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TITLE A Study Of Yeast Growth-Promoting In White Sugar

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Abstract

"A Study of Yeast Growth-Promoting Substances in White Sugar"*

Harlow H. Hall

The extent of multiplication of yeast cells in dilute solutions of white sucrose sugars is shown to be dependent upon the amount of a complex of yeast growth-promoting substances present. Relatively large amounts of substances in a given sugar may influence its use in the manufacture of beverages, fountain sirups, dairy products or other low sugar content liquids which are not heat processed and which are subject to spoilage by yeast. The extent of reproduction of an inoculum of cells of a known yeast culture in a 10-percent solution of the sugar is used as a means for determining the relative amount of yeast growth-promoting substances present.

The direct microscopical cell count technic, using a haemacytometer, is employed to determine the extent of yeast cell reproduction. Each sample of sugar to be tested is made up in amount sufficient to result in 100 ml. after sterilization in 300 ml. Erlenmeyer flasks. When cool 0.5 to 1.5 ml. of a water suspension of a 24-hour culture of the test yeast is added to result in an inoculation of approximately 50,000 cells per ml. of the test solution. After 72 hours incubation at 30° C. the number of cells per ml. is determined. The yeast inoculum multiple is recorded as the number of cells per ml. after incubation divided by the number of cells per ml. initially present.

Approximately 900 composite samples of sugar, representing the annual output of 29 to 87 domestic factories during a period of 11 years were examined. Many samples of sugar did not support the growth of yeast cells while others supported the growth of varying size crops. The average yeast inoculum multiples for yearly campaign composite samples was between 4.3 and 12.4 for the 11-year period. The results also show that the amount of growth-promoting substances may vary between suc-

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cessive strikes of sugar. It may also vary in sugar from the same factory from year to year.

In groups of samples a relationship exists between the relative amount of yeast growth-promoting substances and the amount of ash present. The ash of sugar does not, however, promote yeast growth. Although the growth-promoting factor is organic in nature, there is no correlation with known organic non-sugar impurities. There is likewise no correlation with the amount of the factor and the geographic areas in which the sugar is manufactured or with the manufacturing process employed.

The growth-promoting factor is located at or near the surface of sugar crystals. It is almost completely eliminated by recrystallization of sugar from 80 percent alcohol and may be recovered from it by selective treatment. In the practice of sugar manufacture it is effectively removed by thoroughly washing the crystals of sugar to assure complete elimination of the sirup film surrounding each crystal. The growth-promoting substances are stable to heat and alkali treatment during the process of sugar manufacture and accumulate in the final molasses.

Biotin was found to be present in varying amounts (0.025 γ to 0.445 γ per gram of dry sugar) in alcohol-free extracts which were prepared by recrystallization of individual sugar samples from 80-percent alcohol.

[illegible] OF YEAST PROTEIN-PRECIPITATING SUBSTANCES IN CANE SUGAR

by

HARLAN HOWE HALL

A THESIS

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I. INTRODUCTION

The white sucrose sugars, which include beet and cane, are perhaps the nearest approach to chemically pure substances that are included in the daily diet. As a food commodity, sugar is the cheapest, on a calory a sir, on the American market and is used in quantity in more food products than any other prepared ingredient. Because sugar-containing plants lend themselves to easy cultivation and bulk handling, manufacturing interests have been able, for many years, to recover and sell sugar in sufficiently large quantities to make it an important item in world commerce.

The value of use of sugar as an ingredient in prepared foods is not limited to its energy-giving content, nor to its sweetening property. As a preservative in water-containing foods, it has no equal from the standpoints of maintaining flavor, color, turgidity or keeping quality. Sugar is truly the master preserver.

The utility of sugar, like many other food ingredients, has certain limitations which are governed by the presence or absence of foreign substances. It has been observed that impurities may often be present in food ingredients in relatively large quantities and yet not be of concern when the ingredient is used for a selected food product. However, a trace of another impurity may greatly impair its utility when used in the same product. Sugar is no exception to this observation. The presence of bacteria, yeast and molds in sugar and substances which stimulate their growth in solutions are illustrative. For instance,

[illegible] which would be of no concern when the sugar is for table use. Such sugar would not, however, be acceptable for use in the manufacture of beverages. Likewise, the presence of a relatively large amount of yeast growth-promoting substances in sugar used for table sweetening would be of no concern. However, it would not be desirable to use such sugar for the manufacture of beverages or similar products which must be sterile and contain no yeast living either the sugar itself or from other sources. Because of the extensive use of sugar in beverages, fruit juices, flavoring extracts, and sterilized condensed milk, all of which are subject to spoilage by yeast, the presence of yeast growth- promoting substances in sugar is of vital importance. It is a problem that is entitled to considerable attention from the manufacturers, as well as the users, of sugar.

Scientific workers have, for various purposes or another, delved into the mysteries attending the growth or failure of growth of yeasts in nutrient solutions. Most previous workers have been concerned with the effect of growth-promoting substances on sugar impurities from the standpoint of yeast nutrition requirements. Hall, James and Stuart (7) were the first to consider subject from the standpoint of the commercial manufacture and use of sugar, when it became apparent that there were differences between the biological quality of sugars of the same type, i.e., beet or cane sugar, and that these differences were noticeable in their use in food products, especially beverages.

The data presented here as a result of this research were obtained in the laboratories of the Bureau of Agricultural and Industrial Chemistry of the United States Department of Agriculture. During the course

[illegible] of sugar were received from [illegible]. It was conducted in conjunction with a study of the [illegible] impurities in white sugar. The majority of the sugar samples [illegible] represent individual factory [illegible] composite samples, or bore which constitute a portion of each entire [illegible] sugar [illegible] given sugar campaign. They were furnished in most instances by the beet sugar industry which actively [illegible] this [illegible] investigation of particular [illegible] aspects of manufacturing sugar of uniform biological and chemical quality. Significant factory operating data were made available by the manufacturer of each composite sample [illegible] in the evaluation and interpretation of analytical data. The results of the analysis were, in turn, made available to the manufacturer as a record of factory performance and as a guide for future operations.

The major objectives of this research were:

1. To determine the presence or absence of yeast growth- promoting substances in commercially manufactured white sugar.

2. To develop a method for evaluating the biological quality of white sugar based on the relative amount of yeast growth-promoting substances present.

3. To study the properties of, and identify the growth-promoting substances found.

4. To determine the relationship of the presence of such substances with other known non-sugar substances, especially in sugar of known origin.

5. To develop methods for the removal of such substances during the manufacturing process.

II. HISTORICAL DEVELOPMENT OF THE SUGAR INDUSTRY

CANE SUGAR

During the early centuries, man's chief sweet food was honey. Its replacement by cane-sugar began in the Far East when India developed sugar cane, known originally, as a source of sweets, until Alexander's army in 327 B.C. discovered its excellence. Persia was the next country to cultivate sugar cane; the Arabs and the Moors took it to the Mediterranean countries in the Middle Ages; Columbus and his followers introduced its culture in Cuba and the West Indies; and by 1650, it had been introduced into all the West Indian islands. In 1794, its culture spread to Louisiana where it has continued to the present time.

There was a sugar refinery in New York City as early as 1689, which refined raw sugar imported from the West Indies and Cuba. In the year 1730, Nicholas Bayard erected a refinery on Hall Street, where an unknown expert was able to make, for the first time in America, several types of sugar; that is, single- and double-refined loaf sugar, crushed sugar, brown sugar and a form of rock candy. It is believed that Bayard was the first to establish sugar refining as a separate enterprise in America. His first newspaper advertisement appeared in the New York Gazette for August 17, 1730, as follows:

> "PUBLICK NOTICE is hereby given, that NICHOLAS BAYARD of the City of New York has erected a Refining House for refining all sorts of Sugar and Sugar-Candy, and has procured from Europe an experienced Artist in that Mystery. At which Refining House all Persons in City and Country may be supplied by Whole-sale and Re-tale with both double and single-Refined Loaf

Sugar, s[illegible] Porter and Rhea-Sugars and Sugar-Candy, [illegible] Public Notes."

The initial success of Boyard prompted the construction of additional refineries and by 1795, New York, Philadelphia and Boston were refining 4,[illegible]0,000 pounds of sugar yearly or about 2 percent of the total amount which was consumed annually.

Many new innovations in refining technic and machinery were introduced into the industry during the nineteenth century which enabled the industry further to increase its output of refined sugar manyfold so that the industry supplied three-fourths of the 4.3 billion pounds of sucrose consumed in the United States in 1941. Some of the inventions which led to the establishment of sound processing practices were Watt's steam engine which substituted man and animal power, and the vacuum pan by Howard in 1813. The introduction of modern cane-crushing machinery, juice filtration, carbonation, sulphitation, crystallizers, centrifugals and granulators which are discussed by Meriot (30) in his book "The Manufacture of Sugar from the Cane and Beet", were other innovations of importance. Also contributing to the success and expansion of the industry was the development of modern planting and harvesting machinery.

To supply our cane-sugar needs, there are about 100 refineries and factories which refine imported raw sugar and/or process domestic sugar cane which is grown in Louisiana and Florida.

BEET SUGAR

Sugar beets, Beta vulgaris, were cultured in Europe during the eighteenth century and the first beet-sugar factory was established in Austria in 1769. Napoleon heavily subsidized the industry in France

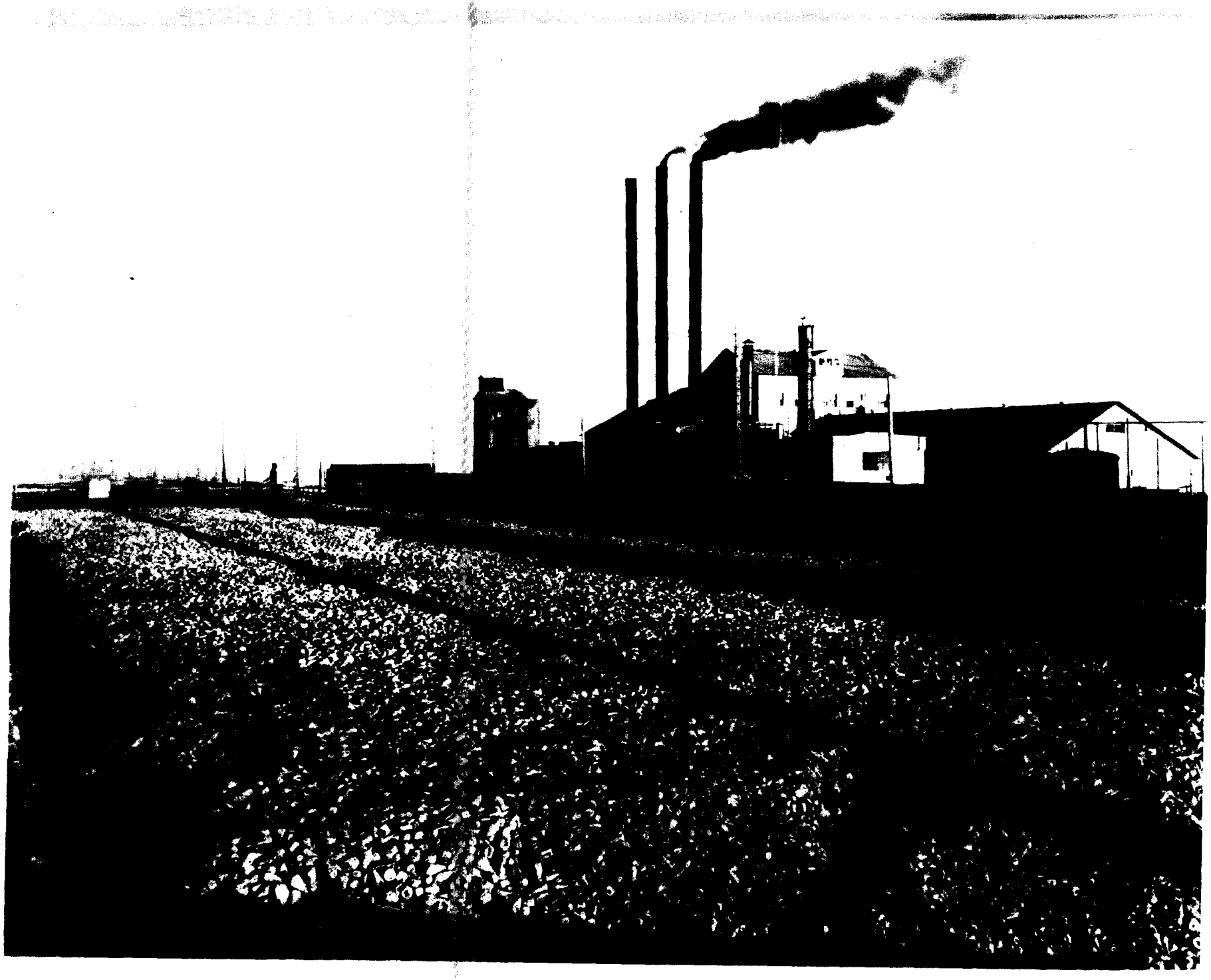


Illustration 1. BEET SUGAR FACTORY [illegible] PILES OF BEETS IN FOREGROUND

in 1810 in order to alleviate the shortage of cane sugar created during the blockade of French ports by the British. The production of beet sugar quickly increased throughout central Europe. In the United States, [illegible] manufacture beet sugar was in their infancy in 1830, followed by attempts in California, Illinois and Wisconsin, between 1863 and 1876. Each met financial failure. The first successful factory in the United States was built at Watsonville, California in 1870. There were probably 50 beet sugar factories in operation in mid-[illegible] and this increased by the year 1900, and by 1920 the number had increased to 87, producing an average of 100 tons of sugar per day for a [illegible] season of about 100 days.

The successful development of the beet sugar industry was likewise dependent upon the development of much of the same modern equipment and methods used by the cane sugar industry. Differences in the methods for processing sugar cane and sugar beets, such as, the diffusion of sugar from beets and the Steffen method for recovering sugar from beet sugar molasses are described in detail by Spencer (30) in his book "The Manufacture of Sugar from the Cane and Beet". Of special importance is the recent development of farm machinery which aids fair to supplant much of the hand work required in thinning, blocking and topping of beets. Still another important development is the domestic production of beet seeds. Although beet seed production is limited to Pacific Northwestern states, there is much evidence available to support the belief that seeds from this area will produce better quality beets throughout the beet-growing states than seeds previously imported from Europe. The capacity of the existing beet sugar factories is limited only by the production of sugar beets.

. DOMESTIC PRODUCTION AND CONSUMPTION OF SUGAR

During the year 1941, there were [illegible] beet sugar factories which produced over one and three-fourths million tons of sugar. In the same year there were 56 cane sugar factories in operation in Louisiana and in Florida which produced about 932,000 tons of raw or direct-consumption sugar.

The consumption of sugar by the beet sugar industry is greater than that produced locally by the cane sugar industry. This is a result of the cultivation of sugar beets throughout the sugar geographic area. The consumption of sugar by the several industries is given in Table I for the calendar year 1939-1941, inclusive, for 1939, 1940 and 1941.

The value of sugar consumed by the several branches of the food industry for 1939 is reported to be $13,6[illegible],910. This includes corn sugar with a valuation of $914,373. The amount of each type of sugar and its value is given for the several branches of the industry in Table I.

The largest user of sugar in the baking industry which amounted in excess of one and one-fourth million pounds in 1939. Following this industry, in order, are the confectionery, canned and preserved foods, beverages, and dairy industries as large users. It is estimated that about 40 percent of the sugar is consumed by industry, with the remaining 60 percent is consumed in the household and in commercial eating establishments.

The annual distribution per capita consumption in short tons and pounds, respectively, of sugar in the United States for the period

TABLE 1. [illegible] ANNUAL Y 1939, 1940 AND 1941*

STATE		PRODUCTION			
		Average 1929-33	1939	1940	1941
Beet Sugar:	No. of Factories**	1,000 short tons	1,000 short tons	1,000 short tons	1,000 short tons
California	10	431	459	454	310
Colorado	17	334	468	313	399
Idaho	9	88	127	145	107
Michigan	12	118	162	168	158
Montana	5	99	140	163	118
Nebraska	7	116	136	115	121
Ohio	4	39	42	45	46
Utah	6	88	130	74	82
Wyoming	5	90	92	93	79
Other States***	10	106	159	191	161
Cane Sugar:					
Florida	2	39	65	94	96
Louisiana	65	467	406	220	323
Total Beet Sugar			1,643	1,761	1,484
Total Cane Sugar			471	314	419
United States		1,605	2,114	2,075	1,903

*Agricultural Statistics, 1941, United States Department of Agriculture

**Number of operating factories in state, 1942

***Including: Iowa and Oregon, 2 each, [illegible], Indiana, N. Dakota, Kansas and Wisconsin, 1 each.

TABLE 2. SUGAR CONSUMED IN SELECTED FOOD INDUSTRIES, 1939*

Industry	Total Consumption (lbs.)	Beet (lbs.)	Cane (lbs.)	Corn (lbs.)	Total Cost
Meat Products	52,290,600	12,627,595	35,528,413	4,134,592	$ 2,344,936
Dairy Products	341,148,674	113,014,935	214,206,863	13,926,876	15,949,016
Canned and Preserved Products	624,571,391	201,284,058	397,718,035	25,569,298	28,158,853
Grain-Mill Products	51,506,887	22,923,405	26,713,602	1,869,880	2,349,901
Bakery Products	1,256,012,386	383,127,668	742,210,826	130,673,892	58,948,204
Confectionery and Related Products	1,052,129,634	290,732,537	736,268,104	25,128,993	47,506,288
Beverages	567,443,543	24,346,816	458,825,909	83,800,818	26,151,764
Miscellaneous**	447,866,044	8,458,899	424,115,139	15,292,006	19,549,993
Aggregate Total	4,634,711,857	1,079,012,493	3,246,632,656	309,066,703	$211,622,919

*Bureau of the Census, U. S. Department of Commerce, Report of March 14, 1941

**Flavorings, vinegar, cider, pharmaceuticals, etc.

1923-1941 are given in Table 3.

The maximum total distribution of 5,090,321 tons in 1941 is the highest on record. This high value, in comparison with previous years, is attributed to the accumulation of large stocks held by some industrial users, and is not the actual amount used during the year. On the basis of the amount distributed in 1941 the war credit consumption was 1,847 tons. This figure is misleading as the true consumption was not materially greater than that shown for 1940.

TABLE 3. ANNUAL DISTRIBUTION AND PER CAPITA CONSUMPTION OF SUGAR FOR THE PERIOD 1913-1941

Year	Total Distribution (tons)	Per Capita (lbs.)	Year	Total Distribution (tons)	Per Capita (lbs.)
1913*	4,677,333	94.9	1933**	6,377,500	101.4
1914	5,[illegible],760	99.9	1934	6,349,[illegible]90	100.[illegible]
1915	5,4[illegible]7,773	107.0	1935	6,633,928	104.0
1916	5,6[illegible],5[illegible]3	108.0	1936	6,706,195	104.4
1917	5,[illegible]49,865	101.5	1937	6,671,40[illegible]	103.[illegible]
1918	5,547,439	103.5	1938	6,6[illegible],153	10[illegible].0
1919	5,746,474	106.4	1939	6,367,518	104.7
1920	5,693,48[illegible]	103.8	1940	6,[illegible]0,667	104.6
1931	5,547,09[illegible]	99.7	1941	6,998,321	1[illegible].[illegible]
1932	5,[illegible]35,134	93.4			

*[illegible]ts Abstract[illegible]r, vol. 2[illegible], No. [illegible], 1933, p. 71

**Values for 1933 to 1941 obtained from Sugar Section, Agricultural Conservation and Adjustment Administration, U. S. Department of Agriculture

III. LITERATURE

YEAST GROWTH IN MINERAL-SALT SUGAR SOLUTIONS

Yeasts have been propagated in synthetic nutrient solutions since 1860, when Pasteur (47) first reported the results of his classic experiments on yeast growth in mineral-salt sugar solutions. Pasteur observed that if yeast the size of a pin head were introduced into a solution of sugar, ammonium tartrate and the minerals in yeast ash, multiplication and fermentation resulted. Almost from the beginning of these studies, until a few years ago, there existed among many workers a controversy regarding the nutritional requirements of yeast. Among the first persons to oppose Pasteur's beliefs was the German chemist, Liebig (41) who, in 1871, contested the statement of Pasteur with regard to the multiplication and fermentation of yeast in a medium free from nitrogenous organic matter. He failed to get the same results when he repeated Pasteur's experiments and vigorously denied the possibility of obtaining either growth or fermentation in a mineral-salt solution.

Other workers also questioned Pasteur's results, although for the following quarter of a century much valuable work was done on synthetic media based on the results of Pasteur. Few papers were published, however, following the controversy between Pasteur and Liebig, each of whom had either formulated or supported separate theories regarding alcoholic fermentation.

EFFECT OF IONS ON YEAST GROWTH

In 1891, Villiers, (58) renewed the interest in this field of investigation by suggesting that a synthetical substance, which he first

found to be present in yeast water, and designated as "bios", was necessary for the fullest development of yeast in synthetic nutrient solutions. His observation that growth was activated by the addition of a filtrate from boiled yeast led him to believe that, although yeast would grow in a synthetic medium without this substance, its development was much better when present. He found that small seedings with Saccharomyces cerevisiae produced no fermentation in this medium composed of water, sucrose, magnesium sulphate, potassium chloride and calcium chloride, while large seedings did produce fermentation. He concluded that growth was stimulated, not because of an increase in the number of cells used, but by some growth-promoting substance which was added when large seedings were employed. Growth of yeast in this medium was activated by the addition of a filtrate from boiled yeast; the evolution of carbon dioxide gas serving as a measure of activation. Wildiers' data on the effect of yeast extract upon the fermentation with small seedings of yeast are as follows:

Time days	cc. boiled yeast emulsion 1	2	3	4	5
	cc.CO_2	cc.CO_2	cc.CO_2	cc.CO_2	cc.CO_2
2	0	0	0.5	1.2	2.5
3	0	0	1.0	3.1	4.7
4	0	0	1.2	3.0	5.6

Some criticism has been offered regarding the title of Wildiers' original paper which was "Une nouvelle substance indispensable au d'evelopement de la levure". Those criticising this title believed

the words "normal development" should have been used, since it has been found that bios is not absolutely necessary for the fullest development of yeast but when absent, small, unhealthy cells result. He proposed the name bios because the substance was unknown chemically and because this name must be satisfactory until the factor was better understood. The Greek meaning of the word bios is life. Wildiers described bios as follows:

1. Soluble in water.

2. Insoluble in absolute alcohol and ether; 80 percent alcohol, however, permitting a good extraction.

3. Not present in yeast ash. It is then not an inorganic substance.

4. Not destroyed by boiling for one-half hour in a 5 percent solution of sulfuric acid. To destroy it, a 20 percent solution was necessary.

5. Bios seemed to be changed by one-half hour boiling in 1.0 percent solutions of NaOH.

6. Not precipitated by lead acetate.

7. Dialyzable.

8. Contained in Liebig's meat extract, commercial peptone and in beer wort.

9. Bios is not present in such substances as urea, asparagin, aniline, tyrosine, nuclein bases, adenine, quinine, thymus nucleic acid, creatine, or pepsic and trypsic digestion products of albumin.

Wildiers' explanation for the differences that existed between the results obtained by Pasteur and Liebig was based on the amount of yeast used as the inoculum. Where Pasteur used a portion of yeast the size

of [illegible] Liebig, used a smaller amount. This difference in the size of the inoculum seemed to Wildiers to be sufficient to account for the failure of growth in Liebig's experiments because, when he repeated Pasteur's experiments, he found that there was a minimum limit to the number of cells of a viable growth and that much depended on the nutrient medium from which the cells were taken.

Wildiers' work, like Pasteur's, continued to become the subject of much controversy based on the amount of inoculum used to seed synthetic media, the carry-over of stimulants in the inoculum to fresh media and, also, the influence of impurities in the media ingredients. Fernbach (14) was the first worker to challenge Wildiers' conclusions by suggesting that his results were due to the presence of toxic substances in the culture media. This opinion was later shared by Lindisch (79) who believed that the presence of copper salts introduced into the media through distilled water, as suggested by Fernbach (14), would inhibit yeast growth. Although Lindisch was one of Wildiers' active opponents, he was able to confirm Wildiers' fundamental observation. However, he preferred not to offer an explanation for it until after further investigation. The possibility that Wildiers' Vios counteracted the toxic effect of copper and other salts, thus permitting growth, was expressed by certain workers. Lindisch's opinion regarding the influence of impurities on yeast growth was furthered when he observed that yeast failed to develop in cane sugar solutions because of the presence of traces of ultramarine which had been used for blueing the sugar.

Wildiers was not without supporters of his views for Amand (4), 1903, defended him against Lindisch and other German workers who believed that impure water was responsible for some of the results obtained. He

showed that water, which was an ingredient of the medium, was free of toxic substances and, therefore, was not a factor. Windisch suggested that the sugar used by Arend contained substances which were poisonous to yeast.

The first reference to sugar as an ingredient inhibiting the development of yeast appears to be by Windisch. Korsowicz, (39) in 1903, was another worker to recognize the possible influence of impurities in sugar on yeast growth, although unlike Windisch, he recognized a stimulative effect of sugar impurities on yeast development. He was able to determine that a difference existed in the stimulative ability of sugar by carefully determining the size of the yeast crop in duplicate experiments in which the sugar was the only variable. The composition of the medium for one of his experiments (third) was as follows:

Water	100 ml.
KCl	0.2 gram
$(NH_4)_2HPO_4$	0.1 "
$MgSO_4$	0.02 "
$Ca_2H(PO_4)_2$	0.02 "
Commercial refined sugar	5.0 "

The composition of the medium of another (fourth) was the same, except that more saccharose was used. In the third experiment there was no development of cells during the first two weeks, but after forty days there were 364 million cells in 100 ml. of the medium. In the fourth experiment, in which the same strength inoculum was used, there was no development during the first three weeks, but after sixty days there were 220 million cells in 100 ml. of the medium. In addition to demonstrating the differences in the biological quality of sugar, Korsowicz contributed otherwise to the subject not only by improving on the methods for estimating the progress of yeast development, but by his studies of the extent

of growth which resulted from variable numbers of cells when seeded into nutrient solutions. Lebedeva concluded in regard to follows:

> "We have shown that commercial sugar stimulates yeast growth and fermentation. Also, in these cases the slight traces of organic impurities in the cane sugar may be responsible for the stimulation. The tests mentioned above do not justify the assertion that for the multiplication of yeast certain organic compounds, aside from sugar, are necessary, although it must be admitted that traces of organic compounds are of vast influence on the possibility of yeast growth. This work establishes the autocatalytic nature of the yeasts."

A number of papers were published following Lebedeva's work on various problems relating to the nature of bios, its origin and its role in yeast nutrition, but workers apparently lost interest in the subject until the vitamin question was opened. Williams' work immediately took on a new significance because it was suggested that bios might be a vitamin-like substance. A number of investigators subscribed to this belief; Funk and Dubin (16) later suggested that the extent of yeast growth in test solutions be used as a measure of the vitamin B content of vitamin-containing materials.

Williams (59), in 1919, was the first to suggest the possible relationship that existed between the vitamin and bios complex, as a result of his observations on the requirements for an unknown organic constituent (or constituents) of yeast or yeast extracts. It was shown:

> "that the substance promoting the growth of yeast occurs in the same materials as those in which vitamine had been found; namely protein-free milk, wheat germ, lactose (Kahlbaum), yeast, egg yolk and pancreatin. The substance is none of the commoner amino acids contained in an acid digest of casein with tryptophane added. It has the same properties of solubility, precipitation by phosphotungstic acid, absorption on fuller's earth, heat stability, and behavior toward acid and alkali, as nearly as we know these properties, of the water soluble vitamine and in addition the two substances so far as we know have no divergent properties.....From the cumulative evidence offered we believe we are justified in concluding that as far as present knowledge is concerned the substance

> [illegible] indicated [illegible] yeast [illegible] indicate [illegible] substances [illegible] animal nutrition [illegible] vitamin [illegible]."

Prior to the publication by Wildiers, the term "bios" was used to designate the vitamin B complex, the enzymes and other substances now ascribed to the "vitamin B complex." The appearance of the trade-mark "Bios" was announced by Effront (47) in a note some years previously after the publication of the [illegible] to prove. Effront's work, in the main, was concerned with the effect of [illegible] on yeast [illegible] yeast extract; however, there was no mention of either bios or vitamin B by [illegible]. In the latter paper, Effront (9) investigated yeast fermentation and stimulators of vitamin-like activity and concluded that:

> "The amount of organic material required in so small and the effect upon the growth and activity of the yeast is so striking that a marked similarity exists between these organic substances and the vitamins required for the normal development of animals."

Prior to these publications which associated vitamins with bios, there were innumerable papers on the bios question; many others followed later.

Tanner (56) in 1935, reviewed the bios question and included a bibliography of 144 references. Miller, (44) and Penkett, (50) also reviewed the subject. Many of the papers concerned the indispensability of bios to the fullest development of yeast, while others described new media designed to test the significance of bios. Still others attempted to decide on the identity or non-identity of bios and the vitamin B complex based on studies on crude plant or animal materials. As a result of these studies, it has become known that the entire bios effect cannot be ascribed to any single substance, and that "vitamin B" activity is

[illegible] found to consist of organic [illegible]. There is [illegible] overlapping of compounds in the two [illegible]. The [illegible] according to Eckstein, the multiple nature of [illegible] (42) and [illegible] from Step II by [illegible]. Miller, [illegible] (45) and Miller, [illegible] (46) [illegible] fraction-[illegible] Step II [illegible] II [illegible] III.

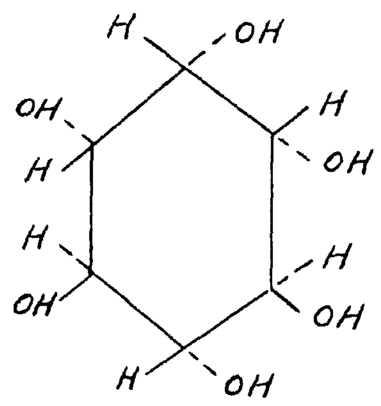
[illegible], (41) [illegible], (61) [illegible], reviewed the subject in considerable detail [illegible] with the identification of the [illegible] yeasts. In addition to reviewing [illegible] specific yeast [illegible] which have been identified and studied, [illegible] contain [illegible] which have arisen regarding interpretation of results previously published. These are (1) the different [illegible] which have been used by various investigators; (2) [illegible] in [illegible] procedures; (3) differences in the source of [illegible]; and (4) unrecognized potency of [illegible] mutrilites.

[illegible] number of specific compounds are now known which influence the growth of yeast. The [illegible] vitamin activities are well determined; [illegible] of them comprising the vitamin B complex. The most important of [illegible] are: inositol, [illegible], pantothenic acid, biotin and pyridoxine. While it is not the purpose of this review to discuss in detail each of the above compounds, brief mention is made of the origin, composition and properties, and role of those factors known to influence yeast growth.

EFFECT OF INOSITOL ON YEAST GROWTH

Inositol, [illegible] isolated by Eckstein (13) [illegible], was the first [illegible] found to possess biological activity. It is the physio-

[illegible] (??) [illegible]. Although its oc-
currence in plant tissues [illegible], its [illegible] significance
is [illegible]. [illegible] acid as
phytin. [illegible] (19) suggests that it is the principal phosphorous
compound in vegetable seeds. [illegible] in ri-
[illegible]. The
molecular formula of inositol ([illegible] hexahydrobenzene) is
$C_6H_{12}O_6$. The [illegible] structure.



It combines with phosphoric acid to form inositol phosphoric acid,
phytin being the calcium-magnesium salt of inositol phosphoric acid.

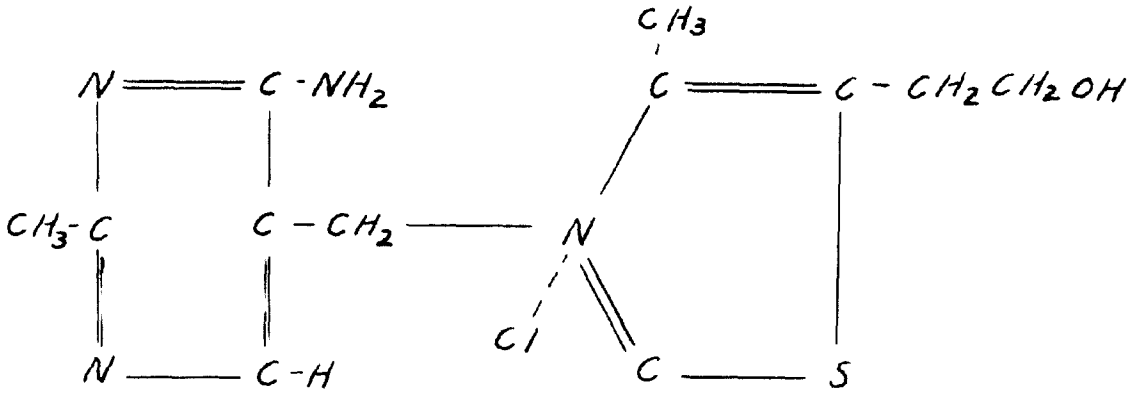
Inositol by itself in synthetic nutrient solution does not ex-
ert [illegible] effect on the growth of yeast. Unlike most other nu-
trient substances it becomes a limiting factor of growth only when
other nutrilites are supplied. Under suitable test conditions it has
been shown to have some effect on all strains of yeast tested. In
her original report [illegible] grew [illegible] yeast without inositol
and determined by the physiological response to the extract of such
yeast that it contained about one-half the normal amount of inositol.
Williams (61) showed that these strains of yeast were able to synthesize
inositol during growth thus indicating that it is essential in yeast

...riology. Jansen (34) found inositol to be indispensable for the ...rowth of Fulleri yeast and Williams, Cain and Snell (62) indicate that "cold process" yeast may require inositol for continued production. Lochhead and Burkholder (41) found inositol to be necessary for the growth of one type of 13 strains of osmophilic yeast.

EFFECT OF THIAMINE ON YEAST GROWTH

Thiamine (vitamin B_1) was first isolated from natural sources by Jansen and Donath, (33) in 1926. The effect of this antineuritic vitamin on yeast growth was conclusively demonstrated by Williams, Waterman and Kerekzuky, (69) who tested the crystalline vitamin prepared by Jansen and Donath. They found that it was effective in concentrations as low as 0.00001 mg. per ml. of culture medium, when supplemented by a residue of rice bran which was unabsorbed by fuller's earth. It is found in a wide variety of foods, but there are few foods of either plant or animal origin that may be considered a potent source of this vitamine. Its importance in nutrition is well recognized, some foods being enriched with thiamine chloride.

The knowledge regarding the chemical identification of thiamine resulted largely from the efforts of Williams and coworkers (7, 61, 69) who determined its empirical formula to be $C_{12}H_{18}N_4OCl_2$. The synthesis of vitamin B_1 was effected by Williams and coworkers soon after determination of the structure of thiamine. It was found to contain a pyrimidine and a thiazole nucleus, the structural formula of thiamin chloride being determined as shown.



The response of yeast to thiamine varies with different cultures. Williams and Boerm (64) recorded varied response by "old process" and yeast No. 574 of the American Type Culture Collection, while several other workers failed to respond similarly under the same conditions (58, 63). Schultz, Atkin and Frey (57, 58) observed that yeast fermentation is greatly enhanced by the presence of thiamine and developed a quantitative method for determining small amounts of thiamine using the fermentation test. By their original method they were able to estimate the presence of 0.5 to 4.0 micrograms of vitamin B_1.

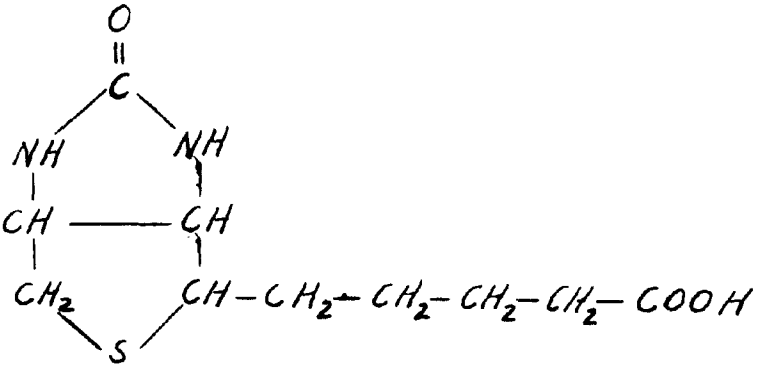
EFFECT OF BIOTIN ON YEAST GROWTH

Crystalline biotin was first isolated in the form of its methyl ester by Kögl and Tönnis (36), in 1936. Its isolation from the yolk of eggs was effected by a lengthy series of fractionations which resulted in the recovery of less than five percent of the total amount present. Because of the extremely small amount present in natural sources and the difficulty in its recovery, Kögl (36) was able to obtain but 70 milligrams of crystals during five years of work. Only a few grams have been isolated since development of a recovery process.

Three groups of workers studied and named a growth-promoting substance which later became identified as biotin. Allison and co-workers (2, 3, 31) found that several species of root-nodule bacteria, Rhizobium, required the presence of a growth factor which was obtainable from yeast, malt and commercial sucrose. This factor, which was found to have a pronounced effect on cell respiration, was designated as a coenzyme of respiration, namely, coenzyme R. Subsequent work by Allison (37) in which comparisons made on the effect of growth by biotin and coenzyme R indicate that they are identical.

A second group, György and coworkers (6, 11, 12, 22, 23, 24, 25) studied a substance occurring in yeast and liver which was capable of preventing the fatal syndrome resulting from feeding large amounts of raw egg white, a syndrome found to occur in all species studied. This substance, which was originally designated as vitamin H, was later recognized as a member of the vitamin B complex, namely, biotin. The third group of workers included Miller and coworkers (45, 46) who fractionated Bios II into Bios IIA and Bios IIB. Kögl and Tönnis (33) indicated the identity of Bios IIB as biotin while studying the properties of crystalline biotin from egg yolk.

Kögl and Price (37) also set the empirical formula of biotin to be $C_{11}H_{18}O_3N_2S$ and du Vigneaud (10) believes its structural formula to be



vidence to support the determination of the structural formula is presented by the latter writer and need not be reproduced here.

Biotin is present in low concentration in most natural substrates; the microbial activity is ordinarily in minute amounts as compared with other substances. Snell, (55) determined that bakers' yeast contains about 0.12ug. of biotin per gram as compared with 10ug. or more of thiamine per gram. Underkofler and Williams (7) found the biotin and thiamine content of dried eggs to be 1.35ug. and 5.4ug. per gram, respectively. They determined the biotin content of a sample of sugar to be 0.304ug. per gram.

Wright showed that only 0.00004ug. of biotin is necessary in 2 ml. of culture medium to produce a 100 percent increase in the crop of bakers' yeast in 8 hours. Snell and coworkers, (55) believe that biotin is the limiting factor in the growth of yeast and showed that the presence of a minute amount of pyridoxine greatly increased its growth- promoting effect. They also believe that yeast is able to synthesize biotin during growth. Lochhead and Hunter in (41) found biotin to be essential to the growth of all strains of osmophilic yeast studied.

EFFECT ON PHYSIOLOGICAL ACTIVITY AND GROWTH

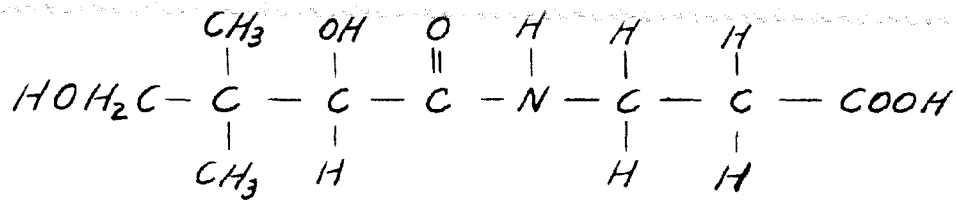
Williams and Bisbey (62) and Williams and Tresselt, (66) in 1931, were the first to indicate that the nutrilite for the "Lactobacillus bulgaricus" was a single substance. Later, (1934), Williams, Burgett and Williams (51) determined its universal presence in plant and animal tissues and assigned to the substance "bios-botheric acid", the name being derived from the Greek meaning <u>life everywhere</u>. Demonstration of the acid properties and the stable nature of the entity of Williams'

cios by a new tool, namely, fractional electrical transport in a d.c. potential field without the use of membranes. In 1935, Williams (60) reviewed the results of this method for the separation of relatively weak acids and bases of low molecular weight. In 1941 he reviewed (61) the entire subject of pantothenic acid.

Williams and his coworkers were solely responsible for the identification of pantothenic acid. Considerable interest is attached to its identification by Williams (62) who states that:

> "The outstanding point of interest in the pantothenic acid investigation is the fact that not only regarding its structure was determined before it was obtained in pure condition. In fact, the complete structure was known and the physiologically active substance was synthesized before pantothenic acid or its salts or other derivatives (excluding cleavage products) were obtained in condition such as to yield the correct analyses."

The empiric formula for calcium pantothenate (the most stable form) is $(C_9H_{16}O_5N)_2Ca$ and the structural formula for the physiologically active acid (combined with ammonia) is:

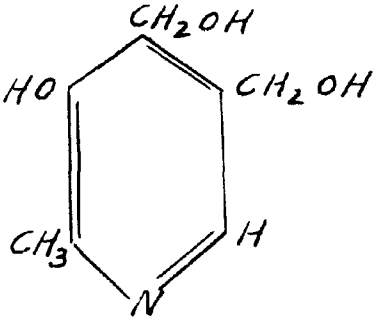


During the course of the investigation of the influence of pantothenic acid on yeast growth the "Gebrüder Mayer" strain was usually used, however, it was found to be an important nutrilite for all of the strains tested by Williams and coworkers (63) and Williams, Lyman and Snell (63). "Gebrüder Mayer" yeast does not appear to be able to synthesize it as nutrilite through the rate of synthesis by other

strains may be a limiting factor for multiplication.

Effect of Pyridoxine on Yeast Growth

Pyridoxine (vitamin B_6) was isolated in crystalline form by Lepkovsky and Stevens (31) and Lepkovsky (47) in 1938. Its vitamin activity, and preventive effect on dermatitis in rats, was recognized by Gyorgy, (20) in 1935. Its curative effect on yeast growth was recognized simultaneously by Snell, Strong and Peterson (54) and Kuhn and Wendt, (18) in 1938. The richest natural source of it is vitamin B complex, yeast. It was synthesized by Harris and Folkers (14) in 1939. Its structural formula is shown as:



The crystalline vitamin was shown by Snell in connection (54) with lactic acid bacteria (18) to be active in amounts down to 0.000 mg. per ml. In order for the B_6 requirement to be maintained, the medium must contain other nutrilites, particularly pantothenic acid and biotin. Vitamin B_6 can be synthesized by yeast during growth, so that its presence is not essential.

Effect of Miscellaneous Substances on Yeast Growth

Numerous other compounds are reported (61) which effect the growth of yeast in solutions. Some are known to be chemical constituents of yeast so that their role in yeast nutrition is assured. They

are: aspartic acid, leucine and other amino acids; uracil, which probably enters into the synthesis of nucleic acid; ethanolamine; nicotinic acid, nicotinic acid, and riboflavin. Some other compounds have certain slight effects are: indole butyric acid, carotene, choline, acetyl choline, colchicine, deuterium oxide, dibenzanthracene derivatives, nitrophenols and follicular hormone. None of these compounds possess activity in such small amounts as those described more fully.

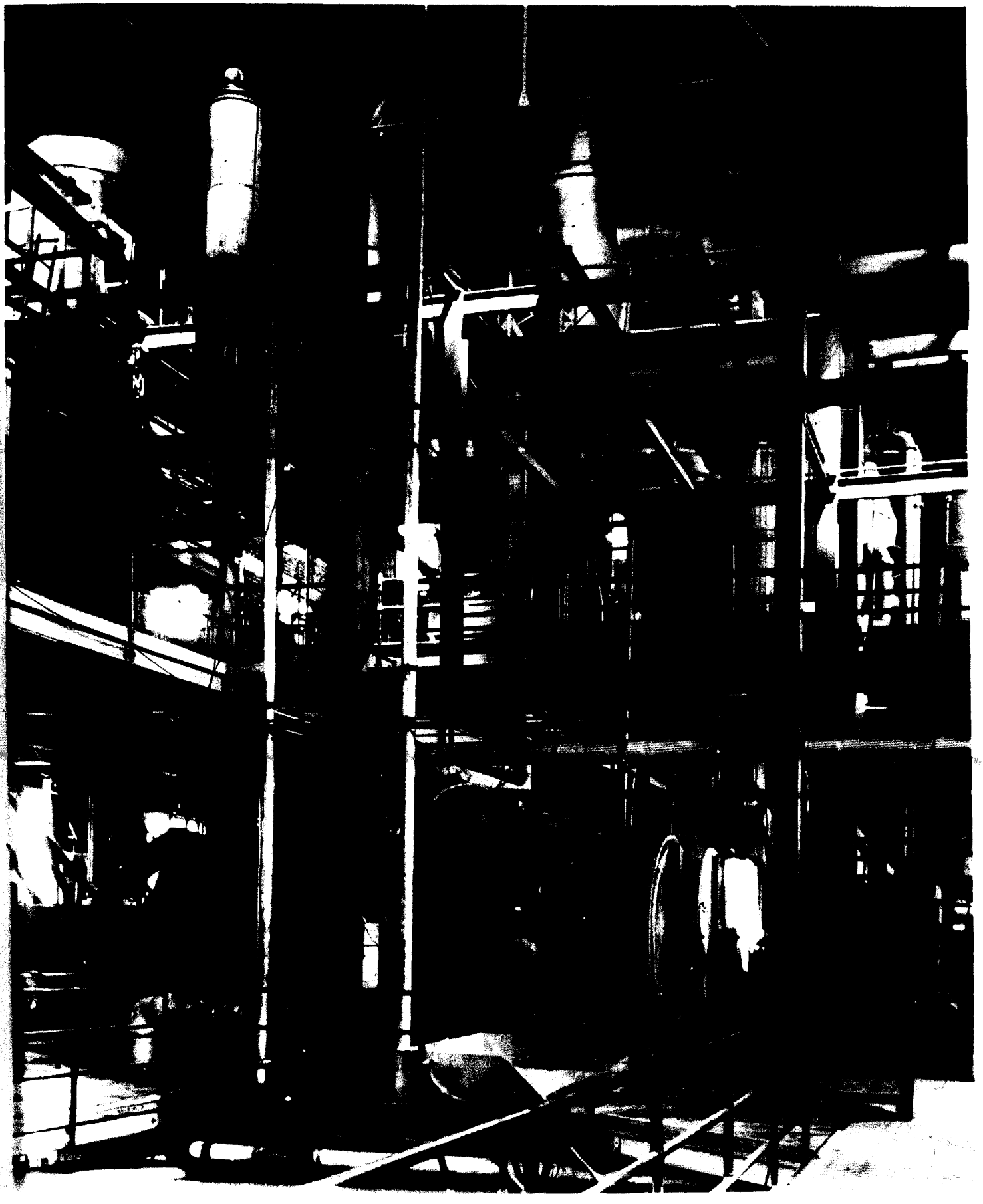


Illustration 2. INTERIOR VIEW OF MODERN SUGAR FACTORY

IV. EXPERIMENTAL

THE YEAST

The individual variations encountered during the isolation of yeast cultures selected for the test. The culture was isolated from a bottle of carbonated beverage in which it had grown and produced a visible sediment. It is identified as a species of the genus Saccharomyces. The culture which is designated as "beverage yeast" in this study was chosen because of its source and the ease of its growth characteristics in media containing stimulating sugar solutions. Upon further isolation, its cultivation in a number of sugar solutions indicated that the extent of multiplication varied in different sugars in part, at least, dependent upon the presence or amount of unknown substances in the sugar. The results also indicated that a good differentiation was obtained in the size of the yeast cells from solutions made from several sugars, when they were inoculated with approximately 50,000 yeast cells per ml. of test solution and incubated at 30° C. for 72 hours. There was greater reproduction of the inoculum in solutions containing 10 percent sugar than in solutions containing lesser amounts as shown in Table 4, Figure 1. The 10 percent concentration of sugar, which supported maximum growth, was chosen for this study, since it approximated that of beverages and also because numerous other workers had used this concentration of sugar in yeast nutrition studies.

Table 4. DEVELOPMENT OF YEAST IN MULTIPLES OF INOCULUM IN 1-15 PERCENT SUCROSE SOLUTIONS

SUCROSE CONCENTRATION PERCENT	YEAST INOCULUM MULTIPLE
1.0	1.4
2.0	10.8
3.0	8.3
4.0	19.1
5.0	19.0
6.0	17.8
7.0	22.8
8.0	26.7
9.0	28.2
10.0	31.4
15.0	30.8

Inoculated with 50,000 cells per ml.

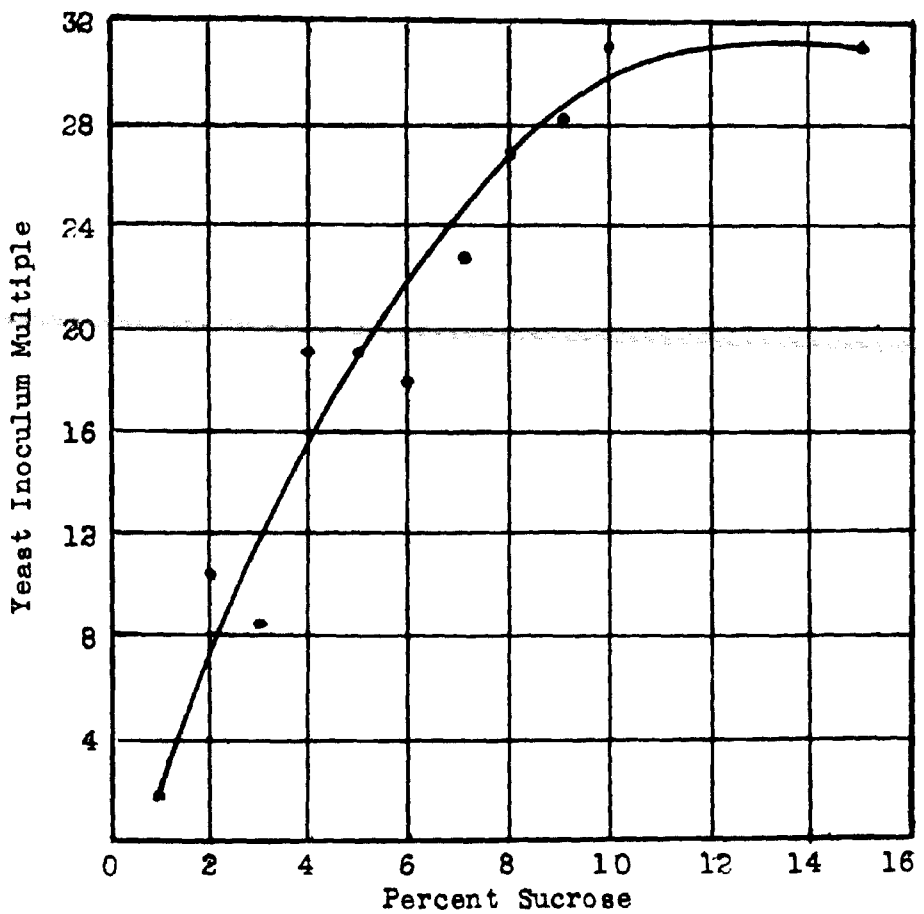


Figure 1. Development of yeast in multiples of inoculum in 1-15 percent sucrose solutions. Inoculated with 50,000 cells per ml.

METHOD FOR DETERMINING YEAST INOCULUM REQUIRED

On the basis of the above observations, [illegible] was developed for estimating the relative amount of yeast growth-promoting material present in samples of soil. A detailed description of the technic is as follows: Ten grams of each sample of soil to be tested are weighed into 300 ml. Erlenmeyer flasks fitted with cotton plugs. Sufficient distilled water is added to give 100 ml. of 10 percent solution. After sterilization in the autoclave at 15 pounds pressure for 30 minutes. Following sterilization and cooling, the solutions are inoculated with the yeast culture. For the purpose of this report, the inoculating suspension is designated as the "inoculum" and is the number of cells introduced per ml. of test solution. The inoculum is prepared from a 24-hour culture grown on a [illegible] agar slant. The mass of cells is transferred with a sterile transfer needle to about 100 ml. of sterile, distilled water. After vigorous shaking, the number of cells per ml. is determined by counting with a hemocytometer. The amount of inoculum necessary to add about 50,000 cells per ml. of test solution is calculated. For extreme accuracy in counting the cells and in setting the inoculum, it should be adjusted to test from 0.5 to 1.5 ml. are used. The inoculated solutions are incubated at 30° C. for 72 hours during which time they are gently shaken at 24-hour intervals. The number of cells that develop is determined by [illegible] in counting 400 small-size fields with a hemocytometer. A few glass beads are added to the flask to assist in breaking up clusters of cells. The number of cells that develop per ml. divided by the number of cells per ml. used as the inoculum gives a value which is

[illegible] from the inoculum [illegible]. The values thus ob-tained from [illegible] (1.2), the value [illegible] the inoculum, is [illegible] the [illegible] growth [illegible] present [illegible] sample.

[illegible]

In order to [illegible] growth-promoting sub-stances [illegible] different [illegible], 22 [illegible] tested. [illegible] from in-dividual [illegible] isolated bacteria. The [illegible] growth-promoting substance [illegible] test-ing [illegible] given in Table 5.

The results show that there was no multiplication of the cells which were inoculated into distilled water [illegible] or so-lution. The [illegible] inoculum multiple is, therefore, in [illegible] 1.0 in these samples, which [illegible] to the inoculum. Mul-tiplication did occur, however, in the remaining [illegible] and varied from 1.1 to 5[illegible] times the inoculum. These [illegible] indicate that there are growth-promoting substances present or available to the inoculum [illegible], but [illegible] present in the [illegible]. The lack of selective [illegible] of the su-gar by [illegible], i.e., [illegible], is an indication that the [illegible] are probably the same or closely related to [illegible] cane sugar. Also,

TABLE 5. [illegible] IN [illegible] WITH [illegible] BY [illegible] INOCULUM [illegible] IN [illegible]

Type Sugar	Yeast Inoculum Multiple	Type Sugar	Yeast Inoculum Multiple	Type Sugar	Yeast Inoculum Multiple
Distilled Water	1.0				
B	1.0	B	2.4	B	16.3
B	1.0	B	3.0	B	18.1
C	1.0	B	3.7	C	11.8
B	1.0	B	4.0	B	20.1
B	1.0	B	4.0	B	20.1
B	1.0	B	6.1	B	20.8
B	1.1	B	6.5	PC	21.4
C	1.1	B	6.8	B	21.7
B	1.2	B	8.4	B	23.1
B	1.4	B	10.4	C	24.1
B	1.4	B	11.1	PC	33.8
B	1.5	B	11.5	B	35.8
B	1.8	C	13.1	PC	55.8
B	2.1	C	14.5	PC	58.5
B	2.2	PC	15.5		

Inoculum 35,000 cells per ml.
B - Beet Sugar
C - Refined cane sugar
PC - Plantation cane sugar

the presence of growth-promoting substances in some of the refined [illegible] effective to [illegible] slices. The [illegible] cocultivation multiplication was obtained [illegible], whereas the [illegible] supporting evidence for this period. There is no [illegible] differentiation between [illegible] cells. [illegible], [illegible] resulting from [illegible] factory [illegible], i.e., [illegible] non-toxic method of [illegible].

The [illegible] to [illegible] suggested the possibility of the presence of toxic substances in the [illegible] growth-promoting substances. This possibility was investigated by [illegible] proportions, [illegible] which [illegible] support multiplication. It was assumed that if toxic substances were present, multiplication of cells would not occur, regardless of the amount of substances added by another tumor. If toxic substances were not present, then multiplication would occur, the extent depending upon the amount of growth-promoting substances added through another tumor.

The results obtained fail to support the idea of the possibility of the existence of toxic substances. Other tests, in which resorcin cyanine (C. A. Lawrence) was used, failed to give any indication of the presence of toxic substances in the tumor. Yeast cells, which were known to [illegible] were cultivated in the tumor to support their multiplication, were not stained by methylene blue, thus indicating that death of the cells had not occurred.

[illegible]
[illegible] TH— [illegible]

Following the [illegible] of the presence of [illegible] amounts of [illegible] substances in sugar [illegible] development of [illegible] [illegible], the investigation [illegible]. [illegible] of [illegible] extensive [illegible] sugar. Also, [illegible] of complete [illegible] of the [illegible] will [illegible] comparison [illegible] tive amounts of growth-promoting [illegible] present in [illegible] sugar.

[illegible] year [illegible] of the sugar manufacturer [illegible] campaign, when [illegible] received [illegible] tory, a portion [illegible] in [illegible] solution for [illegible]. In order [illegible] es, [illegible], [illegible] sterilized under the same conditions. [illegible] year-to-year [illegible] of the [illegible] culture [illegible]. In the [illegible] the [illegible]. [illegible] being 100 ml. of distilled [illegible] control. [illegible] preparation, [illegible] were similarly [illegible] according to the previously [illegible] method. [illegible] for [illegible] days, [illegible] sample. This

examination [illegible] 1941 crop [illegible] and [illegible] 1941 crop [illegible] at least in 1937, 1939 or 1943 are given in Table 6, Chart [illegible]. The most favorable values [illegible] for the preceding years.

Twenty-five grain [illegible] preceding [illegible] in 1941. [illegible] ranged from [illegible] to 7.0, [illegible] 6.6. [illegible] to 6.4, [illegible] 1.9 for [illegible] in 1939. It should be noted, however, that the maximum value of 30.4 for sample No. 61 greatly exceeds the value of 19.4 for sample No. 7. A study of the [illegible] values of the chemical analysis (the constituents, organic and inorganic compounds) of the varieties of wheat may be considered as information that would account for the high value. The maximum values of 1.0 to 30.4, which were obtained for the 1941 samples received in 1942, [illegible] the previous years. The average multiple value of 6.9, as compared with 4.9 for the previous year, is the best record of any of the nine [illegible] years and the number of samples in 1933.

The results obtained in the examination of the individual varieties composite samples are presented in Tables 6, 7 and 8 are typical of those for the [illegible] of this study. Therefore, [illegible] the results [illegible], which are not [illegible] for the remaining [illegible] from 1934 to 1941, [illegible] with re-

TABLE 6. [illegible] IN [illegible] BY YEAST INOCULUM MULTIPLES

Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple
1	1.0	16	4.0
2	1.0	17	4.0
3	1.0	18	6.1
4	1.0	19	6.5
5	1.0	20	6.9
6	1.1	21	8.4
7	1.4	22	10.4
8	1.4	23	11.1
9	1.5	24	12.6
10	1.8	25	15.7
11	2.1	26	18.1
12	2.3	27	20.1
13	2.4	28	20.1
14	3.0	29	23.0
15	3.7		

Number of cells in inoculum, 35,000 per ml.

TABLE 7. [illegible] SUBSTANCES IN BEET SOIL IN 1932 CAMPAIGN AS POINTS OF [illegible] YEAST INOCULUM MULTIPLES

Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple
1	1.0	15	1.8	29	3.4	43	6.2	57	17.0
2	1.0	16	1.8	30	3.4	44	6.2	58	18.4
3	1.0	17	2.0	31	3.6	45	7.0	59	15.0
4	1.0	18	2.4	32	3.8	46	7.2	60	17.6
5	1.0	19	2.6	33	3.8	47	7.4	61	21.8
6	1.0	20	2.6	34	3.8	48	7.8	62	23.0
7	1.0	21	2.8	35	4.2	49	8.2	63	25.0
8	1.0	22	2.8	36	4.2	50	8.4	64	25.0
9	1.2	23	3.0	37	4.6	51	8.8	65	33.0
10	1.2	24	3.0	38	4.6	52	9.6	66	35.8
11	1.4	25	3.0	39	4.8	53	10.8	67	39.2
12	1.4	26	3.2	40	4.8	54	11.2	68	80.8
13	1.6	27	3.2	41	5.2	55	11.6		
14	1.6	28	3.4	42	5.4	56	12.0		

Number of cells in inoculum, 47,[illegible] per ml.

TABLE 8. [illegible] GROWTH-[illegible] [illegible] IN 1953 [illegible]

Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple	Sample	Yeast Inoculum Multiple
1	1.0	15	4.5	29	3.4	37	[illegible]	47	12.4
2	1.2	14	4.6	20	3.6	38	[illegible]	50	12.6
3	1.2	15	4.7	27	4.1	39	6.8	51	14.1
4	1.2	16	4.8	22	4.4	40	[illegible]	52	14.4
5	1.3	17	4.8	24	4.7	41	7.1	53	14.8
6	1.5	18	4.8	30	4.7	42	7.7	54	16.4
7	1.6	19	4.8	31	5.0	43	[illegible]	55	19.9
8	1.8	20	5.0	32	5.2	44	[illegible]	56	20.0
9	1.8	21	5.1	33	5.7	45	[illegible]	57	27.6
10	2.2	22	5.3	34	6.1	46	10.0	58	30.4
11	2.4	23	5.3	35	6.2	47	10.4		
12	2.4	24	5.4	36	6.7	48	12.4		

Number of cells in inoculum, 50,000 per ml.

vious results for each year in Table 9.

The average yearly yeast inoculum multiple values show a marked variation over the 11-year period. Beginning with the campaign year 193[illegible], there are three distinct periods during which the average multiple values showed yearly [illegible] only to be followed by sudden increases which are again followed by yearly [illegible]. It [illegible] that [illegible] for correlation [illegible] factors [illegible] due [illegible] variation [illegible]. However, [illegible] were noted. A possible explanation for the [illegible] tion [illegible] to be quantitatively [illegible]. It is significant that the [illegible] not exceed values that are considered to be [illegible] limit [illegible] of the sugar in [illegible] with less than 40, [illegible] provide [illegible] ments are not [illegible] ingredients.

Nearly [illegible], [illegible] ting [illegible] increases, [illegible] result of [illegible] tion [illegible] operation of new [illegible] or of old [illegible] moved to new locations. [illegible]. During this period of time, enormous improvements [illegible] which greatly increased their efficiency. [illegible] it, there was also noted an improvement in the general quality of [illegible] from nearly every factory as reflected by [illegible] amount of non-sugar substances. One of the significant [illegible] the continued reduction of the ash content of the sugar. A correlation exists, although not exact, between the

TABLE 2. [illegible] 1931-1941

Year	No. Samples	No. Cells Inoculated per 1.	For all Locations of Wells Average	Minimum	Maximum
1931	29	39,000	6.6	1.0	23.0
1932	61	47,000	7.5	1.0	37.8*
1933	58	50,000	6.9	1.0	30.4
1934	59	46,000	4.8	1.0	13.4
1935	66	49,000	11.4	1.0	59.6
1936	70	47,000	8.6	1.1	37.4
1937	70	51,000	5.0	1.6	19.2
1938	77	50,000	7.6	1.4	35.1***
1939	76	53,400	9.1	1.9	40.1**
1940	77	55,000	5.0	1.1	23.4
1941	87	50,000	6.6	1.0	16.6

*only one sample above 30.0

**only one sample above 34.2

***only one sample above 13.2

amount [illegible] the [illegible] relative amount [illegible] growth-promoting substance [illegible]. [illegible] divided into two groups, i.e., [illegible] multiple value, [illegible] in the [illegible] [illegible], [illegible] com- [illegible] inoculum multiple values, [illegible], the [illegible] content [illegible] giving the high- [illegible]. [illegible] for [illegible] given in Table 10.

The relationship [illegible] in [illegible] inoculum multiple values in- [illegible] growth-promoting substance is directly rela- [illegible] of the [illegible]. [illegible] stimulation [illegible], however, be- [illegible] stimulative effect on the yeast.

Since [illegible] relationship [illegible] to exist between the [illegible] content and the yeast inoculum multiple for the [illegible] composite samples examined [illegible], this relationship [illegible] on the basis of individual samples over the 11-year period. It [illegible] direct correlation might be found between these values [illegible] estimation of the amount of growth-promoting substances from the ash content of the samples. By using the ash content of the sugar, which is generally determined daily during its manufacture, as a basis for estimating the relative amount of growth-promoting substances, information would become available immediately regarding this quality of the sugar. In order to [illegible] relationship, the yeast inoculum multiple [illegible] ash content of individual fac- tory campaign composite samples [illegible] basis. Typi-

TABLE 10. [illegible] 1931 - 1941

Year	Wheat Inoculum Infectible Average	Rust* Average %	Percent in Classes Below Average Infectible	Above Average Infectible	Number of Plots Below Average Infectible	Above Average Infectible
1931	6.6	.0874	.0244	.0343	19	10
1932	7.5	.0245	.0119	.0246	50	18
1933	6.9	.0197	.0136	.0133	40	18
1934	4.9	.0142	.0100	.0160	36	23
1935	11.4	.0155	.0116	.0156	47	19
1936	4.6	.0135	.0130	.0191	34	34
1937	5.0	.0116	.0095	.0151	37	13
1938	7.6	.0159	.0106	.0216	47	33
1939	9.1	.0110	.0104	.0117	37	33
1940	6.0	.0099	.0095	.0104	45	42
1941	6.6	.0091	.0082	.0101	49	38

*The [illegible] in bins [illegible], [illegible] list, [illegible] plots [illegible] Twenty-five [illegible] part of [illegible].

[illegible] given in table 11.

[illegible] solution [illegible] growth-promoting sub- [illegible], [illegible] obtained [illegible] from [illegible] growth-promoting [illegible] more [illegible] .

In [illegible] inoculum [illegible] content [illegible] The [illegible] few [illegible] in the [illegible] content [illegible] samples [illegible] for [illegible] There is [illegible] or [illegible] inoculum [illegible] values to correspond [illegible] from Victoria, [illegible] there is a downward trend in both values of the years 1932, [illegible] 1934. In [illegible] values of [illegible] inoculum values [illegible] in comparison with the [illegible] content for the remaining [illegible] particularly significant. It is concluded, therefore, that the [illegible] content of the [illegible] cannot be used [illegible] of [illegible] growth-promoting substances [illegible]. [illegible] is: (1) The stimulative substances [illegible], therefore, [illegible] of the [illegible], [illegible] (2) the amount of stimulating substances is too small to be quantitatively correlated with other [illegible], [illegible] estimated by this method.

TABLE 11. [illegible]

Factory		1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941
A*	Multiple	X	10.8	6.8	1.6	11.4	4.7	5.4	7.8	6.5	3.0	5.0
	Ash %		.0456	.0134	.0048	.0498	.0105	.0[illegible]	.0[illegible]76	.0[illegible]	.0[illegible]	.0[illegible]3
B	Multiple	X	7.2	4.7	2.6	7.1	4.3	7.2	6.6	5.[illegible]	[illegible].3	10.0
	Ash %		.0206	.0140	.0095	.0[illegible]3	.0102	.0113	.0[illegible]8	.0[illegible]25	.0104	.0095
C	Multiple	X	12.8	14.1	7.8	13.[illegible]	11.3	9.6	12.[illegible]	12.[illegible]	9.4	6.4
	Ash %		.0213	.0318	.0[illegible]6	.0138	.0[illegible]1	.0175	.0[illegible]0	.0147	.0163	.0132
D	Multiple	11.1	5.[illegible]	4.1	3.6	5.3	3.7	[illegible].[illegible]	6.2	3.[illegible]	1.1	5.0
	Ash %	.0160	.0106	.0160	.0066	.0056	.0106	.0055	.0[illegible]0	.0066	.0[illegible]3	.0083
E	Multiple	5.4	4.6	4.7	3.3	[illegible].3	6.[illegible]	3.8	3.4	[illegible].[illegible]	6.7	6.6
	Ash %	.0180	.0[illegible]3	.0086	.0100	.0110	.0086	.0[illegible]5	.0[illegible]38	.0[illegible]86	.0[illegible]7[illegible]	.0078
F	Multiple	1.4	3.4	4.4	3.4	[illegible].[illegible]	[illegible].4	[illegible].[illegible]	5.6	[illegible].1	[illegible].[illegible]	4.[illegible]
	Ash %	.0[illegible]0	.0[illegible]	.0115	.0[illegible]0	.0060	.0[illegible]57	.0[illegible]49	.0[illegible]70	.0[illegible]56	.0[illegible]47	.0[illegible]62
G**	Multiple	X	[illegible].[illegible]	3.3	1.[illegible]	[illegible].[illegible]	4.[illegible]	[illegible].4	7.6	[illegible].[illegible]6	[illegible].[illegible]5	[illegible].6
	Ash %		.0[illegible]44	.0[illegible]72	.0[illegible]75	.0[illegible]3	.0[illegible]95	.0[illegible]90	.0[illegible]61	.0[illegible]7[illegible]	.0[illegible]3	.0[illegible]7[illegible]
H	Multiple	2.4	3.0	5.7	1.6	3.3	[illegible].[illegible]	1.6	4.6	[illegible].3	6.6	[illegible].4
	Ash %	.0170	.0[illegible]3	.0145	.0[illegible]73	.0[illegible]90	.0[illegible].[illegible]	.0[illegible]85	.0[illegible]70	.0[illegible]76	.0[illegible]7[illegible]	.0[illegible]3[illegible]
I	Multiple	X	3.8	[illegible].[illegible]	1.0	[illegible].3	[illegible].[illegible]	9.4	4.8	4.[illegible]	[illegible].[illegible]	[illegible].[illegible]
	Ash %		.0[illegible]4	.0051	.0[illegible]40	.0[illegible]16	.0[illegible]7[illegible]	.0[illegible]70	.0[illegible]70	.0[illegible]92	.0[illegible]34	.0102
J	Multiple	1.0	[illegible].4	3.[illegible]	X	[illegible]	7.[illegible]	6.0	5.[illegible]	[illegible].5	[illegible].5	X
	Ash %	.0[illegible]50	.0156	.0[illegible]35			.0[illegible]35	.0[illegible]35	.0[illegible]60	.0[illegible]39	.0[illegible]47	
K	Multiple	[illegible].[illegible]	[illegible].8	6.[illegible]	3.[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	7.6	7.3	X	X
	Ash %	.0[illegible]7	.0[illegible]93	.0[illegible]73	.0[illegible]1[illegible]	.0[illegible]48	.0[illegible]20	.0110	.0110	.0[illegible]75		
L	Multiple	[illegible].0	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	6.6	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
	Ash %	.0[illegible]10	.0[illegible]47	.0[illegible]73	.0[illegible]10[illegible]	.0[illegible]85	.0[illegible].[illegible]	.0[illegible]20	.0[illegible]35	.0[illegible]34	.0[illegible]5[illegible]	.0[illegible]66
Average on factories	Multiple	6.6	[illegible].[illegible]	6.[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	7.6	[illegible].[illegible]	[illegible].[illegible]	6.6
	Ash %	.0[illegible]24	.0[illegible]25	.0[illegible]37	.0[illegible]37	.0[illegible]37	.0[illegible]35	.0[illegible]16	.0[illegible]1[illegible]	.0[illegible]11	.0[illegible]44	.0[illegible]41

*-F, [illegible] factories; **G-, [illegible] factories; X, [illegible] receives or [illegible] not [illegible].

[illegible] obtained from individual consecutive [illegible] the amount [illegible] in consecutive [illegible]. [illegible] may [illegible] information regarding [illegible] uniformity of opera- tion of [illegible] factories [illegible] determining [illegible] deviation [illegible] from [illegible] respective factories. [illegible] samples [illegible] study. The [illegible] inoculum multiple values [illegible] determined for the individual samples [illegible] the average (mean) [illegible] deviation values were calculated. The re- sults [illegible] given in Table 12.

The [illegible] inoculum multiple values [illegible] samples from factories [illegible] 7.9, 5.3 [illegible] 4.7, respectively. Correspond- ing [illegible] deviation values of [illegible].4, 1.[illegible] and .[illegible], respectively. [illegible] these values [illegible] numerical index of the uniformity of operation, [illegible] value zero for perfect [illegible] factory [illegible] to have the best operation. The operation of factories [illegible] less uniform in that order.

[illegible]

[illegible] sugars did not [illegible] multi- plication of yeast cells in 10 percent solutions, while others stimu- lated [illegible] multiplication, attention [illegible] to [illegible] study of the nature and properties of the stimulants. [illegible] number of sugars, some of which [illegible], were extracted with alcohol [illegible] similar to that described by [illegible], (6) [illegible] observe high activity in Merck's (highest purity) [illegible]. The test sugars were dissolved in hot, distilled water until saturated solutions resulted. After the solutions had cooled,

TABLE 12. [illegible] TYPE STRAINS IN CHEMOSTAT [illegible]

FACTORY

A		B		C	
Strike No.	Least Inoculum Multiple	Strike No.	Least Inoculum Multiple	Strike No.	Least Inoculum Multiple
[illegible]	8.3	394	5.8	184	4.2
221	6.4	395	6.2	185	3.8
222	8.0	396	6.9	186	4.3
223	10.1	397	5.8	187	5.8
[illegible]4	[illegible].1	398	3.2	188	5.1
229	12.9	399	5.6	189	4.3
241	11.6	400	3.3	190	5.0
242	9.3	401	5.6	191	4.4
244	15.[illegible]	402	6.4	192	5.4
245	19.9	403	3.2	193	5.4
246	11.0	404	4.1	194	5.8
247	6.0	405	4.9	195	5.2
248	5.6	406	4.8	196	5.8
249	9.3	407	3.0	197	4.7
250	11.5	408	4.1	198	4.8
		409	7.1	199	3.6
		410	6.0	200	3.4
		411	5.2	201	4.4
		412	6.3	202	4.9
		413	5.1	203	4.3
		414	7.1	204	4.9
		415	6.4	205	4.6
		416	7.3	206	5.0
		417	5.8	207	4.4
		418	7.5	208	5.4
Average (Mean) 9.9		Average (Mean) 5.8		Average (Mean) 4.7	
Mean Deviation 2.4		Mean Deviation 1.0		Mean Deviation 0.53	

the sugar-free filtrates were recovered, and to them 95 percent ethyl alcohol was added to result in an alcoholic concentration of 80 percent. The sugar crystallized out from these solutions and was usually completely within 96 hours. The alcoholic liquor was then removed from the crystallized sugar by decantation and preserved for future studies. The sugar was dried, ground in a ball mill, and alcohol-purified sugars were then tested with those of the unpurified sugars in 20 percent solutions. The results given in Table 12 show the influence of alcohol purification in reducing the amount of stimulants.

A substantial reduction was obtained in the amount of stimulants in all samples which originally stimulated the growth of yeast. These reductions are similar to those obtained by Fulmer and Freedman (13, 14), who showed that the size of the yeast crop in their synthetic medium was reduced from 6.1 cm. to 1.5 mm. when ordinary sugar was replaced with sugar recrystallized from alcohol. These workers believed that the growth-promoting substance was vitamin B, and that without its presence, the strain of yeast used in their studies was unable to synthesize vitamin B and was therefore unable to grow.

Fulmer and Nelson (11) and Lampen and Silva (20) did not obtain reductions in the size of yeast crops when alcohol-purified sugar was substituted for unpurified sugar in synthetic media. It is apparent that they were using a higher grade of sugar, such as A and B, Table 11, for their experiments than that used by Fulmer and Freedman.

FRACTIONATION OF ALCOHOLIC EXTRACT

The solubility of the growth-promoting substance in 80-percent alcohol, its presence in sugar, resistance to autoclaving treatment

TABLE 13. [illegible] OF [illegible] BY CHEMICALLY PURIFIED AND 80-PERCENT ALCOHOL

Sample	Yeast Inoculum Unpurified Sugar	Yeast Inoculum Multiple Purified Sugar	Sample	Yeast Inoculum Unpurified Sugar	Yeast Inoculum Multiple Purified Sugar
A	1.0	1.0	G	17.0	9.5
B	1.0	1.0	H	21.0	10.0
C	3.0	1.0	I	24.5	1.3
D	11.3	6.6	J	44.0	3.0
E	22.6	3.0	K	59.0	12.0
F	[illegible]	9.6	L	87.3	1.0

(during the manufacturing process of sugar) suggested that it might [illegible]. The alcoholic extract which remained after crystallization of the sugar were purified according to the method described by Lucas (22) [illegible] fractionation from salt combings. Two fractions were then prepared and the remainder of the test yeast to the solutions were observed to determine if the stimulant contained blos properties similar to those in previously studied blos preparations. Two fractions designated as Blos I and Blos II were then prepared from the alcoholic liquors in the following manner:

Fractionation of Blos

The alcoholic liquor from 1,100 grams of crystallized sugar is distilled in vacuo to remove the alcohol. The volume of the residue concentrate is 750 c.c. of a straw-colored liquid. To the concentrate is added 700 c.c. of a hot, saturated aqueous solution of barium hydroxide $Ba(OH)_2.8H_2O$ to effect complete precipitation of uncrystallized sugar residing in the concentrate. The quantity of barium hydroxide required is estimated by previous determinations on similar material. A white precipitate is formed which is not removed. Alcohol, 95 percent, is then added in volume equal to that of the sum (900 c.c.) of the volume of the concentrate and the barium hydroxide solution; at first a gummy, sticky precipitate forms which adheres to the walls of the container and to the stirring rod, but which later becomes loose and granular. A few drops of concentrate barium hydroxide are added to a small quantity of the clear liquid to determine if sufficient amount of barium hydroxide has been added. The precipitate, after it is filtered off, is washed with dilute alcohol (2 volumes of 95 percent alcohol to one volume of water) and the filtrate, together with the washings, is reserved for Blos II as described later. (Smaller quantities of sugar may be crystallized from alcohol, thus requiring proportionally smaller quantities of reagents).

Blos I

The barium precipitate, after washing, is stirred with three or four successive portions of boiling water, room temperature, filtering between each. The undissolved precipitate which contains no blos is rejected. Blos I liquor (Lucas) is prepared from the combined filtrates by saturation with carbon dioxide, and then boiled in vacuo to remove the excess of the gas, after which it is filtered. The precipitate con-

[illegible] Bios. To the filtrate, heated to about 65° C., is added 2.5 % sulfuric acid until no further precipitate forms. This is then filtered off by the use of Celite and removed with the [illegible] in excess of acid in the solution, although [illegible] is obtained. The precipitate, which contains no Bios, is rejected and the filtrate is retained as "Crude Bios I solution", (Lugs). For the purpose of this study this fraction is considered as Bios I. The volume is about 2 percent of the original concentrate. (Note: In the preparation of Bios I from malt sprouts, the addition and subsequent precipitation of the "Crude Bios I solution" with lead acetate is omitted in order to eliminate glucose. This step is not necessary when the fraction is prepared from brewer's yeast autolysate).

Bios II

The Bios II liquor is [illegible] concentrate, the excess [illegible] boiled off in vacuo. The precipitate is filtered off on diatomite and contains most of the Bios II fraction. The filtrate is evaporated in vacuo at the lowest possible temperature until about 10 percent of the original volume remains. At this concentration, large bubbles form which nearly fill the container. The viscous liquid is treated with about 1,500 ml. of acetone or sufficient to effect complete precipitation. The acetone solution is decanted off, the precipitate washed with acetone and the [illegible] added to the solution. The solution is concentrated in vacuo until a gummy residue remains which is taken up in water. This solution, the so-called Bios II, is made up in volume to that of the Bios I.

A number of [illegible] extracts from purified yeasts were fractionated by the [illegible] and were tested in different solutions of the purified yeasts from which they were obtained. The amounts of individual fractions were [illegible] to give maximum stimulation of the yeast inoculum [illegible] ml. each of Bios I and Bios II solutions, respectively. The results obtained from four typical samples having initial yeast inoculum solids [illegible] from 1.4 to 57.2 are given in Table 14.

Crystallization of samples [illegible] from 50 percent alcohol resulted in complete removal of the stimulant. Since only a slight amount of stimulant was present in the original extract, very little

TABLE 14. [illegible] OF [illegible] FROM [illegible] AND [illegible] OF PURIFIED [illegible] IN SOLUTIONS OF PURIFIED SUGAR

Treatment	Yeast Inoculum Multiples for Samples			
	A	B	C	D
Unpurified sugar	1.5	1.4	41.8	57.4
Purified sugar	1.0	.8	3.1	5.4
Purified sugar plus Bios I (10 ml.)	1.1	1.1	7.1	17.1
Purified sugar plus Bios II (3 ml.)	1.0	1.4	18.6	31.0
Purified sugar plus Bios I (10 ml.) and Bios II (3 ml.)	1.1	1.7	33.3	44.5
No. yeast cells in inoculum, per ml.	50,450	46,300	21,050	50,450

effect [illegible] isolated from the various clarification. Lactic acid [illegible] substantially to the growth-promoting effect of yeast cells introduced into the test solution after fermentation. The results obtained [illegible] the use of Brewer's Yeast Extract Concentrate C and [illegible] the effect of the [illegible] are summarized in Figure [illegible].

Optimum levels of the two Concentrates [illegible] 0.3 and 0.4 percent by weight, respectively, of the yeast in culture solution. The stimulation of the fraction by the Plus I solution alone was relatively [illegible], but when fermentation was followed by the Plus II solution alone. [illegible] in the individual results, a still greater [illegible] was obtained. The relationship is graphically illustrated in Figure [illegible], in which the yield of yeast in multiple volumes for Concentrates C and D is reduced to [illegible] times that for [illegible] 0. Although the multiple [illegible] are lower [illegible] obtained in the commercial plant, the trend [illegible] in the same order as those obtained under laboratory conditions. It is, therefore, concluded that a [illegible] stimulation activity similar to that of other [illegible] is present in sugar. Further work in this [illegible] laboratory was discontinued in 1933 in favor of [illegible] the problem of the small amount of sugar free from yeast growth-promoting substances.

[illegible] AND [illegible] IN [illegible] PRODUCTS [illegible] ITS SUGAR

The reason for the failure of [illegible] to show growth-promoting activity, while others showed wide [illegible] variations, was investigated from the standpoint of the manufacturing process in the

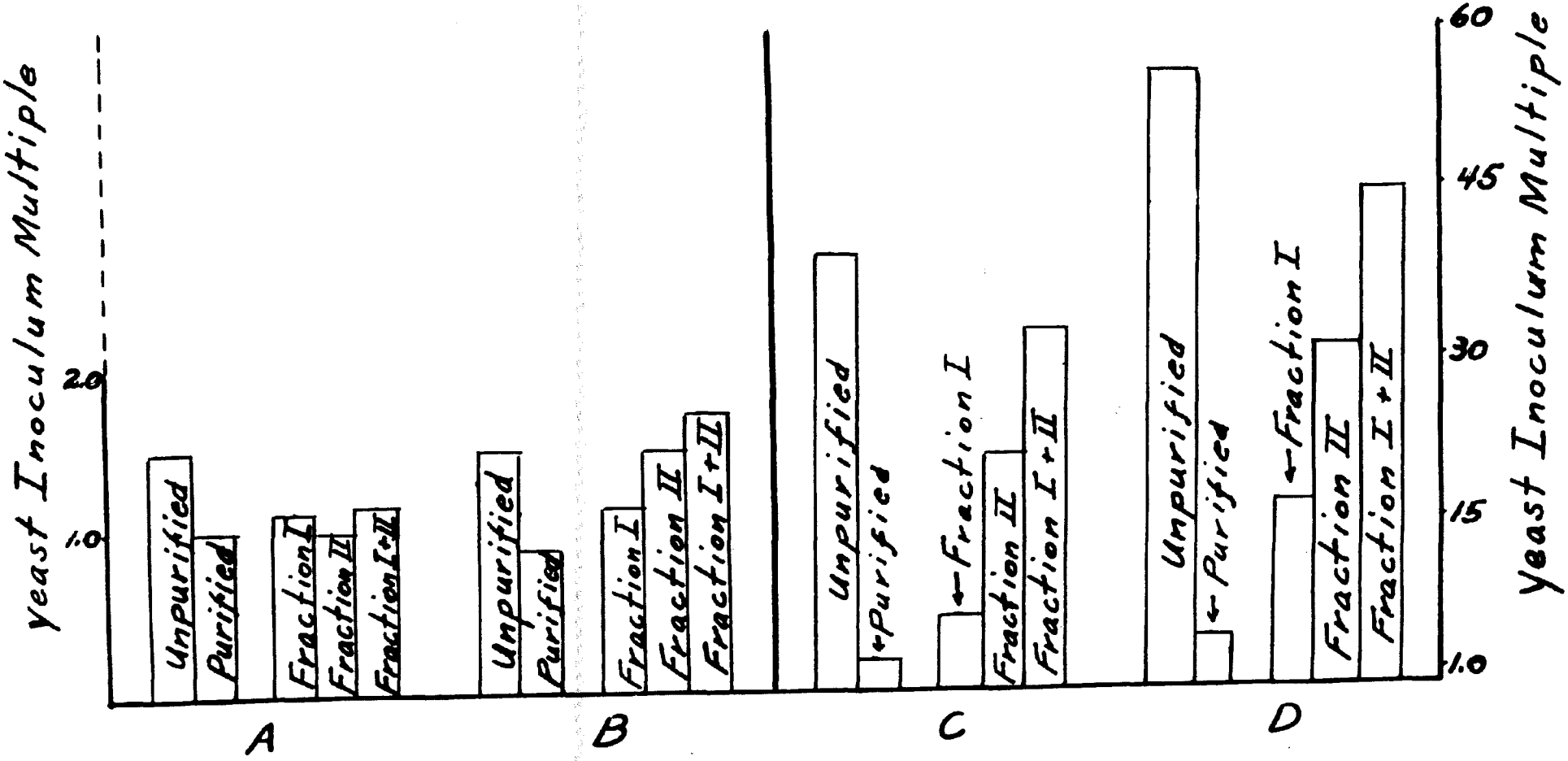


Figure 2. Purification of Sugars by Crystallization From Alcohol and Addition of Purified Fractions to Solutions of Purified Sugar.

have cast doubt on the validity of the method. The stimulant from [illegible], [illegible], rice, [illegible], and [illegible] are somewhat similar to those from [illegible]. [illegible] All of these sources [illegible] the yeast in solution. The excess of yeast growth obtained by the addition of [illegible] factors is not easy to explain. The stimulating substances are believed to have their origin in the [illegible] which extracted during the sugar recovery process.

Since the most active fraction of the yeast occurred in the diffusion-sugar liquor, which is the most concentrated form of sugar juice obtained in the process, it is evident that there is no substantial concentration of the stimulant in the product. The effect of the relative amount of growth-promoting substances in white sugar prepared from beets was examined in a chain of design consisting of sugar from 12 Steffen factories and 12 non-Steffen factories. [illegible] was made up in 10 percent solutions of sugar and the yeast increment multiplied determined. The results obtained are given in Table 15.

There is no uniform relationship between the relative amount of stimulants in the sugar and the ash content. An example of the disparity which exists is illustrated between samples 2 and 3 from the Steffen factories. The sugar from factory 2 has a multiple value of 10.0 as compared with 3.3 for that from factory 3, but its ash content has a value of 230.0 as compared with 24.0 from factory 3. Similar relationships also exist between the samples obtained from the non-Steffen factories. The failure to eliminate the stimulant from the

TABLE 25. [illegible]

STEPPE			NON-STEPPE		
Factory	Total Inoculum Unsuitable	Multiple M. F	Factory	Total Inoculum Unsuitable	Multiple M. F
A	92.0	10.0	A	168.0	1.6
B	134.0	11.6	B	172.0	1.6
C	156.0	7.0	C	201.0	5.6
D	164.0	9.4	D	216.0	6.6
E	130.0	6.6	E	254.0	10.4
F	172.0	4.7	F	256.0	4.8
G	124.0	16.6	G	260.0	4.8
H	118.0	8.8	H	271.0	4.4
I	224.0	6.3	I	278.0	10.2
J	160.0	9.0	J	284.0	9.2
K	250.0	8.6	K	316.0	9.6
L	252.0	9.0	L	660.0	11.4
M	376.0	9.0	M	842.0	6.6
N	414.0	11.2			

sugar while forming the precipitate is only small in the presence in the sugar. The mechanism for the action of [illegible] [illegible] [illegible] [illegible] [illegible] precipitates.

[illegible] [illegible]
TO CRYSTAL SURFACES

The [illegible] which exists between the yeast function [illegible] by yeast in the presence of [illegible] were made from the present test (1) some of the stimulus might be occluded in the crystals, thus making its [illegible] impossible by centrifugation and washing; or, (2) that an insufficient amount of water was used to wash the stimulus after allowing time for the stimulus to diffuse from the precipitate. It seemed desirable, therefore, to determine the location of the stimulative substance with respect to the crystal surfaces. This knowledge was obtained by selecting 1 samples of sugar with a range of yeast inoculum multiples of 1.4 to 11.6 and [illegible] the crystals with sugar solutions of densities below saturation. In this manner a portion of each crystal was dissolved away, the extent of dissolution being determined by the initial density of the solvent solution. The stained crystals were removed from the slides, washed with a small amount of distilled water, and dried. The yeast inoculum multiple values were then determined and corrected to zero from the curve [illegible] before [illegible] ling. The results of this treatment, which were previously reported by Will and Jones, (26) are given in Table 16.

Substantial reductions were obtained in all but two samples. The reduction was 50.0 percent or greater for 13 of the 14 samples examined, and in general, the greater the amount of stimulant present,

TABLE 16. [illegible] OF GRAM-POSITIVE AND GRAM-NEGATIVE [illegible]

Sample	Mean Bacterial Count per Milliliter		
	Non-Treated	Treated	Percent Reduction
1	11.6	7.0	34.1
2	11.4	7.4	30.6
3	10.0	1.0	81.0
4	6.4	1.6	75.0
5	6.0	2.2	63.3
6	5.4	2.2	60.7
7	3.6	1.0	72.2
8	3.4	1.0	70.6
9	2.2	1.0	54.5
10	2.0	1.0	50.0
11	1.8	1.2	33.3
12	1.4	1.0	28.5

[illegible] is

[illegible] (A) [illegible]

[illegible] (B) [illegible] the

distribution of [illegible] in [illegible] from 4.2 to 10.0

[illegible] 50

percent of [illegible], [illegible] over 5 percent of the crystal, i.e., in

the [illegible] color, [illegible] more uni-

formly distributed throughout the whole crystal. The difficulties

attending the elimination of the water film by centrifuging milling in successive amount of water in the centrifuge is pointed out as an aid to the study of commercial sugars. From these observations it is concluded that in order to manufacture a "better grade" granulated sugar, the film of mother liquor surrounding each crystal [illegible] as well as part of the crystal itself must be eliminated. Also, it is [illegible] difficulties may be experienced in eliminating certain [illegible] types of uniform size from smaller crystals.

INFLUENCE OF QUANTITY OF CENTRIFUGAL WASH WATER ON THE PHYSICAL PROPERTIES

In order to determine if greater elimination of non-sugar substances could be obtained from crystal surfaces during centrifugation by increased quantities of centrifugal wash water, the quantity of water was varied in successive baskets of a recently installed [illegible] centrifugal. One machine was selected in each of four factories, and for four successive baskets the quantity of water normally used was increased or decreased by two-fourths. A sample of finished sugar was collected from the machine after each basket of the regular operating lower grade sugar to be used as representative of the entire basket. The usual granulation analysis was made for each sample of sugar. These results, together with the quantities of wash water used, are given in Table 17.

There was a decrease in the yeast fermentation [illegible] values for each variable (except one variable from factory C) when the quantity of wash water was increased by two-fourths per machine for successive baskets of sugar. Conversely, when the normal quantity of water was decreased by one-fourth per basket, the yeast fermentation values

TABLE 17. [illegible] OF [illegible] OF CERTAIN [illegible] WATER

Factory A		Factory B	
Quarts Water at 38° C	Yeast Inoculum Multiple	Quarts Water at 72° C	Yeast Inoculum Multiple
14*	22.7	16*	13.4
16	20.5	18	9.6
18	17.4	20	7.4
20	14.8	22	3.6

Factory C		Factory D	
Quarts Water at 86 C	Yeast Inoculum Multiple	Quarts Water at 86 C	Yeast Inoculum Multiple
15*	8.8	24*	3.8
17	8.8	22	3.4
19	6.4	20	6.0
21	4.8	18	7.8

*Quarts normally used.

(except one c[illegible]) increase. [illegible] the [illegible] growth-promoting [illegible] is [illegible] of [illegible] by increasing [illegible]. [illegible] the speed of the [illegible] [illegible] [illegible]. Since [illegible], several [illegible] [illegible], [illegible] (95° C. [illegible]) [illegible] [illegible]. [illegible] improvements are reported to [illegible] the elimination of [illegible] surface, non-[illegible] impurities. The [illegible] [illegible] to include yet a growth-promoting substance, [illegible] that similar improvement in elimination likely [illegible].

It is suggested that for the manufacture of "beverage" grade [illegible] coarse grain [illegible] of the [illegible] with which the crystal surface, [illegible], therefore, a large percentage of surface impurities, may be removed. Although it is felt that the [illegible] produced by the present [illegible] is generally satisfactory for use in beverage [illegible] tool product, an extra effort should be made to insure the elimination of the most terrible amount of non-[illegible] impurities. It is recognized that their presence in sufficient quantities in beverage can satisfy the nutritional [illegible] of contamination [illegible] and permit the levels [illegible] are sufficiently large to [illegible] turbidity or sediment in the solution.

V. [illegible]

Early in 1947 [illegible] shortly before [illegible] this [illegible] a number of bent [illegible] were [illegible] impurity, non-

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[illegible] Office of Technical Services, U.S. Department of Commerce, Washington, D.C.

tothenic acid, a growth-promoting [illegible] folic acid. Biotin was found to be present in very low amounts (0.025 to 0.445 [illegible] per gram of raw sugar) in alcohol free extracts which were prepared by recrystallization of individual sugar samples from 80 percent alcohol. The assay was conducted [illegible] Cline. This is a microbiological procedure employing Lactobacillus arabinosus with a modified culture medium (see Table [illegible]). [illegible] insufficient number of samples [illegible] to permit [illegible] definite conclusion, the results [illegible] possible [illegible] the amount of biotin present [illegible] the growth-promoting ability of the sugar as measured by yeast inoculum [illegible]. Preliminary examination of the extracts [illegible] the presence of other growth-promoting substances. Existing conditions did not permit further investigation of this subject; however, it is hoped that in the future this study can be resumed in order to make a complete examination for other substances which might be present in sugar.

VI. SUMMARY

The results of this research show that:

1. There are present in beet and cane sugar non-sugar substances which stimulate the growth of yeast in solutions.

2. [illegible] method developed for testing the relative amount of yeast growth-promoting substances shows them to be present in [illegible] sugars and present in them, in varying quantities.

3. The relative amount of substances is roughly correlated with the ash content of the sugar but it is not a part of the ash. It is organic in nature.

4. The stimulative substances are soluble in [illegible] percent alcohol and

[illegible] from [illegible] precipitation [illegible] ethanol.

5. [illegible] crystalline [illegible] relatively [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] centrifugation.

6. [illegible] [illegible] [illegible] Martin.

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Illustration 2. [illegible]

VITA

[illegible] Bushnell [illegible], was born October 1, 1904, at Fort Wayne, Michigan. [illegible] elementary education [illegible] (1918), and the high school education at [illegible], Michigan (1921). Five years later he graduated [illegible] and was granted the Michigan State College, receiving the Bachelor of Science degree in the School of Applied Science. During the following [illegible] he was employed [illegible] Dairy Chemist and Bacteriologist, in the laboratories of the Michigan Department of Agriculture, Lansing, Michigan. In August 15, 1930 he became employed by the present Agricultural Research Administration, Bureau of Agricultural and Industrial Chemistry, United States Department of Agriculture, Washington, D. C. [illegible] Assistant Bacteriologist, where he did some research work in the bacteriology on sugar, sirups, molasses, starch, fish and egg products. Promotions were granted to Associate Bacteriologist (1936) and later (1942) to Bacteriologist, the position which he held at present. As the result of part time studies at the Michigan State College, the University of Maryland, College Park, Maryland, and the United States Department of Agriculture Graduate School, Washington, D. C., the degree of Master of Science was obtained at the former school in 1936. Fraternity and society memberships are held as follows: Sigma Xi, Society of American Bacteriologists, American Public Health Association and the Institute of Food Technologists.

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