in the form of mono-glycerides, alone, or in combination with di-glycerides. When used to comprise 20 percent of the total amount of fat in the formula, the mono- and di-glycerides exercise a softening effect upon the bread by a mechanism not yet fully understood.

2. Dough

Thirty or more years ago a great deal of the bread made in the United States was made by the straight dough process. Then fermentation was considered the all-important factor contributing to the production of uniformly high quality bread. The sponge and dough process came into vogue later, and for some time great emphasis was placed on the proper adjustment of fermentation in relation to finished bread quality. With advancing experience and knowledge and because of intensification of production, it became apparent that this process embodies great tolerance and lends itself well to high-speed, mechanized production practices.

The baking industry has virtually standardized on the sponge and dough process and ahead lies the probable utilization of "outside ferments" and all that that device implies with respect to simplification and automation of the bread-making process. Curiously, the sponge and dough process has not been subjected to much organized scientific study. It has come into almost universal use, however, because it is so tolerant that nominal variations of time, percent sponge, and temperature do not greatly affect the finished product. Experience has developed that with the currently available types of yeast, a level within the region of 2 percent (compressed yeast basis) is adequate to support fermentation. Much has been learned that permits the intelligent application of the diastatic and proteolytic enzymes, as well as the oxidizing maturing agents. It has already been mentioned that the typical white bread formula utilizes generous proportions of enriching ingredients. In general, therefore, the baker is operating in such an environment of sufficiency with respect to the factors associated with fermentation that attaining bread quality should not be too difficult. Only when some element has been inadvertently overlooked should fermentation be responsible for lapses in quality.

In recent years, much has been learned about physical dough properties and their modification. In order to turn out bread of consistently good quality, emphasis today is being placed upon the mechanical modification of the dough, and fermentation has been relegated to a secondary role. This works hand in hand with the objectives of intensified production and stepped-up efficiency. It calls for a closely controlled shop so far as the adjustment and operation of the dough-

handling equipment is concerned. It paves the way for maintaining a close time schedule. It also develops among the personnel a sense for closely replicating operations from dough to dough, day after day, which of necessity will be reflected in the uniformity and quality of the bread produced.

3. Stages in Bread Production

It may be pertinent to review in their logical order the several stages in the production of bread, keeping in mind their effects upon the finished loaf. Since ingredients have already been discussed, it will be in order to cover briefly the formation of the sponge and its conditioning through fermentation.

This is not a very critical step because sponge mixing time, percent sponge, sponge temperature and sponge fermentation time, and fermentation room temperature may be selected somewhat arbitrarily within the rather well-defined ranges for each of the factors that have evolved as good practice. In forming the sponge, absorption may be adjusted to give average consistency, a soft sponge or a stiff one, according to one's choosing. Variations in sponge consistency result in differences in the rate and extent of rise of the sponge as well as the time at which the sponge will drop, but these variations are not significantly influential in determining the character and quality of the bread. What is desirable is to select a level that is convenient and adhere to it consistently. Replication of conditions and effects in every stage of the breadmaking process is the essence of snop control. To mix the sponge, all that is necessary is to insure that the ingredients are well incorporated. Some attention must be given to controlling the temperature of the sponge from the mixer. Again, it is more a matter of control than effect that should determine choice of the initial sponge temperature. There are some who recommend cool sponges and others who prefer warm sponges. Good bread may be produced from both. Of course, abnormally warm sponges should be avoided to get away from production of secondary fermentation products which may be noted critically in bread flavor. The important thing is to induce mixing room personnel to achieve the preferred initial sponge temperature consistently. Of all the variables involved in the sponge stage, temperature appears to be the most influential one, probably as it affects the rate of enzyme activity rather than because of its influence on yeast activity and the production of carbon dioxide.

The character and quality of the bread does not respond at all sensitively to variations in percent of sponge. The selection of the level at which to work with this factor can very well be left to such considerations as the rate of flow of dough through the makeup units, the size of the dough, and finally, the limitations imposed by floor space in the fermentation room and trough size. It is better to use a percentage of sponge and size of sponge which at maximum rise will be

contained by the trough than to be bothered by knocking down sponges or to incur losses by having sponges overflow the trough.

Sponge time is another factor toward which considerable tolerance is manifested by bread quality. Studies on the effect of this variable on the finished bread by means of many plant- and pilot-scale tests teach that though sponge times from 2 hours, 30 minutes to 6 hours were observed, bread quality is only nominally affected, if at all, so long as the sponge time exceeds 3 hours. Consequently, the sponge time adopted may be the one most convenient to use, provided it is greater than 3 hours.

The temperature rise developed within the sponge is the composite effect of the initial sponge temperature, the heat developed by the biochemical reactions taking place within the sponge, the ambient temperature in which the sponge resides, the area of the sponge available for transfer of heat into or from the environmental atmosphere, the heat transfer capacity of the material from which the trough is fabricated (and which by conduction and radiation removes heat from or introduces it into the sponge) and evaporation from the sponge. It can be appreciated from this that no absolute value can be set. Rather, for a given set of conditions the normal range of experience is established. Then constant observation to note whether or not the normal temperature rise is being experienced is a means only to determine if, up to this point, the shop is under control and conditions are being satisfactorily reproduced.

Some consideration must be given to atmospheric conditions within the fermentation room. The temperature factor has already been discussed, and humidity can be disposed of by pointing out that it should be maintained at a level adequate to prevent crusting and to hold evaporation losses to a minimum. These are merely factors of good practice and exercise little, if any, effect upon bread quality. In February 1942 there was announced a method of fermenting sponges in a restricted space. It was designated "cabinet fermentation." Early reports claimed that cabinet fermentation reduced sponge time to about 2 hours, 15 minutes; permitted the sponges to develop more expansion; resulted in 2 percent extra absorption in the dough; and, in general, yielded bread of better quality. A study of cabinet fermentation by the author (*3*) and his co-workers led to the conclusions that none of the claims could be realized; that the bread resulting from sponges fermented in a cabinet is, for practical purposes, no better in quality than the bread made from sponges fermented in open troughs; but that the cabinet is effective in insulating the sponges against significant fluctuations in fermentation room temperature and automatically provides sufficient humidity to prevent crusting of sponges.

It is in the dough stage where things begin to happen which call for closer adjustment and control. The precise adjustment of absorption is important. It provides the foundation for dough consistency and,

within limits, influences bread yield. As has already been pointed out, it is influenced by the absorptive capacity of the flour and the effects exercised upon it by the various ingredients. It is interrelated with dough mixing time. As absorption is increased, the dough mixing time is lengthened. While there is less hazard from impairment of bread quality by keeping on the conservative side of absorption, there are presented for consideration such factors as accuracy of scaling and machining properties of the dough at the molder. On the other hand, one may not go too high on absorption for then one might encounter stickiness in the makeup, resulting in "doubles" and "pickups," the use of excessive dusting flour, stickups at the molder and the conversion of extra moisture into steam in the oven—each contributing its adverse effect on bread quality. The optimum to which absorption should be adjusted consistently by some objective means is the highest level which, without sacrifice in bread quality and dough machining properties, gives maximum shop efficiency in converting raw materials to finished product under the particular set of conditions that prevail.

Dough-mixing time for development of the extensibility, plasticity, and machinability of the dough is most important. In fact, there is reason to believe that the proper adjustment of this variable is the crux to making bread of uniformly good quality. It determines how well the dough will lend itself to the action of the makeup machinery. Undermixing makes the molder's job more difficult and brings the dough to the oven too resistant to yield easily but effectively to the forces of expansion in the early stage of baking. An underdeveloped dough, in consequence, will manifest a wild break and shred along one side of the loaf, accompanied by a coarse-celled irregular crumb texture. Overmixing results in a dough that will tend to tear at the molder and whose gas retention may be so impaired as not to permit the expanding gasses in the proofbox and the oven to impart to the finished loaf the desired attributes of volume and symmetry. The loaf from such a dough may lack in volume and boldness of symmetry. The crumb will reflect a number of nonuniform cells, and the cells in general will be heavy walled, round, rather than elongated, while crumb color will take on a dull, greyish cast. Moreover, J. C. Baker (*2*) and his coworkers have established that the origin of the gas cell in dough and bread is primarily the air that has been intimately dispersed throughout the dough during mixing and the mechanical modification experienced through the makeup units. The gasses of fermentation do not add to the number of gas cells in the dough but merely migrate to the original gas cells. It can readily be appreciated, therefore, that mixing time in the dough stage has an important bearing on crumb texture. The cells in bread can be no more regular or no finer than the extent to which the air, attached to the flour particles and mechanically incorporated during mixing and makeup, has been dispersed uniformly and intimately throughout the dough mass.

In the dough stage, the dough from the sponge and dough process acts very much like a rapidly acting straight dough. Floor time appears to be essential, perhaps not so much to get gas production under way, as to allow the dough to relax after mixing, so that it can assume a degree of plasticity which will permit the makeup machinery to handle it to best advantage. Excessive floor time must be avoided in order not to bring the dough to the divider with gas production too far along, for this interferes with accurate scaling. Neither must floor time be too short. This would bring the dough to the makeup in a "bucky" state which generally is reflected in a finished loaf with characteristics of underdevelopment. The temperature of the dough out of the mixer, provided it does not exceed the range of accepted practice, is a compensating device available to the baker to effect minor adjustments to the dough to circumvent the effects of a relatively long floor time or to accentuate relaxation in conjunction with a relatively short floor time.

There is no purpose to be served by giving much attention to the divider, rounder, and intermediate proofer except to point out that these machines, too, effect a degree of mechanical modification of the dough. The primary function of the divider is obvious; that of the rounder is to seal up the dough after the divider; while the intermediate proofer relaxes the dough in preparation for the molding operation. For best quality bread, dusting flour should be used frugally at the makeup machinery and intermediate-proof time should be adequate for relaxation of the dough, but not so prolonged as to increase the degassing job required of the sheeter rolls of the molder.

Much of the provision for bread quality built into the dough in the mixer can be lost if proper adjustment of the molder is not realized and maintained. In the course of sheeting, a good job of degassing must be accomplished without tearing the dough, to be followed by a reasonably tight curl and subsequent compression which gently and progressively, but effectively, seals and extends the dough cylinder. An inadequate job of molding can throw the bread characteristics over to the underdeveloped type of texture while over-zealous sheeting, accompanied by tearing of the dough, yields crumb texture symptomatic of overdevelopment. One cannot overemphasize the fact that bread quality is critically influenced by molder performance. More and more, mechanical panning devices are being used. They do not, however, relieve the baker of the responsibility to see that this operation is done properly—otherwise, loaf shape and symmetry may be adversely affected. The dough cylinders must be centered in the pan lengthwise and the seams must be down so as not to present a ready path of exit when the gasses expand in the oven.

In the proofbox, where pan proof takes place, the temperature employed is secondary provided it falls within the range of accepted practice. What is more important is that proofbox conditions be

uniform as to temperature and humidity to assure uniform proof height and prevent crusting of the dough. What is of transcendent importance, and frequently overlooked by bakers, is that the dough must be proofed to just the right height. Much bread suffers from abuse in the proofbox. There it is that the potentialities for good bread, built into the dough in the mixer, can be wiped out. If the dough be underproofed, the loaf will burst along the side while texture will be course and irregular. When the dough is overproofed the loaf will take on a flat symmetry and the texture becomes open, round celled and crumbly. Careful control of the proofed height of the dough must be practiced if uniform bread quality is to be realized.

Vigilance by the baker cannot be relaxed while the dough is in the oven. Here important changes take place. As the temperature of the dough rises, the starches dextrinize so that the greatly stepped-up activity of alpha amylase has something on which to act. At the same time, forces of expansion begin to act through the carbon dioxide and moisture vapor present. When the dough temperature rises further, yeast and enzyme activity is arrested. Then the gluten is "set" by coagulation. Ultimately, the crust is formed and is then caramelized. Care must be exercised not to form the crust prematurely, else volume is throttled, the side bursts, and texture becomes coarse and irregular. The bake should progress cautiously for about the first 5 to 7 minutes to encourage the dough to attain its full volume and symmetry. It is in the oven where the fruits of all previous effort pointed toward peak development of the dough are enjoyed. The objective is to achieve such a measure of extensibility, such an ultimate and intimate dispersion of air in the dough, and such a measure of gas retention that as the temperature of the dough rises in the oven, it will react sensitively to the expansive forces permitting them to do their work effectively and yet so progressively, that close, even elongated cell structure results. These qualities are built into the dough in the mixer, are improved if the subsequent processing steps have been satisfactorily applied, and will be realized in the oven only if diligent control has been maintained.

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Role of Active Dry Yeast in Commercial Bread Manufacture

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THE MATTER COVERED IN THIS PAPER has been presented in three publications of the *American Society of Bakery Engineers.* In Bulletin No. 137, Merritt covered the role of active dry yeast in the baking industry. In the proceedings of the Twenty-ninth Annual Meeting of the American Society of Bakery Engineers, E. Harley Shirley presented *Active Dry Yeast in Bread and Roll Production.* In the same volume of proceedings, Richard Prince presented *Active Dry Yeast in Sweet Goods Production.*

In this paper I shall present the gist of the three papers just cited.

The variety of yeast used in the manufacture of active dry yeast is *Saccharomyces cerevisiae.* While it is the same variety as that of compressed yeast it is a different strain. It is one that has been selected from a large number of strains or it may be one that has been bred for the purpose. It has been, and still is, our practice to seek or to produce by genetic methods a better strain of yeast for drying. Such a search will probably continue as long as yeast is dried.

The growth of yeast for drying is a special process, too. It requires a special diet supplied at a carefully controlled rate and over a longer period of time than that for compressed yeast. The very latest and best practices of sanitation must be followed because the medium in which yeast is grown, the nutrients, and conditions of temperature and aeration all favor the growth of bacteriological contaminants. Only constant vigilance and the best practices can ensure a clean and satisfactory product.

Once the yeast has been grown and has been shown by chemical and bacteriological testing to be suitable for drying it is handled in the customary way of washing, centrifuging, and concentrating for passage through the filter press. The press cake is adjusted with water to just the right consistency for extrusion through a die to form noodles. These noodles are deposited in a uniform layer on a continuous, moving metal belt which conveys them through the dryer.

The dryer is divided into chambers where conditions of temperature and humidity are maintained so that the moisture of the yeast noodles is removed at a rate found, by trial and error methods, to preserve the greatest amount of activity of the yeast. At the end of the drying process, which requires a few hours, the moisture content of the yeast

has been reduced from 65 to 70 percent down to about 8 percent. This latter figure appears to be optimum. If yeast contains more than 8.5 percent moisture it loses keeping quality. If it is dried to less than 7.5 percent moisture it loses in baking strength when freshly made.

When the yeast leaves the dryer it is packed in various sized containers for the ultimate user. These sizes vary from the 2-pound can for export to the 300-pound fiber drum for commercial bakers. The length of time the yeast is kept before it is used determines the method of packing. For domestic commercial use the yeast may be packaged under an atmosphere of air. If the period of shelf life is much longer, as for export, the yeast may be packaged under either nitrogen or a good vacuum. For practical purposes a vacuum is as good as a nitrogen atmosphere. However, a vacuum requires the use of a rigid container such as a metal can.

While active dry yeast is partly dormant it is not entirely so. Because it has a propensity for absorbing water it must be prevented from doing so by suitable packaging. As yeast absorbs water it becomes less dormant and both heat and oxygen then take their toll of its original strength. Consequently, packaging of the yeast and storage of the packaged yeast become very important. The manufacturer can take care of the former, but the user must see to its proper storage. It is wise to protect it until used but also to use it within a reasonable time. It is recommended that deliveries be made once or twice a month.

Active dry yeast is a free-flowing product that is easily scaled off in any necessary amount, usually 40 to 45 percent of the weight of the compressed yeast it replaces. Most bakeries have the scales or balance suitable for weighing it to the nearest ounce or half ounce. It is as easy to weigh as salt and far more accurate than counting or breaking cakes of compressed yeast. It can be scaled in advance as other dry ingredients are. There is no sanitary problem of wrapper disposal.

Active dry yeast should be rehydrated in about four times its weight of water. It is always better to add yeast to water than to add water to yeast. Larger quantities of water do no harm but are not necessary. The water should have a temperature of about 110° F. While water must be removed from yeast slowly, its return must be rapid. Cold water penetrates the yeast cell too slowly. Extremely hot water may injure the yeast. Water at 110° F., plus or minus a few degrees, has been found by practice to be the optimum temperature to use.

When dry yeast is poured into water at 110° F. it requires about 5 minutes for rehydration to be complete. If rehydration is done in a mechanical agitator the best results are secured if the water is in motion when the dry yeast is added. Then the time of rehydration is less than 5 minutes.

As soon as rehydration of the yeast is complete and can be suspended by agitation or stirring it is ready to use. Nothing is gained by giving it more time. The mixer should be charged with the other ingredients

for the sponge or straight dough and the yeast suspension poured or pumped into it.

When active dry yeast is used, a slight adjustment in the formula is usually necessary in order to get the most out of the yeast. An increase in the quantity of water equal to 3 or 4 pounds for each pound of dry yeast is advisable. Two of the pounds merely replace the water accompanying one pound of yeast solids in compressed yeast. The other pound or two of water have been found necessary for better working properties of the dough. These extra pounds account for part of the increased yield.

The yeast food has been increased from 1 to 2 ounces per hundred pounds of flour, depending, some, on the flour. The yeast food acts more as a dough conditioner and, as such, helps to regulate the fermentation.

The time required to mix a sponge with dry yeast is about the same as with compressed yeast. In the dough stage it has been found that the dough reaches full development in a shorter period of time if dry yeast is used. The mixing time can usually be shortened by 20 to 30 percent of the time required with the compressed yeast because of the mellowing action of the yeast on the sponge. In straight doughs the mixing period may also be shortened because of mellowing action during mixing.

The temperature of the sponge or dough mixed with dry yeast can well be 1 or 2 degrees higher than is customary with compressed yeast. Dry yeast is more tolerant to higher temperatures and does a better job if sponges are set about 80°F. and doughs at 82°F.

The fermentation with dry yeast is essentially the same as with compressed yeast. Perhaps it starts out a little slower during the first half hour with dry yeast. Then it catches up. There is a double effect with dry yeast, part of which is due to the dry yeast itself and part to fermentation alone. Because of the mellowing effect of dry yeast in sponges they usually do not rise so high in troughs nor pick up so many degrees of heat during the fermentation. These effects are both to the good. Too often sponges overrun the troughs. Warmer sponges at remix time require more refrigeration capacity. This latter condition is also made easier because dry yeast doughs do not have to be mixed so long for full development.

In doughs remixed from sponges the fermentation or floor time is about the same as for compressed yeast doughs but is more tolerant if interruptions in the schedule occur. Less tendency toward "buckiness" is apparent in doughs made with dry yeast. Floor times have been varied from 30 to 60 minutes with little apparent effect either on the machining properties or the characteristics of the final loaf.

A dough that has been brought along as described above, mixed properly and given the right floor time, is found to have excellent properties at the divider. Its plasticity, dryness, and lack of "buckiness"

permit even dividing and separation on the way to the rounder.

In the rounder it balls up quickly and easily with a minimum of "pilling" and doubling. This is a great relief to the operator responsible for getting the dough into the pockets of the intermediate proofer. The time of relaxing in the intermendiate proofer is normal, running around 6 to 10 minutes, depending on the shop.

Because of the excellent properties built into the dough made with dry yeast it goes to the moulder relaxed and extensible yet dry enough to require a minimum of dusting flour. It forms a loaf uniformly moulded and makes panning a simple operation whether hand or automatic. Twist bread lends itself very well to this operation.

No special conditions in the proofbox are necessary. The bread proofs at about the same rate as if made with compressed yeast. The relative rate of proof depends on the quantity of yeast used, other things being equal. When compressed yeast is replaced with 42 to 44 percent its weight of dry yeast the proof rate with dry yeast would equal or exceed that of compressed yeast.

Conditions in the oven are the same for both types of yeast. Ovenspring is normal and uniform. Break and shred are fully equal or superior to that of compressed yeast. Crust color may be a little redder.

Dry yeast has various effects on doughs that have been touched on and may now be summarized. In the drying of yeast some cells die—a very small percentage of all the cells if drying is carefully controlled. These dead cells and perhaps some of the material on the surface of live cells exert a softening or mellowing effect on the gluten of the dough. This becomes evident during the mixing period when development time is reduced and also in the fermentation when sponges mellow without extreme gas production. This mellowing effect produces a more machinable dough that results in better handling. Because it has less "buckiness" the pan flow is better, filling the pan before proofing is completed. This effect results in a more uniform job of proofing. Inequalities in panning are evened out in the proof.

The natural effect of uniform proof is an even ovenspring. The mellowing effect on the gluten permits formation of thin walled cells of uniform grain and silky texture. Such bread has superior keeping qualities. Bread made of good materials and fermented right will have a good flavor and aroma. The bread made with active dry yeast is no exception. While flavor is something that is hard to define it is something that must be present if the bread is acceptable.

Because of the mellowing effect of dry yeast the machining properties of the dough are very good, being dry but extensible, yielding to dividing, rounding and moulding with a minimum use of dusting flour. Shirley noted this effect with dry yeast and attributed the finer texture and thinner cell walls of the bread to the better mellowing of the gluten. He noticed that there was more pan flow with dry yeast but about the same proof time as with compressed yeast. His resulting loaves

had good break and shred and plenty of tolerance with dry yeast. They had improved flavor and increased keeping qualities, due, he said, to more thorough mellowing of the gluten.

In his paper Prince compared the effects of dry yeast with compressed yeast in standard Danish sweet doughs under carefully controlled conditions as follows:

1. Doughs were retarded at 34°F. after being rolled in.
2. Madeup 8-ounce pieces were rested at 70°F. for 45 minutes.
3. Pieces were proofed at 93°F. and 85 to 90 percent relative humidity for 100 minutes.
4. All pieces were baked at 390°F. for 20 minutes. Baking tests were made after retarding periods of 9, 24, 57, 81, and 105 hours.

Comparisons were made between dry and compressed yeast on the basis of volume, texture, color, and pH value.

Both types of yeast produced goods of about equal volume after 9 hours, but from 24 to 105 hours retarding the dry yeast produced the better volume, indicating greater tolerance.

There was no significant difference in texture between the two types until after the 81-hour period. At a 105-hour retarding period the dry yeast products had texture superior to that of the compressed yeast goods.

Color of the products was the same for both types of yeast at the same retarding period. The actual color score of both types of products decreased as the retarding period was lengthened from 24 to 105 hours.

The effect of both types of yeast on the pH of the finished goods was essentially the same, the difference being 0.1 pH units for 4 of the 5 periods of retarding. In the case of dry yeast the overall pH decrease was 0.55 and 0.60 for compressed yeast.

Among the many advantages of active dry yeast are the following:

a. Requires no refrigeration.
b. Several weeks supply may be brought in one delivery.
c. Is accurate and convenient to weigh.
d. Simple and easy to rehydrate.
e. Efficient in mellowing gluten and fermenting carbohydrates.
f. Economical in first cost and in production of dough.
g. Uniform and stable over necessary period of storage.
h. Improved sanitation control.

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IV. PROBLEMS IN PRODUCTION

General Discussion

CHAIRMAN LIGHT:

THE MEETING is now open for discussion. May I ask that anyone posing a question or offering comments please give his name and affiliation so that we can have a record made of it. Who will be the first discusser?

DR. SKOVHOLT (Quality Bakers of America):

In connection with dry yeast, I noticed that Dr. Merritt stressed the better machinability often obtained because of the activity of the dead yeast cells and so forth. However, does not that occasionally pose a problem of less machinability because of stickiness?

DR. MERRITT:

I would like to suggest that Mr. Gohde who has had a lot of practical experience in the shops answer that question.

MR. RAY GOHDE (Red Star Yeast):

Over the past 4 years in commercial production we have found at no time in working with dry yeast that we have had any stickups in our fermentation. As Dr. Merritt says, there are slight fermentation adjustments that have to be made. We have found that we have drier doughs all the way through with dried yeast than compressed yeasts. Why, I am not able to say. But these are things that have followed from the fermentation room right on through all the machines. In no instance that I know of have we had that trouble.

CHAIRMAN LIGHT:

Who else has a question?

DR. D. K. TRESSLER:

What is the optimum humidity for dehydrating active dry yeast?

CHAIRMAN LIGHT:

Dr. Merritt, are you the active dry yeast expert here, or would you call on someone else?

DR. MERRITT:

I couldn't answer it myself. I think Dr. Frey helped establish the precise specifications for temperature and humidity. Maybe he would like to answer it.

DR. CHARLES N. FREY:

Mr. Chairman, that is a very difficult question. I was hoping somebody else would take it. I don't think we can answer that directly, Dr. Tressler. The curve indicates a changing temperature, humidity, and so your curve using a belt drier (I don't happen to have the curve

here that I used to follow) shows you have a period in the first portion of the dehydration where you are taking off the moisture rather rapidly. The temperature, that is, the yeast temperature, is lowered by the rapid rate of evaporation. Then you gradually come down to a point where the moisture is coming off rather slowly, and the yeast temperature has a tendency to rise. To get your moisture down to 7 or 8 percent you have to be very careful so that you don't overheat your yeast. If you can keep the temperature below 70 degrees—the yeast temperature itself—why you are very fortunate. The lower you can get it, the better.

When we first started, we couldn't get a dry yeast that had any commercial value unless we froze the yeast and dehydrated while it was practically in the frozen state, that is, just a few degrees above. We dried in 22 hours in order to avoid destruction of the enzymes, and this question of Dr. Spiegleman's came up as to balancing up the enzymes. You are likely to upset the enzyme balance if you allow autolysis to take place by rising temperatures.

CHAIRMAN LIGHT:

Thank you very much. I think that was a pretty tough question. I suspect you could get a number of answers. If you wish to set an optimum condition, I doubt whether you could get any unanimity of opinion as to what the optimum is.

DR. TRESSLER:

Perhaps the question should be phrased some other way. If you use real low humidity in dehydrating, is it going to run into a drying rate which will reduce the vitality of your active dry yeast?

CHAIRMAN LIGHT:

Can we get some opinion from the audience on this question?

DR. FREY:

I think I can add one thing. If you had the relative humidity so low that you dehydrated in, say, a few minutes, and you can do this, it would be harmful. No one, as far as I know, has been able to develop a good stability in a yeast which has been dehydrated in a few minutes. This refers to belt or tray driers.

CHAIRMAN LIGHT:

Are there any other comments on that question?

DR. MAX MILNER (Kansas State College):

Just one question. This is pointed to Dr. Frey. What is the limiting moisture content of enzymatic activity in yeast?

DR. FREY:

Maybe there is some information that has been developed since I was connected with the experimental work, but if you took the moisture down to 5 or 6 percent, you reduced the activity of the yeast, and if you took the moisture down to only 10 percent, the yeast didn't keep very well. So there is some place around 7 or 8 percent, it seems

to me, that is about right for tray or endless belt drying and this percent falls within the Army specifications.

Now, if you change something else, perhaps you could lower the moisture. In other words, it may be a different rate of drying at lower temperature or a change in some ingredient; for example, you might take out some of the salts, thereby lowering the salt content or some similar change. You might then be able to get the moisture down lower than the 7 to 8 percent figure and retain the life of the cell. But under the conditions of our ordinary operation, that is what we found.

CHAIRMAN LIGHT:

I will offer a comment on this question. We have dried yeasts down to 4 or 5 percent, but when we do, it requires a special rehydration before this yeast will reactivate fully, but the enzymes are not destroyed at those moisture levels. Is that your question?

DR. MILNER:

I would like to state that perhaps the question is an impractical one. I can see that as you dry these proteins, which happen to be enzymes also, you reach some stage of hydration where they would no longer be catalytic. They would lose their catalytic effect. This question is purely theoretical. What reduction in moisture does one need to obtain in these proteins before they lose their catalytic effect even though it might be a reversible effect?

DR. SPIEGELMAN:

I think one can go much further down in humidity without losing activity. We routinely isolate enzymes and dry them, I believe, more effectively and more crudely than what I understand by inference to be the method employed in industry. I can't be sure, but I would say that the problem being faced here is what Dr. Frey refers to as a matter of balance rather than a matter of enzyme destruction.

CHAIRMAN LIGHT:

Are there any other comments or questions?

DR. RUDOLPH SANDSTEDT (University of Nebraska):

Mr. Chairman, surely someone has dried dry yeast by, say, a freeze-dry method and dried it clear down to an exceedingly low moisture without destroying the enzymes. We are sure that there are instances where enzyme systems will exist with exceedingly low moistures, so that is probably true in yeast also, isn't it?

CHAIRMAN LIGHT:

Yes, I think it would be true.

DR. CHARLES FERRARI (Short Milling Company):

I would like to suggest since you are drying an intact cell with cell walls containing mineral substances, that during the dehydration process you are building up the mineral concentration. As you do that, the very fact that the concentration ratio of minerals to protein is increasing might have some effect on proteins because it has both an

effect on solubility and precipitation, depending on what the minerals are.

DR. SPIEGLEMAN:

I believe that what Dr. Sandstedt mentioned is relevant here. If we induce a yeast to make some enzyme and dry it down, we do so from the frozen state using a megavac pump on a small volume for a period of about 10 hours. I believe that we get down well below the 5 percent moisture. However, we maintain full enzyme activity and, to my knowledge, do not lose any under these conditions. If instead of drying the cells down you isolate the enzyme and purify it, you can repeat this lyophilization process from the frozen state on the purified enzyme without losing activity. It would seem then that with respect to the specific enzymes we have examined, the concentration of internal factors in cells like mineral elements does not lead to their destruction. I should like, however, to point out that what you are asking the yeast to do is far more complicated than exhibiting the activity of one enzyme. You are asking the dried preparation to carry through the entire Meyerhof-Embden cycle. There are lots of things that could go wrong that would interfere with its functioning.

CHAIRMAN LIGHT:

When you have dried yeast down by freeze drying, is the cell still viable?

DR. SPIEGELMAN:

Oh, no. You get something in the order of 1×10^{-1} to 1×10^{-3} survivors. It's pretty low.

CHAIRMAN LIGHT:

Essentially killed.

DR. H. J. PEPPLER (Red Star Yeast):

I would like to answer all those questions with reference to some data which is now in the press on spray-dried yeast, that can recover even though they have attained moisture content of about 2½ percent. The yeasts are viable, and they will make a loaf of bread.

MR. PAUL HOLTON (The Holton Company):

What special spray dry technique does it employ?

DR. PEPPLER:

I can't tell you.

DR. HERMAN PFAFF (University of California):

In connection with Dr. Milner's question, we might perhaps also have taken into account that some of the water is present as water of crystallization of trehalose since dry yeast is known to contain 15 or 16 percent trehalose. It means probably about 1½ percent of water is present as water of crystallization, if the trehalose is present in the crystallized form in the dried yeast.

CHAIRMAN LIGHT:

Are there other questions or comments?

DR. MILNER:

What is the importance, if any, of the presence of enzymatic substrates in the dry yeast at the time of drying on the ultimate viability of the yeast? In other words, can we conceive that all that is necessary is simply the protein in dry and active state which can go back to work upon rehydration or must they have, as in the case of trehalose, all substrates in the Myerhof cycle to support the activity to initiate it at the proper time and to keep the cycle going?

CHAIRMAN LIGHT:

Do you have any comment, Dr. Spiegelman?

DR. SPIEGELMAN:

I think you are certainly right. If one introduces glucose into a freshly prepared yeast extract (i.e., a Lebeder juice), there is a lag before the onset of the fermentation. The reason for this is that in making such preparations you dilute the hexosediphosphate, and possibly some other intermediates, to concentrations too low for the cycle to get started. The lag period is the time required for the preparation to synthesize enough hexosediphosphate to permit the cycle to initiate activity. The same situation would, of course, be true for any intermediate. Thus, even if the drying procedure left the enzymes completely intact, you might still get an inactive preparation by virtue of having leached out or lost one of the intermediates or cofactors in the middle of the cycle. It would have low activity until the missing factor was brought up to the proper level.

CAIRMAN LIGHT:

Does that answer your question?

DR. SANDSTEDT:

I want to ask a question about this system. We ruled out the precursor as a possibility, but then the RNA was necessary. How did you distinguish between the RNA as a precursor and as the template—I guess that was what you called it—for the building of an enzyme?

DR. SPIEGELMAN:

RNA is composed of units completely different from enzymes. We know from the isolation of the protein that enzymes are composed only of amino acids so RNA couldn't be the precursor. Therefore, it must be the template.

CHAIRMAN LIGHT:

Will you clarify one other point while you are up? Did you supply amino acids to yeast cells which were completely intact, with internally available free amino acids?

DR. SPIEGELMAN:

I neglected to mention this point. It is clear that the experiment which was performed to decide between hypotheses 2 and 3 could not have been done with yeast without running into difficulties of interpretation since yeasts possess an internal pool. What we actually employed then was *E. coli* which has virtually no free internal amino

acids. With this system one can perform an uncomplicated experiment and get a definitive answer.

CHAIRMAN LIGHT: But you supplied the amino acids needed to make the enzyme from the outside which were not labeled.

DR. SPIEGELMAN:

We let the cell make them from ammonia and lactate.

DR. HARRY E. GORESLINE (QMFCI):

You made the statement that when the RNA had become depleted, you no longer had this template effect. Now, does it disappear during this template effect? In other words, as your enzymes are formed, do you have a decrease in your RNA, or is it merely a catalyst? As you described it, I visualized it as a welder puts all the pieces down here, and then all of a sudden he turns on the juice, and they all go together.

DR. SPIEGELMAN:

The welder doesn't disappear.

DR. GORESLINE:

That is what I am getting at.

DR. SPIEGELMAN:

This much is clear. It cannot be that an RNA template of the size of protein molecule falls completely to pieces every time it makes an enzyme molecule because the turnover rate of RNA calculated on a molecular basis is just too low. Therefore, we are inclined to believe that in the template functioning, a small portion of it is destroyed—not much, but enough to make the RNA unusable for the next step until it is repaired.

Maybe some of the bonds between phosphate and ribose are broken —not many. Nucleotides do not come in and go out with any great rapidity involving a large percentage of the molecules. I might point out this, by the way, that logically, we can make a distinction between these 2 possibilities: (1) every time an RNA template functions, it suffers sufficient damage to require repair via nucleic acid metabolism; (2) the only time the RNA can make a protein molecule is when it is making part of itself; either of these 2 would explain our data, namely, that RNA synthesis is mandatorily coupled to protein synthesis.

There is a way of making a distinction between these two experimentally, but only in an *in vitro* system. You can state the way, but it is going to be hard to do it. If the first hypothesis is right, it should be possible, by getting enough of the right kind of RNA into these fragmented or protoplasmic preparations, to have them make some enzyme even without any new nucleic acid synthesis going on. They will do what they can and then fall apart. If the second hypothesis is true, it should never be possible to have enzyme formation under those conditions unless you supply RNA precursors at the same time. Thus, in principle we should be able to settle that issue.

DR. PFAFF (University of California):

I would like to ask you a question, too. How, in the light of present

findings, do you explain the earlier finding of competitive interactions between enzymes like fructosidase and glucosidase in yeast?

DR. SPIEGELMAN:

We can explain at least certain aspects far better now than we could then. It is a very interesting situation and probably when these investigations are put to practical use, it is this level of the system which will probably turn out to be the more important. If you ask a cell to make two enzymes at once, it generally can't do it. Thus, if 2 inducers are presented simultaneously, in general only 1 enzyme is fabricated and only when the first inducer is used up or removed is the second enzyme synthesized. There is evidently some kind of interference, and the interference seems to involve some nitrogenous compounds.

This type of competition which exists turns out to be different for different enzyme-forming systems. For example, if we consider the case of maltase and galactozymase, it turns out that the maltase is a much better competitor for nucleotides than is the galactozymase. Thus, if the nucleotides are in short supply, it is the maltase which is preferentially formed even though both inducers are present. If you elevate the nucleotide supply artifically, both enzymes can be formed. You can demonstrate this competition for nucleotide supply very rapidly and prettily in certain forms. Thus, one can, by throwing in an excess of preformed amino acids, depress temporarily the internal supply of nucleotides. Under those conditions, the formation of certain enzymes is completely eliminated even though the additional supply of amino acids should in principle make enzyme synthesis easier. In any case, it can be shown that in certain cases the explanation for the competitive interaction does lie at the level of nucleic acid precursors. In other cases it appears to involve competition for available amino acids. A number of these interacting systems will have to be studied carefully before the whole picture will emerge. There may be other factors which we don't know about and which have yet to be pinpointed and identified.

MR. WELKER J. BECHTEL (American Institute of Baking):

I would like to inquire whether anyone has tried bread fermentation using enzyme systems separate from the living yeast cells, and if so, would that be a possible means of getting a stable fermenting material?

CHAIRMAN LIGHT:

Dr. Frey, would you want to discuss this question?

DR. FREY:

That is an awfully hard one. It has been tried, but it is similar to our attempts to dry yeast down to a very low percentage of moisture. To dry down to zero and retain viability has not been possible. To separate the fermenting enzymes from the cell is a possibility, but nobody, as far as I know, has succeeded in doing it effectively. That is, I know the European laboratories have been concerned with it, and they have tried to extract all the enzymes, the whole complex, but they

didn't succeed in getting them all. In such experiments usually one or several enzymes are lost and this may result in either accelerating or inhibiting certain reactions. The experiments were not successful; at present we have no complete explanation. Somebody, I think, is going to do it sometime.

CHAIRMAN LIGHT:

Are there any other comments on that question?

DR. SANDSTEDT:

Mr. Chairman, in the early days when we were first starting work on some of these problems, I thought that that should be an easy thing to do because in the literature you read that they can extract the enzymes from yeast and make them work, but if you go to the laboratory and try it, you will find that the amount of active fermentation that you get without any yeast cell material being present is going to be exceedingly low.

I think some of the things that Dr. Spiegelman was talking about give the answer, because of the fact that it is just practically impossible to prepare good active yeast extracts that will give any fermentation.

CHAIRMAN LIGHT:

Do you have a question, Dr. Mitchell?

DR. MITCHELL:

I had a question of Dr. Spiegelman about the relative proportion of RNA and DNA in the yeast. It is my understanding that there is very little DNA in yeast.

DR. SPIEGELMAN:

Fifty to one. It is about 50 parts RNA to 1 part DNA.

DR. MITCHELL:

Fifty to one.

DR. SPIEGELMAN:

The amount of DNA is lower than in other cells proportionally to RNA. This is what has made yeast genetics rather difficult because it is difficult to do good cytology on chromosomes so small that most people can't agree even where they are, let alone number.

DR. MORRIS MEAD (National Yeast):

I want to ask Dr. Spiegelman what is the source of the amino acid pool in the yeast. As yeast absorbs nitrogen, I believe the tendency is for it immediately to synthesize protein.

DR. SPIEGELMAN:

No.

DR. MEAD:

It always has to keep a reserve of amino acid?

DR. SPIEGELMAN:

Oh, yes. We have looked at a rather wide variety of different yeast types, both haploid and diploid, and we have found no exceptions to

the rule that about 5 percent of the total amino acid content can be extracted with cold trichloracetic acid.

We have been able to demonstrate the existence in this extractable pool of 18 of the usual 20 amino acids that you find.

CHAIRMAN LIGHT:

Did you tell us how you depleted your free amino acid pool?

DR. SPIEGELMAN:

We accomplished that by vigorous aeration of yeast suspended in phosphate-buffer-glucose solution. The free amino acid pool disappears very quickly. Within about 80 minutes it is down to about 10 percent of normal.

To get rid of the other 10 percent is quite a job. In fact, we have never succeeded in getting it below 2 percent of normal. There is always a residue that just doesn't go anywhere.

DR. FREY:

What is the protein content of the yeast when you get through?

DR. SPIEGELMAN:

Not much higher.

DR. FREY:

The protein content has been lowered. You have taken it down. Is that what you are saying?

DR. SPIEGELMAN:

Ninety percent of the free amino acid pool has gone into the protein fraction so, essentially, what you have done is to increase your protein fraction by a factor of about 3 percent by this kind of starvation procedure.

DR. PFAFF: You must increase your carbohydrate during that time.

DR. SPIEGELMAN:

Yes.

DR. PFAFF: The relative percentage is lower.

DR. SPIEGELMAN: The relative percentage is lower. That is true.

DR. FREY: You can often bring the protein down, as you probably know, by fermenting sugar with aeration from 50 percent down to 40 percent in a very short time, and the carbohydrate content probably has gone up.

DR. SPIEGELMAN:

Yes, the absolute amount of protein per cell has increased, but the percentage has decreased because you are piling up polysaccharides.

CHAIRMAN LIGHT:

Do you have any idea what the drop is in the apparent protein as nitrogen, that is, the amount of nitrogen you have in the cells after feeding the sugar?

DR. SPIEGELMAN:

None of it leaks out.

CHAIRMAN LIGHT:

What is your percentage, on a solids basis? You put in carbohydrates and the nitrogen is the same.

DR. SPIEGELMAN:

I just don't remember offhand.

DR. PFAFF:

I would like to ask Dr. Mitchell—you mentioned you isolated yeast from samples that were stored at high temperature. Did these isolated cultures have any better storage qualities than the original yeast?

DR. MITCHELL:

The stability stresses have not been conducted on any of these cultures which I talked about this morning.

CHAIRMAN LIGHT:

Are there other comments or questions?

DR. FERRARI:

Mr. Chairman, not being a dried yeast expert, maybe I can stick out my neck by making an observation and asking a question. Most methods for drying yeast are the application of heat or freeze drying, and as yeast can only stand a certain amount of heat energy before you hurt it, perhaps some other drying method might be preferable. Now, I am thinking of some such thing as this. If yeast were suspended in proper salt solutions, of proper concentration, you might dehydrate the yeast quite considerably in that sort of a medium after which you could separate it by filtration and then the amount of water you would have to remove by heat would be very considerably less. Has that ever been done?

CHAIRMAN LIGHT:

Whom do we have on our panel that is a good expert on that? How about you, Dr. Merritt?

DR. MERRITT:

I don't think I could answer it.

CHAIRMAN LIGHT:

You are working overtime on this, aren't you, Dr. Frey?

DR. FREY:

I am the most ignorant! Some of these things have been tried, but until you try them all under the proper conditions, you can't tell whether they work. The use of a number of salts was tried many years ago, and we thought at one time that this method was going to work successfully, but it did not. To go back to Dr. Spiegelman's statement, his question, that is, whether some of these substances—enzyme systems—are actually destroyed or inhibited, I can't answer. We did find that in many of the types of yeast used and with the experimental methods employed, we could never rejuvenate certain of the enzyme systems. Your inverting action sometimes just disappears, although you couldn't say it is destroyed. It may have been in the cell but inactivated in some way. We couldn't get the system to work together again. Where you have such a vast system of enzymes that have to be in balance and work together, you can see that if you inactivate just one or two in that system, they are all off balance.

CHAIRMAN LIGHT:

It seems to me Dr. Spiegelman's presentation today probably points to the explanation for some of the practical experiences in the yeast field. One, for example, involves making a dry yeast which has a low percentage of viable cells. You believe the enzymes of the yeast should be active, but the viability is poor. In the process of making this enzymically active dry yeast, you have depleted your free amino acid pool. Without an adequate free amino acid pool, and without the viability of the cell to replenish the pool, you have no mechanism for making enzymes, even though your cells are put into a nutrient medium and you have the proper substrate, the inducers available.

DR. SPIEGELMAN:

Isn't it true that actually the sugar they have to get used to is different from what they are normally grown up in?

DR. FREY:

Yes. For instance, if you use a yeast that is grown in maltose, it develops quite a little maltase and ferments maltose readily in the dough, but how do you explain your lactose-maltose picture here in connection with Michaelis's constant, Michaelis's equation, for fructose and glucose, for example, preference for glucose over fructose when the two are given together?

DR. SPIEGELMAN:

On the enzyme.

DR. FREY:

That is, a preferential fermentation of glucose over fructose.

DR. SPIEGELMAN:

I think the reason for the glucose-fructose story is simply that in the case of fructose, the cell has to make a fructose kinase. Unless they have that enzyme, they can't use the fructose.

DR. FREY:

It seems to follow that pattern.

DR. SPIEGELMAN:

It lowers the old isotherm. Anything that has to absorb will follow that law, whether a substrate or inducer.

DR. FREY:

That puzzles me because you would think under certain conditions it would not follow the laws. If it happened to be an environmental condition previous to the addition that conditioned it, or if adjusted to both sugars, prior to that time, you would think the system would not be expressed mathematically according to Michaelis's equation.

DR. SPIEGELMAN:

As a matter of fact, we did such experiments in order to try to examine the question of the role of inducers. The question was: does the inducer have to combine with enzyme in order to function? What we did then was to examine the Miachaelis's constant of a substance as an inducer and as a substrate. If in acting as an inducer it also acted

as a substrate complex, then the two constants should be the same. The answer is they are widely different, about two-hundred-fold apart. But the shape of the inducer concentration *versus* rate of enzyme synthesis curve is a typical Michaelis plot.

DR. FREY:

It followed the curve.

DR. SPIEGELMAN:

Oh, yes.

DR. FREY:

I know that it does in the case of fructose and glucose, and you state that it does in this case also.

DR. PFAFF:

You must consider that fructose is mostly in an unfermentable form. There must be equilibrium. The fermentable fraction can be taken up by the yeast, so if you put fructose in high concentration, it is fermented as fast as glucose.

DR. FREY:

These are all at low concentrations.

DR. PFAFF:

I know.

CHAIRMAN LIGHT:

I have one more question for Dr. Merritt. In your report, you mentioned the fact that your active dry yeast starts out slowly and later catches up. Does that tie in with Dr. Spiegleman's idea that you would be depleting your yeast of its free amino acid pool? You would need a little time to regenerate that free amino acid pool before enzymes can be formed. Would that tie in?

DR. SPIEGELMAN:

Yes.

DR. GORESLINE:

We know that this active dried yeast when it is first dried, we will say, has 100 percent activity. Over the period of time of storage this activity is slowly lost. I was wondering, Dr. Spiegleman, whether there is any explanation for the disappearance of something that you might call the spark of energy of life? I realize it is a protoplasm proposition. I wondered if certain enzyme systems become depleted to the point where they cannot regenerate is one explanation why these cells die because they just slowly lose ability to start over again. As I understand it, you can demonstrate that the enzyme system is still there, but something is lacking to give them the spark to actually start on the regeneration. Have you any explanation as to what part of the system it is that would become depleted?

DR. SPIEGELMAN:

No.

DR. GORESLINE:

If we knew, we might protect it.

DR. SPIEGELMAN:

I would be willing to guess that there is more than one. The thing that can be lost and be irreplaceable is anything which is involved in its own formation; there are lots of such things in a cell. Take a trivial case every bacteriologist meets up with when he tries to grow an organism which requires CO_2. Deprive them of CO_2 and they will not grow. But give them a little CO_2 and carbohydrate and then they get started. They can then keep going since they now can manufacture their own CO_2.

DR. GORESLINE:

Is there a possibility, with the amount of moisture that is actually present, that there is a slow enzyme change that eventually depletes one of these systems? We think of it as being down to a dry state where it won't work. We were wondering whether it slowly depletes itself because of low moisture content.

DR. SPIEGELMAN:

It could be.

DR. FREY:

You have a curious thing in connection with fermentation. It is theoretically an anaerobic process. If you add a little oxygen, just a little amount, it activates the cell and the rate increases, and there is no lag period. So that sometimes the very opposite thing, the inhibitor as you said, is a stimulator.

DR. SANDSTEDT:

I think what Dr. Goresline wants to know is what can we do to keep from growing old. Isn't that the answer? (Laughter).

CHAIRMAN LIGHT:

Are there more questions? We have had good discussion so far. Dr. Sandstedt has given us a most fitting final remark! I want to express the appreciation of the committee to all of the speakers participating in the program and to all the people who have attended and entered into the discussion. I think it has been a very much worthwhile meeting.

V. APPENDIX

Abstracts of the Papers
History of the Development of Active Dry Yeast

CHARLES N. FREY
Scarsdale, New York

THE DEVELOPMENT OF THE MODERN CONCEPT of fermentation is discussed and some of the first attempts to manufacture dry yeast are mentioned. The influence of the vitalistic theory of fermentation in stimulating research and the influence of cogent thinking concerning the metabolic activities of yeast and the mechanism by which the cell accomplishes the transformation of sugar and other nutrients into cell substance and carbon dioxide is discussed briefly.

Drying yeast in order to retain its quality and fermentation rate has been a long and arduous research. Many scientific and industrial researchers have given years of activity which contributed greatly to the successful fulfillment of this endeavor.

The physiological state of the cell has been found to play a role in determining the stability of the cell during and following dehydration. Some of the factors that have been found to be involved are the type of cell—genetically, its maturity, the nitrogen content of the cell, glycogen content, and the nature of the carbohydrates of the cell wall and reserve tissue. Mineral nutrition also plays an important part in the life and stability of the cell.

The dehydration of yeast must be conducted in such manner that very little impairment of the normal state of the cell takes place. The delicate balance of the complicated enzyme systems must remain essentially unaltered. The cell must function normally in the dough; that is, be free from reducing action and the production of abnormal byproducts. Its ability to reproduce, and to ferment sugar in the dough should be essentially like that of the undehydrated cell from which it is derived.

Genetic Principles As Tools for the Taxonomic Study of Yeasts

LYNFERD J. WICKERHAM
Northern Utilization Research Branch
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Peoria, Illinois

THE MOST PRIMITIVE SPECIES of the genus *Hansenula* live in association with trees and bark beetles. They are exclusively haploid, their colonies mucoid, their ability to ferment and to produce vitamins is slight. More recently evolved species are almost exclusively diploid. As the percentage of diploid cells increased, the species became associated with deciduous trees, and later species became free-living in soil. The colony appearance changed from mucoid to glistening butyrous, and finally to mat; more sugars were fermented and with greater rapidity, fewer vitamins were required, with the predominantly diploid species needing none from their environment. No two species of *Hansenula*, regardless of how closely related, have been hybridized. *Hansenula* is compared with a related genus to be proposed under the name *Dekkeromyces*. Species of the latter genus hydridizes readily, and a very simple procedure allows the hybridization of such sexually dissimilar species as a heterothallic haploid with a homothallic diploid. The analysis of segregation of parental characteristics which occurs in hybrids at sporulation is likewise effected by very simple procedures.

Conditioning Yeasts for the Production of Enzymes

S. SPIEGELMAN
University of Illinois

THE EVOLUTION OF A PROCEDURE for conditioning yeast to make specific enzymes in adequate amounts presupposes an adequate understanding of the factors controlling enzyme formation. The major purpose of the present discussion is to summarize the knowledge which has been accumulated in the past several years relevant to this problem. In principle, the study of enzyme synthesis breaks down naturally into attempts to provide answers to the following three questions.

1. What is the nature of the precursor material converted into active enzyme?
2. What is the part of the cell machinery that performs this conversion?
3. What are the roles of the chemical agents known as "inducers" which are apparently required to stimulate specifically the synthesis of the particular enzymes?

The data available are discussed from the point of view of the light

they throw on these questions. The evidence clearly demonstrates that the cell fabricates enzyme molecules directly from the simplest components of proteins, i.e., the amino acids. No evidence for a complex precursor or intermediate stage emerged from these studies. Further, the experiments indicate that ribonucleic acids are the critical components of the machinery which puts the enzyme molecules together.

Role of Yeast Fermentation and Baking in Flavor Development in Baked Products

JOHN C. BAKER
Monrovia, California

DOUGHS WERE HEATED as small dumplings and as a resistor in an alternating current. Yeastless dough changes flavor in such heatings when chemical reactions occur. Freshly added yeast causes no other appreciable change. When allowed to ferment, flavors develop which largely distill off during continued heating.

Bread cooked conventionally forms a crust which contributes much additional flavor and thus develops the characteristic taste of bread. Yeast competes for the same substances that react to give crust color. The reactions occurring and compounds formed during fermentation and baking are discussed.

Evaluation of the Bread Baking Properties of Various Genera and Species of Yeasts

J. H. MITCHELL, JR.
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OF THE 79 CULTURES TESTED in bread doughs, 17 did not leaven the dough as determined by lack of an expansion during an incubation period of two hours at a temperature of approximately 90°F. The 62 other cultures produced bread ranging from very acceptable to nonacceptable. Criteria used to judge quality of the loaf were volume, flavor, odor, texture, and "eating quality," a term used to describe the feel of the bread in the mouth. Evaluations were made by an "expert" panel of six people.

When all of the yeasts had been tested, a priority list of cultures was set up (table 1), for detailed propagation and storage studies. Group I shows those having highest priority, followed in order of priority by Groups II, III, and IV. The organisms included in these groups total 49. Seventeen additional yeasts did not cause an expansion of the dough in baking tests, while 13 others did not produce acceptable bread.

In comparison with the known growth rates of *S. cerevisiae* the cultures in Group I may be divided into subgroups, table 2. Subgroup A consists of 4 yeasts having growth rates similar to that of *S. cerevisiae;*

subgroup AA, 1 yeast having a growth rate of 10 percent faster; subgroup B, 7 yeasts, having growth rates 20 percent lower; subgroup C, 3 yeasts, having growth rates 30 percent lower; subgroup F, 1 yeast, having a growth rate 60 percent lower; and subgroup I, 1 yeast having a growth rate of 90 percent lower.

Stability evaluations of the promising yeasts discovered in this screening operation have not yet been made. It is anticipated that this work will go forward within the near future.

Table 1. Priority List of Yeast Cultures

Group I	Group II	Group III	Group IV
H–37 (a hybrid)	*S. carlsbergensis var. polymorphus*	*S. fragilis* 665	*Z. fermentati*
H–44 (a hybrid)	Colony #10 (a hybrid)	*S. intermedius*	*S. fragilis* #1109 (a hybrid)
Distillery yeast 1341	*S. logos*	*S. chodati*	H–87d
QM top	*S. cerevisiae* 684	*S. cerevisiae* #B–1779	*S. pombe*
QM 6b	*S. cerevisiae* 132	#B–898	*T. colliculosa*
Z. lactis	*S. cerevisiae* 978	*S. tubeformis*	*T. dattila*
S. carlsbergensis mandshuricus	*S. cerevisiae* 1188	*S. fragilis* 876	*H. subpelliculosa*
S. chevalier	*S. carlsbergensis*	*S. ellipoidius thermophilus*	*C. arborae*
Z. drosophilae	*Hawaiian food* 254	*S. oviformis*	
S. cerevisiae thermophilus	*S. cerevisiae* 643	*C. tropicalis* 1767	
S. cerevisiae 567		*S. osmophilus*	
S. cerevisiae 1386		*S. diastaticus*	
C. pseudotropicalis			
S. italicus			
Undetermined B–932a			
S. fragilis #610			
S. marxianus #1052			
C. tropicalis 1410			

Table 2. Subgroups Based on Comparative Growth Rates

Subgroup A	Subgroup AA	Subgroup B	Subgroup C	Subgroup F	Subgroup I
QM–top	*Z. drosophilae*	*C. pseudotropicalis*	H-37 (1140 x 610)		
QM–6b		*S. marxianus* 1052	H-44 (1140 x 1052)	Unidentified B–932a	*S. italicus* 1373
S. cerevisiae 567		*C. tropicalis* 1410	Distillery 1341		
S. cerevisiae 1386		*S. carlsbergensis mandshuricus*			
		S. chevalier			
		S. cerevisiae thermophilus			
		S. fragilis 610			

Practical and Theoretical Aspects of Brew Fermentation

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THE BREW FERMENTATION process for the production of yeast-leavened bakery products can be compared to a conventional sponge-dough process in which flour is omitted from the sponge and to which a source of yeast-fermentable carbohydrate is added. During the brew-fermentation period compounds responsible for a substantial part of the flavor of the finished bread are formed by the action of yeast on the fermentable carbohydrates.

The effects of a number of variables in the brew and dough stages have been experimentally determined and the results will be discussed with emphasis on the necessity for regulating the brew acidity in the neighborhood of pH 5. Brew stability (for a practical length of time, i.e., 24 hours) is strongly influenced by pH control. Means for controlling brew pH include incorporation of substances having buffering action, i.e., inorganic compounds (calcium carbonate) or proteins (nonfat dry milk solids) in the brew.

Brew bread is comparable in keeping quality to conventional bread, and as presently produced by some bakeries it is equivalent in overall bread score, including flavor.

Practically all yeast-leavened goods, as commonly produced by the baking industry, can be prepared by the brew process.

Relationship of Yeast Fermentation, Ingredients, and Bread Processing Methods Upon Finished Bread Quality

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THE RELATIONSHIP OF INGREDIENTS, yeast fermentation, mechanical modification of dough, and processing steps to finished commercial bread quality are discussed.

Commercially produced bread is almost universally made over the sponge and dough process. It is an inherently tolerant process and adapted to mechanized, high-speed-production methods. Whereas formerly, yeast fermentation was considered the all-important factor contributing to the production of uniform, high quality bread, advancing experience and knowledge provides for yeast fermentation to go forward in an environment of sufficiency and has focused attention on physical dough characteristics.

Now the physical development of dough, chiefly by mechanical modification in the mixer, is the major factor in determining bread quality. By reducing the dough to a state of yielding extensibility (without impairment of gas retention), by dispersing air intimately and uniformly throughout the dough, the groundwork is laid down for the attainment of a loaf with ample volume, good, bold symmetry, with a crumb texture characterized by myriads of thin-walled, elongated cells evenly distributed throughout the bread.

Subsequent processing steps, through that of baking, must be carried out to conserve or improve the potentialities for quality bread rather than to wipe them out by improper dough handling.

Role of Active Dry Yeast in Commercial Bread Manufacture

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YEAST CULTURE used in the production of active dry yeast is Saccharomyces cerevisiae variety. It is fed special nutrients and is especially conditioned for drying. The yeast approaches a partially dormant state with removal of moisture to a level of about 8 percent.

Initial activity of dry yeast is preserved with good packaging and cool and dry storage. Use during a safe period is advised, depending on various factors.

Methods of use and procedures to follow are described. Results with dry yeast in both bread and sweet goods are cited.

Reasons why dry yeast has met with acceptance are summarized as convenience, accuracy, economy, and performance.

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