1927

A study of the preparation of sirups

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14518

A STUDY OF THE PREPARATION OF SIRUPS

By

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A Thesis Submitted to the Graduate Faculty
for the degree of

DOCTOR OF PHILOSOPHY

Major Subject: Food Chemistry

Approved

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

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1927

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ACKNOWLEDGMENT

The writer wishes to express his sincere appreciation for guidance and criticisms of this work to Professor John H. Buchanan and Doctor Max Levine, under whose supervision this problem was undertaken and completed.

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I. INTRODUCTION

A. Historical.

One of the most important problems confronting the manufacturer of bottled carbonated beverages is the making of a product which will keep without deteriorating or spoiling over a long period of time. If such a product is to be prepared, either (1) the material entering into its composition must be sterile and handled aseptically, or (2) conditions within the bottle must be such that there will be no growth of micro-organisms which may have been present in any of the materials going to make up the finished product.

The Fellowship established at Iowa State College by the American Bottlers of Carbonated Beverages with the Departments of Chemistry and Bacteriology cooperating has been studying the spoilage of bottled carbonated beverages with the purpose of determining the principal causes of spoilage and possible methods for their elimination. Of the 734 samples of spoiled carbonated beverages examined in 1925-26, 83.3% were found to contain yeast. In many cases even casual examination showed flocculent sediments which, when examined microscopically, were found to consist principally of yeast cells.

In order to determine whether the principal source of

this yeast contamination was from improperly cleaned apparatus and pipe-lines, from the sugar or other ingredients, or from casual contamination from the air, a series of bottling experiments was carried out in the bottling plant which was equipped by the American Bottlers of Carbonated Beverages. It was found that a complete sterilization of apparatus and pipe-lines did not guarantee a finished product which would not spoil.

As a confirmation of these results, complete series of samples including simple sirup, flavored sirup, raw and filtered water, carbonated water, and finished product were studied from bottling plants which had had yeast trouble. In nearly all of these series, if yeast cells were found to be present in the finished product, they were also found in the simple sirup. Questionnaires were sent to the firms which supplied these series of samples. The replies to these indicated that the cold process of making sirups was employed by over 90% of the bottlers.

In order to study the sugar as a possible source of yeast contamination, since these series of samples usually showed yeast as absent from the water although present in the simple sirup, it was desired to have samples of sugar which would be representative of the sugar supply of the

bottling industry. Accordingly, sterile sample bottles with instructions for proper sampling were sent out to 125 representative bottlers in 28 states who had previously expressed a willingness to cooperate on this problem. Of the 132 samples of sugar obtained, 62 (47%) were found to contain yeast.[1] Since the prevalent method for preparing sirups is by the cold process, conditions are usually suitable for the survival of these yeasts which may be present in the sugar and their subsequent growth in the finished beverage. Owen[2] says that the process of refining sugar does not leave the sugar sterile and says that there are organisms capable of withstanding the temperature of the processes and which survive in the form of spores. An investigation of the resistance of these spores of the species found in sugar showed that they could survive a 3 to 4 hour exposure to streaming steam. Owen[3] also showed that this sugar when inoculated into sterile 10% sirup yields gum-forming species of bacteria almost exclusively. When grown in 25% sugar solution, unequal numbers of yeasts and bacteria appear with the latter predominating. In 50% sugar solutions, a mixed growth

1. McKelvey, C. E., J. Bact. 11, 98 (1926).
2. Owen, W. L., Centr. Bakt. Parasitenk., II Abt., Bd. 40, 247 (1914).
3. Owen, W. L., Louisiana Agr. Expt. Sta., Bull. 146, 57, May, (1914).

of yeasts and bacteria is also obtained but the yeasts are predominating. Owen makes the following statement with regard to less highly refined sugars--"That the sugars used in these experiments were not abnormal in respect to their mixed flora of bacteria and yeast appears evident from a direct microscopical examination of about 70 samples of different qualities of raw sugars, about 40% of which were found to contain yeast cells." Dubourg[4] reported a yeast which was still active at a concentration of 80% sugar. Owen[5] isolated Saccharomyces zopfii from heavy sirup and characterized this species by (1) its ability to ferment highly concentrated sugar solutions and (2) its resistance to heat. McKelvey[6,7] has shown that yeast cells in the vegetative form would die off rather quickly in sirups of high density but this does not hold true for spores. Usually however, the sirup is not stored for any great length of time before it is diluted to make up the flavored sirup which subsequently is diluted again upon being bottled. Thus the yeast

4. Dubourg, Rev. de Viticult. 1897, p. 467.
5. Owen, W. L., Centr. Bakt. Parasitenk., II Abt., Bd. 39, 468-82 (1913).
6. McKelvey, C. E., The Beverage Journal 61, 58, April, (1925).
7. McKelvey, C. E., The Beverage News 17, 18-19, Dec. (1925).

cells or spores which may have been originally present in the dry sugar find conditions favorable for germination and growth.

The examination of samples of spoiled beverages by the Fellowship has shown that the acid of the beverage and the carbon dioxide pressure are effective in keeping bacterial and mold growths to a minimum or eliminating them entirely. As far as these two agents of spoilage are concerned, it is usually possible to use enough acid or have a great enough carbon dioxide content to prevent them from growing without making the beverage objectionable because of its sourness or high carbonation. Many spoiled products having a high acidity as well as good carbonation have been found to contain yeasts. It seems improbable that the use of as great quantities of these agents as is feasible, without seriously affecting the flavor and taste of the beverage, would eliminate this type of spoilage. Weldin[8] reports that with agar media it took a pH range from 2.6 to 3.3 to inhibit growth of yeasts while broth media showed that a pH range from 2.3 to 2.7 was necessary to inhibit the growth of these same organisms. This latter represents the limit of acidity in

8. Weldin, J. C., Proc. Iowa Academy Science 37, 95 (1925).

a beverage which will be pleasing to taste. Obviously then, some method must be found to eliminate these yeast cells and spores from the simple sirup unless the bottler wishes to resort to the pasteurization of all of his bottled products.

A chemical change which takes place in beverages which are stored for any length of time is the slow inversion of the sucrose by the acid of the beverage. This is one of the principal changes which takes place in the aging of ginger ales--a practice which is said to mellow them. The invert sugar which is produced by the decomposition of the sucrose is less sweet than the sucrose from which it is formed, different investigators evaluating its sweetness at 78 to 95 on the basis of sucrose as 100.[9,10,11,12]

There seems to be some disagreement concerning the sweetness of levulose,[13] values having been reported ranging from 103 to 150. Spengler and Traegel[14] assign a value of 108 for its sweetness which is in good agreement with Paul's value of 103.3. Bates[15] on the contrary agrees quite close-

9. Deerr, Intern. Sugar J. 24, 481 (1922).
10. Paul, Z. Untersuch. Nahr. Genuss-mit. 43, 137 (1922).
11. Sale and Skinner, Ind. Eng. Chem. 14, 522 (1922).
12. Buchanan, J. H. and Beard, P. J., Unpublished data.
13. Intern. Critical Tables, Vol. 1, 358, (1926).
14. Spengler and Traegel, Z. Ver. deut. Zuckerind. 77, 1-12 (1927).
15. Bates, Facts About Sugar 21, 250-1 (1925).

ly with Deerr, and Sale and Skinner, on a sweetness value near 150 for levulose. These same workers agree more closely on the sweetness value for dextrose assigning it a sweetness value of 50 to 60 on the same basis. No matter which values of sweetness are taken, an artificial invert sugar made from equal parts of dextrose and levulose would be less sweet than an equivalent quantity of sucrose. This loss of sweetness caused by inversion accounts for the change of taste of beverages upon long storage and is entirely independent of the growth of micro-organisms.

It was thought advisable to investigate the practicability of the bottler inverting his simple sirups in the process of its sterilization and preparation. By using an inverted sirup there would be an unchanging sweetness and a uniform product would result for the manufacturer. It also might be found that the time of aging could be cut down or possibly eliminated altogether.

The hydrolysis of sucrose has been a much investigated subject but, unfortunately, nearly all of the work has been done with dilute solutions of sucrose and at low temperatures. The primary object has been to investigate the mechanism of the reaction rather than the production of a large amount of invert sugar. Caldwell[16] gives rather a

16. Caldwell, R. J., Brit. Assoc. Advancement Sci. Rept. 1906, 267-92.

complete bibliography up to 1906 on the hydrolysis of sucrose.

Spencer[17] studied the effect of citric, tartaric, phosphoric, and sulfuric acids on the rate of inversion of sucrose. She gives a review of the literature up to and including 1925. Spencer worked with sucrose and acid solutions at the concentrations in which they are found in the finished beverage. She concludes that (1) The hydrogen ion concentration does not change appreciably during the course of the reaction. (2) In a given solution, increasing the concentration of sucrose increases the velocity of the reaction to a small extent. (3) For tartaric, citric, and phosphoric acids, the rate of reaction decreases as the reaction proceeds until a point of equilibrium is reached. This point of equilibrium depends for a given acid with a definite concentration of sucrose upon the pH of the solution. (4) For a given pH and a given concentration of sucrose the rate of reaction varies with the kind of acid, being considerably greater for phosphoric acid than for citric or tartaric acids. Tartaric acid inverts slightly faster than

17. Spencer, Grace G., M.S. Thesis, Dept. of Chemistry, Iowa State College Library, 1925. Unpublished.

citric acid but the difference is scarcely appreciable. Many workers[18,19,20,21,22,23] have tried to fit the hydrolysis of sucrose to the general equation for a monomolecular reaction by using activities instead of concentrations for sugar and hydrogen ion, and assuming that the sucrose molecules were hydrated with five, six or more molecules of water. Scatchard[24] says that uncertainty as to the activity of the H ion in solutions containing sucrose makes inconclusive the attempts to determine the mechanism of the inversion process by fitting the rates of reaction quantitatively with formulae containing these activities. Even the difference between the change of liquid junction potential with flowing and with stationary junction amounts to 11% in the hydrogen activity between 0 and 700 grams of sucrose per liter while the change in water activity in this range is less than 9%. Obviously the number of mols of water that enter into the reaction cannot be accurately determined.

18. Pennycuick, S. W., J. Am. Chem. Soc. 48, 6-19 (1926).
19. Corran and Lewis, J. Am. Chem. Soc. 44, 1673-84 (1922).
20. Jones and MacLewis, J. Chem. Soc. 117, 1120 (1920).
21. Clark, R. H., J. Am. Chem. Soc. 43, 1759 (1921).
22. Rosanoff and Potter, J. Am. Chem. Soc. 35, 245 (1913).
23. Kolthoff, I. M., Verslag. Akad. Wetenschappen. Amsterdam 35, 281-295 (1926).
24. Scatchard, George, J. Am. Chem. Soc. 48, 2034 (1926).

Since very few of these investigators worked with sucrose solutions concentrated enough to be called sirups and much of the foregoing work was inapplicable because it was done with such a low temperature that inversion took place only slowly, it was decided to use citric and tartaric acids in the concentrations usually present in the flavored sirup and to use a temperature near to the boiling point of the sirup.

Siebel[25] contends that the original source of yeast contamination is from bottles which have not been adequately washed rather than from the sugar. Accordingly it was deemed advisable to investigate the resistance of yeast spores when subjected to the action of hot alkali solutions at temperatures generally recommended for the washing of bottles.

B. Statement of Problem.

The preceding discussion led to the experimental consideration of the following problem which may be grouped under three sub-heads.

1. Relation of the time of exposure and temperature in sterilization of sirups contaminated with yeast spores.

25. Siebel, Carbonator and Bottler 44, 56-58, March (1927).

2. Relation of the temperature, concentration of alkali, and period of exposure to the death rate of yeast spores.
3. Rates of hydrolysis at 90°C and 100°C for different sirups with varying concentrations of citric and tartaric acids.

II. THE RELATION OF THE TIME OF EXPOSURE AND TEMPERATURE IN STERILIZATION OF SIRUPS CONTAMINATED WITH YEAST SPORES

A. Preparation of Reagents.

All of the sirups used in this series were prepared from a highly refined sugar whose normal solution gave a reading of 99.9 when polarized in a 200 mm. tube on a saccharimeter. The moisture content of this sugar was 0.028%. These sirups were made up on the density basis by using the proper amount of distilled water to funish the density of sirup desired. Three densities of sirup were used in this series--24° Baume, 30° Baume, and 36° Baume. These are equivalent to specific gravities at 20°C/20°C of 1.1984, 1.2609, and 1.3303 respectively.

The spores were prepared from cultures of 28 spore-forming yeasts which had been isolated from spoiled carbonat-

ed beverages.[26] A special medium of carrot agar was used as this was found to be especially suitable for the formation of spores by yeasts.[27] This carrot calcium sulfate agar was prepared by grinding up about four pounds of carrots (saving the juice) and extracting the pulp with about two liters of boiling distilled water. The total volume of juice and extraction water was two liters. This liquid was saturated with calcium sulfate and 2% of agar agar was added. This was autoclaved at 15 pounds steam pressure for 20 minutes.

About 50 cubic centimeter quantities of this medium was transferred to each of 28 Kolle flasks. These were then rather heavily plugged with cotton and autoclaved for another 20 minutes. Slants of carrot agar were made in 28 test tubes at the same time. After cooling and solidification of the agar had taken place, the Kolle flasks were inoculated evenly over the surface by the use of sterile swabs. One Kolle flask was used for each of the 28 cultures of the spore-forming yeasts. The slants of carrot agar were also inoculated with the same cultures. The Kolle flasks and tubes were incubated for 17 days when numerous spores were demonstrated by making spore

26. Turner, W. R., M.S. Thesis, Dept. of Bacteriology, Iowa State College Library, 1925. Unpublished.
27. McKelvey, C. E., J. Bact. 11, 98 (1926).

stains of the cultures which were grown in the tubes. The yeast growths on the surface of the Kolle flasks were scraped off by means of a hoe-shaped Monel metal tool which had previously been sterilized in a flame. The scrapings were all mixed together in a sterile evaporating dish and dried at 45°C in a vacuum oven. After drying, the spores were ground up in an agate mortar under aseptic conditions and screened to pass a 200 mesh sieve. The dry weight of the mixture of the 28 yeast spore cultures was 39289 g. A count of 72,000,000 per gram was obtained by plating out a small portion. Since it would be rather difficult to get approximately uniform suspensions by attempting to weigh out equal quantities of this spore mixture with such a high count, it was diluted with 9 times its weight of sterile lactose. Lactose was used because very few yeasts can utilize it directly and because the lactose would have no toxic effect on the yeasts as might have been the case if a salt had been used instead of the lactose. The spore mixture and lactose were homogenized by stirring with a spatula and sieving twice through a 200 mesh sieve.

B. Method of Procedure.

The method of procedure in this series of experiments was to measure 100 cubic centimeters of sirup of the density

desired into a 3-necked, round-bottomed Pyrex flask of 200 cubic centimeters capacity. A one-hole cork was fitted with a suitable stirring rod and placed in the middle neck of the flask. The other two necks were plugged with cotton. This flask was then sterilized in a steam autoclave at 15 pounds of steam pressure for 20 minutes. After being sterilized, the flask was immersed in a DeKhotinsky water bath which had previously been regulated to the proper temperature. The stirring rod was connected to a stirring motor and set in motion. After allowing it to remain in the bath for about 30 minutes, the contents of the flask would be at the desired temperature. Then one cubic centimeter of a 10% suspension of the spore mixture was added to the warm sirup by means of a capillary pipette taking care not to contaminate the sides of the neck. At stated intervals, 5 cubic centimeters of the sirup would be withdrawn with a sterile pipette and put into 45 cubic centimeters of sterile malt extract broth. Counts were obtained by plating out in duplicate one cubic centimeter and 0.1 cubic centimeters of this broth using wort agar as the medium for growth.

The initial count was obtained by inoculating 100 cubic centimeters of sterile tap water with the same capillary pipette. After thorough shaking to insure an even distribu-

tion, a 5 cubic centimeter portion of this was withdrawn and transferred to 45 cubic centimeters of the malt extract broth. One cubic centimeter and 0.1 cubic centimeter quantities of this broth were plated out. Thus the count on the one cubic centimeter plate should represent the initial count for one cubic centimeter of the broth which had received 5 cubic centimeters of sirup, since the same dilutions had been made in both cases.

The Wort Agar, dehydrated, which was used in these experiments was prepared by the Digestive Ferments Company of Detroit, Michigan and has the following composition;

Maltose, Technical, Difco	12.75
Malt Extract, Difco	15.00
Dextrin, Difco	2.75
Glycerin, C.P.	2.35
Di-Potassium Phosphate, C.P.	1.00
Ammonium Chloride, C.P.	1.00
Bacto Peptone	0.78
Bacto Agar	15.00

50.63 grams of this dehydrated material are supposed to be dissolved in 1000 cubic centimeters of distilled water and autoclaved at 15 pounds of steam pressure for 20 minutes. The final pH is given as 4.65. This wort agar was modified by the addition of 5 grams more of agar agar per liter of

media. The same media may be prepared by using 30 grams of Difco Malt Extract Broth, dehydrated, with 20 grams of agar agar and one gram each of di-potassium phosphate and ammonium chloride per liter of distilled water.

The composition of the malt extract broth was the same as this latter method of preparing the wort agar with the exception that the 20 grams of agar agar were eliminated.

Counts were made at the end of 48 hours after incubating the plates at a temperature of 25 to 28°C. Smears were prepared and examined from the colonies on the plates when the survivors numbered a dozen or less per plate. This was to insure that contamination had not occurred and that the survivors were actually yeasts. The flasks of broth were incubated for 4 days and observed at the end of 2, 3, and 4 days for turbidity and sediment. This was designed to serve as a check on the plating process. The sediment from the last turbid flask in a series was always examined to determine whether or not the turbidity was due to yeasts.

Owen[28] in speaking of effective temperature for cane sirup sterilization states that this temperature varies in-

28. Owen, W. L., U. S. Dept. of Agr., Bull. 1370, 59, Oct., (1925).

versely with the volume of the cans in which the sirup is canned. He gives temperatures varying from 71°C to 81°C for Number 10 to 1⅓ cans but gives no hint as to the time required in the heating of the sirup to this temperature nor to the time required for cooling. Owen[29] says in regard to the resistance to heat of Saccharomyces zopfii that, instead of a 67°C thermal death point, he found that a temperature of 90°C for 10 minutes was not sufficient to kill this yeast. He further adds that, while the resistance of the yeast to high temperatures does not vary directly with the density of the solution in which it is grown, it does seem to retain its power of fermentation more persistently when subjected to such temperatures when grown in thick sirup than when grown in more dilute solutions.

McKelvey[30] states that, in general, the higher the concentration of sugar the longer was the time required to kill the yeast spores under examination. At 70°C he gives times ranging from 2 to 5 minutes as being necessary to kill the yeast spores in sugar sirups whose densities varied from 24° to 36° Baume.

C. Experimental Results and Discussion.

29. Owen, W. L., Centr. Bakt. Parasitenk., II Abt., Bd. 39, 468-82 (1913).
30. McKelvey, C. E., J. Bact. 11, 98 (1926).

Taking McKelvey's work as a basis, runs were made at 70°C but it was found that an hour was not long enough to sterilize either distilled water, or light sirups, or heavy sirups which had been inoculated with a suspension of dried yeast spores. About one-third to one-half of the number of spores originally inoculated had disappeared at the end of an hour but after the first four minutes there was little reduction in count.

As the sterilization of sirups apparently was not being effected at 70°C, the temperature was raised to 100°C. Results typical of the several runs are shown in Table I. It will be noticed that the higher the concentration of sugar in the sirups, the longer was the time required to kill the yeast spores which had been inoculated. As samples were removed in 5 cubic centimeter quantities, counts are given in terms of this 5 cubic centimeter unit.

TABLE I.

Temperature 100°C.

Initial contamination--36,000 per 5 cc. of sirup.

Density of sirup	Time required to kill 99.9% of yeast spores.
Distilled water	2-4 minutes
24° Baume sirup	6 minutes
30° Baume sirup	8-10 minutes
36° Baume sirup	28 minutes

Detailed data are given in Table II for the death rate of the yeast spores in the 36° Baume sirup. From these data the curves on Plate I were constructed.

TABLE II.

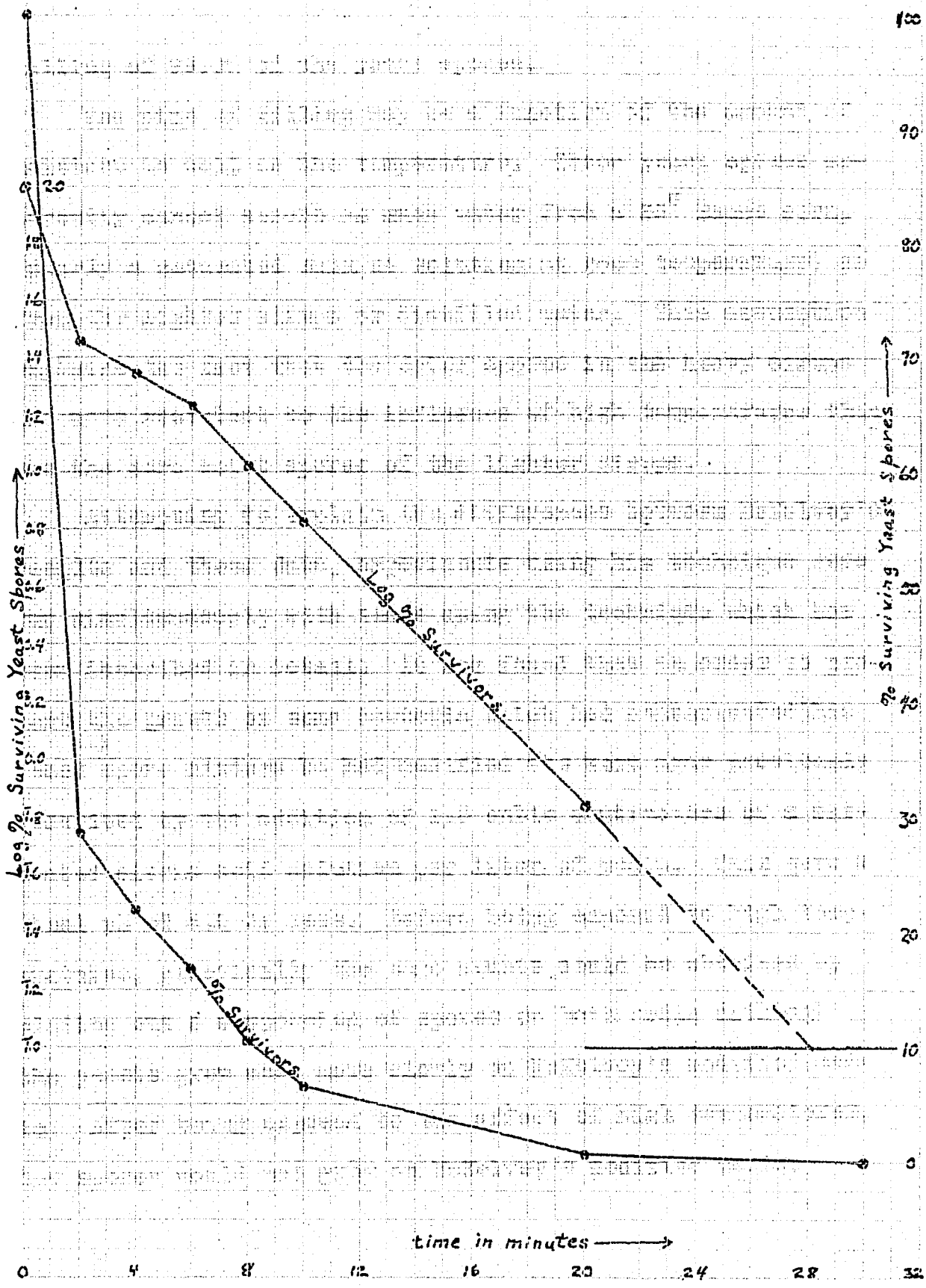
Temperature 100°C.

36° Baume sucrose sirup. (Sp. Gr. at 20C/20C = 1.3303).

Time of exposure in minutes	Counts per 5 cc. unit of sirup--run A	Counts per 5 cc. unit of sirup--run B	Average count per 5 cc. unit of sirup	Average % survivors	Log of % survivors
0	28,500	43,000	35,750	100.0	2.000
2	11,250	9,250	10,250	28.65	1.457
4	7,900	7,750	7,825	21.9	1.341
6	5,900	6,100	6,000	16.8	1.225
8	3,750	3,750	3,750	10.5	1.020
10	2,500	2,250	2,375	6.65	0.822
20	250	250	250	0.7	$\bar{1}$.845
30	0	0	0	0	---

The sudden decrease in the first two minutes is probably explained by the death of the weakest cells. After the weak cells have been killed, the death rate is more or less regular for the rest of the experiment. Extrapolation at the same rate of death between 20 and 30 minutes as was shown between 10 and 20 minutes shows 28 minutes as the time for the

Plate I.


Log % Surviving Yeast Spores
% Surviving Yeast Spores
Log % Survivors
% Survivors
time in minutes
0
4
8
12
16
20
24
28
32
100
90
80
70
60
50
40
30
20
10
0

killing of 99.9% of the yeast spores.

The time of killing may be a function of the amount of moisture as well as the temperature. These yeast spores apparently cannot absorb as much water from a 36° Baume sirup (nearly a saturated sucrose solution at room temperature) as from the lighter sirups or distilled water. This assumption explains the fact that the drier spores in the heavy sirups are more resistant to the influence of high temperatures than are the more moist spores of the lighter sirups.

Attempting to explain the differences between McKelvey's results and these data, experiments using his technique were run simultaneously with those using the technique which has been described in detail. It was found that in order to prevent the growth of some bacteria which had contaminated his yeast spore mixture he had modified the wort agar previously described by the addition of 1.5 cubic centimeters of a saturated citric acid solution per liter of media. This gave a final pH of 4.0 or less. Before being exposed to high temperatures, practically the same counts could be obtained by plating out a suspension of spores on both media although the yeasts grew much more slowly on McKelvey's modified media. After being exposed to the effect of high temperatures, the spores would not grow on McKelvey's modified media.

These results were checked several times and after exposure to a temperature of 100°C no growth was obtained on McKelvey's media while the wort agar previously described gave counts which decreased directly with the time of exposure.

In order to reduce the time of sterilization for heavy sirups, a small amount of acid was added to the 36° Baume sirup. One cubic centimeter of a 7.074 N solution of citric acid (equivalent to the bottler's one ounce of 50% citric acid solution per gallon of sirup) was added per 100 cubic centimeters of sirup. At 100°C no growth was obtained after 2 minutes exposure to this temperature. This is in contrast with the 28 minutes required to sterilize the same sirup without acid.

D. Conclusions.

1. Modification of wort agar by the addition of 1.5 cubic centimeters of saturated citric acid solution per liter of media is not advisable since yeast spores which have been exposed to high temperatures do not develop readily upon it.

2. The greater the concentration of sugar in the sirup, the longer was the time required to kill the yeast spores.

3. Addition of acid to a sirup materially reduces the

time required to kill the yeast spores at any definite temperature.

4. A temperature of 100°C for 28 minutes is sufficient to sterilize. In actual practice, the sterilization may be effected by bringing the sirup to a boil and boiling for 5 minutes.

III. THE RELATION OF THE TEMPERATURE, CONCENTRATION OF ALKALI, AND PERIOD OF EXPOSURE TO THE DEATH RATE OF YEAST SPORES

A. Preparation of Reagents.

The preparation of the dried yeast spores was described in the section preceding this.

The solutions of alkalies used were sodium hydroxide solutions and solutions of a commercial alkali consisting of a mixture of sodium hydroxide and sodium carbonate which will be designated as Alkali A.

The sodium hydroxide solutions were freed of their carbonate content by being made up in a 50% stock solution at the first. Sodium carbonate is practically insoluble in this concentration of sodium hydroxide. After cooling, the solution was filtered through a Gooch crucible using a pad of asbestos which had previously been prepared according to the Munson-

Walker method for gravimetric sugar determinations. This filtered sodium hydroxide solution was then diluted with freshly boiled distilled water until its titration value showed it to be at the concentration which was desired.

The solutions of Alkali A were made up from a stock solution of approximately 5% strength. The titration value of this was determined and the proper dilutions were made with carbon dioxide-free water.

Table III shows the titration values of both the sodium hydroxide and Alkali A solutions. Titrations were made with 0.2519 N HCl using phenolphthalein and methyl orange as indicators.

TABLE III.

Titration Values of Alkali Solutions.

Solution	Titration Values: cc. .2519 N HCl = 5 cc. alkali P.P.*	M.O.*	% Alkali calculated as NaOH	g. Na_2CO_3 per 100 cc.	g. NaOH per 100 cc.
1% NaOH	4.95	5.00	1.01	0.027	0.989
2% NaOH	9.90	10.00	2.01	0.053	1.975
3% NaOH	14.70	14.89	3.00	0.101	2.924
4% NaOH	19.65	19.90	4.01	0.134	3.909
1% Alkali A	3.45	4.96	0.999	0.806	0.391
2% Alkali A	6.88	9.91	1.997	1.621	0.776
3% Alkali A	10.37	14.80	2.982	2.366	1.197
4% Alkali A	14.10	19.93	4.018	3.113	1.667

*P.P. = phenolphthalein used as indicator.
*M.O. = methyl orange used as indicator.

B. Method of Procedure.

Essentially the same method of procedure was followed in this series of experiments as in the sterilization of sirups. One hundred cubic centimeters of alkali solution were substituted for the sirup. Forty-five cubic centimeters of a sterile acid solution were substituted for the 45 cubic centimeters of a malt extract broth. Each flask of this acid solution contained a drop of methyl orange solution and just enough acid to neutralize 5 cubic centimeters of the alkali which it was to receive. If the indicator showed that the end-point had not been reached after the addition of the 5 cubic centimeters of alkali solution, the end-point was adjusted with a sterile 1% sodium hydroxide solution. One cubic centimeter quantities from these flasks were plated out in duplicate using the wort agar described in the previous section. The initial count was obtained in the same manner and the same precautions were taken to insure that the colonies on the plates (when only a few were left) were yeasts.

Duplicate runs were made at temperatures of 50°C, 60°C and 70°C. At 50°C, 2%, 3% and 4% solutions of both Alkali A and NaOH were used. At 60°C, 2%, 3% and 4% solutions of Alkali A and 1%, 2% and 3% solutions of sodium hydroxide were used. At 70°C, 1% and 2% solutions of Alkali A and a

1% solution of NaOH were used. Distilled water was also run for comparison.

C. Experimental Results and Discussion.

Table IV gives the time in minutes required to kill 99.9% of the yeast spores when exposed to the action of the different alkali solutions at 50°C, 60°C and 70°C. It will be noticed that the time required to kill 99.9% of the yeast spores may be reduced either by increasing the concentration of the alkali or by increasing the temperature. It is also apparent that it takes somewhat less than twice the concentration of Alkali A to be as effective at any given temperature as it does of the sodium hydroxide solution. In other words, the germicidal efficiency of the sodium carbonate content is less than that of an equivalent amount of sodium hydroxide. The germicidal efficiency of a sodium carbonate and sodium hydroxide mixture however is greater than can be accounted for by its sodium hydroxide content above.

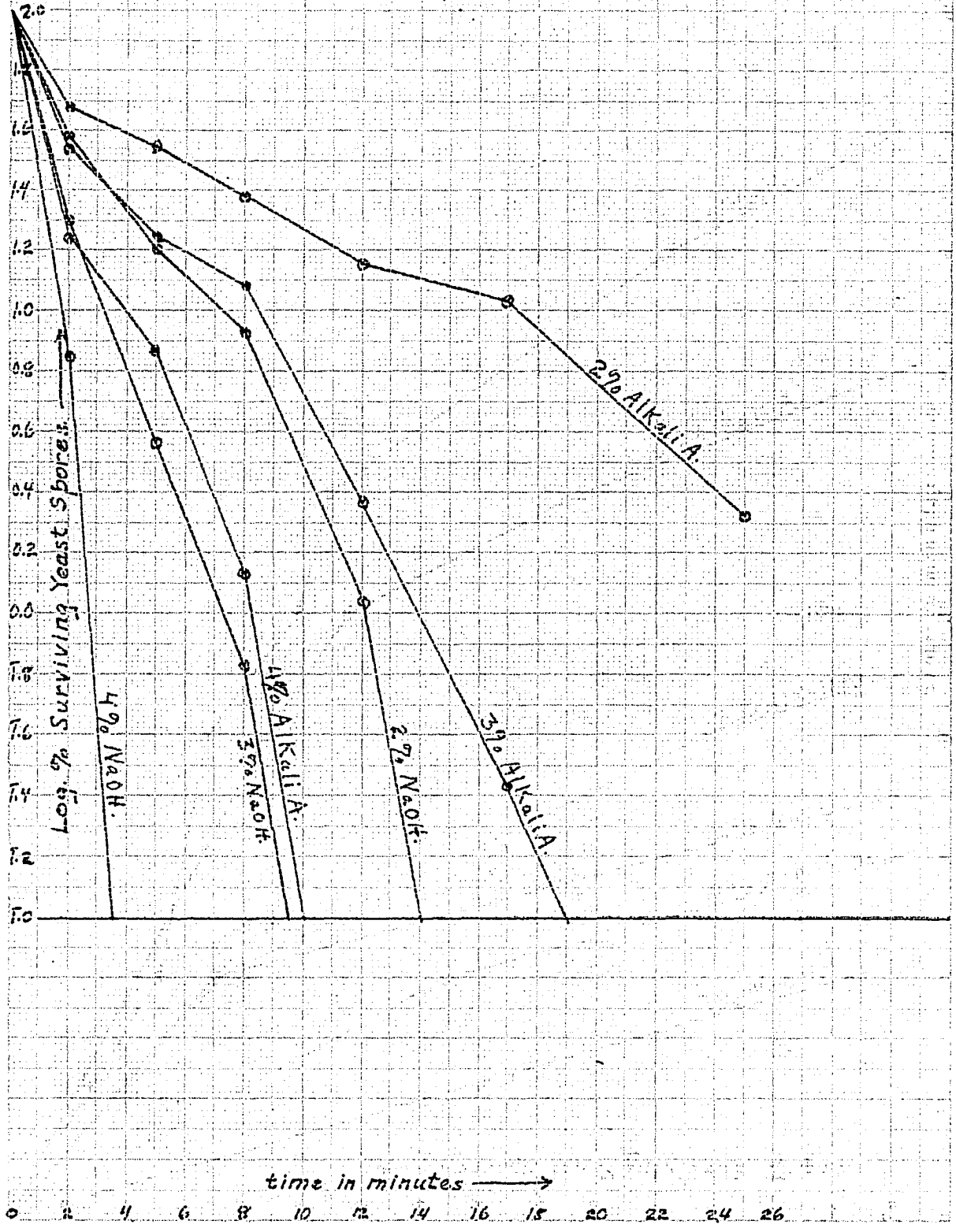
TABLE IV.

Alkali Solution	Temperature °C	Time in minutes required to kill 99.9% of yeast spores
2% NaOH	50	14
3% NaOH	50	9.5
4% NaOH	50	3.5
2% Alkali A	50	more than 25
3% Alkali A	50	19
4% Alkali A	50	10
1% NaOH	60	9
2% NaOH	60	3.5
3% NaOH	60	2
2% Alkali A	60	7
3% Alkali A	60	4.5
4% Alkali A	60	3
1% NaOH	70	less than 2
1% Alkali A	70	less than 2
2% Alkali A	70	less than 2

Plate II at 50°C and Plate III at 60°C show the rates at which the yeast spores are killed off. From these curves the per cent of surviving yeast spores in any of the alkali solutions after different periods of time may be calculated. The large initial drop in the count during the first two minutes which was commented upon in Plate I is again quite apparent in Plate II although not so noticeable in Plate III.

The rate of killing of yeast spores in distilled water at 70°C was determined for the sake of comparison with those of the alkali solutions. Table V shows the per cent of sur-

Plate II.


Log % Surviving Yeast Spores.
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.8
1.6
1.4
1.2
1.0
2% Alkali A.
4% NaOH.
4% Alkali A.
3% NaOH.
3% Alkali A.
2% NaOH.
time in minutes ⟶
0
2
4
6
8
10
12
14
16
18
20
22
24
26

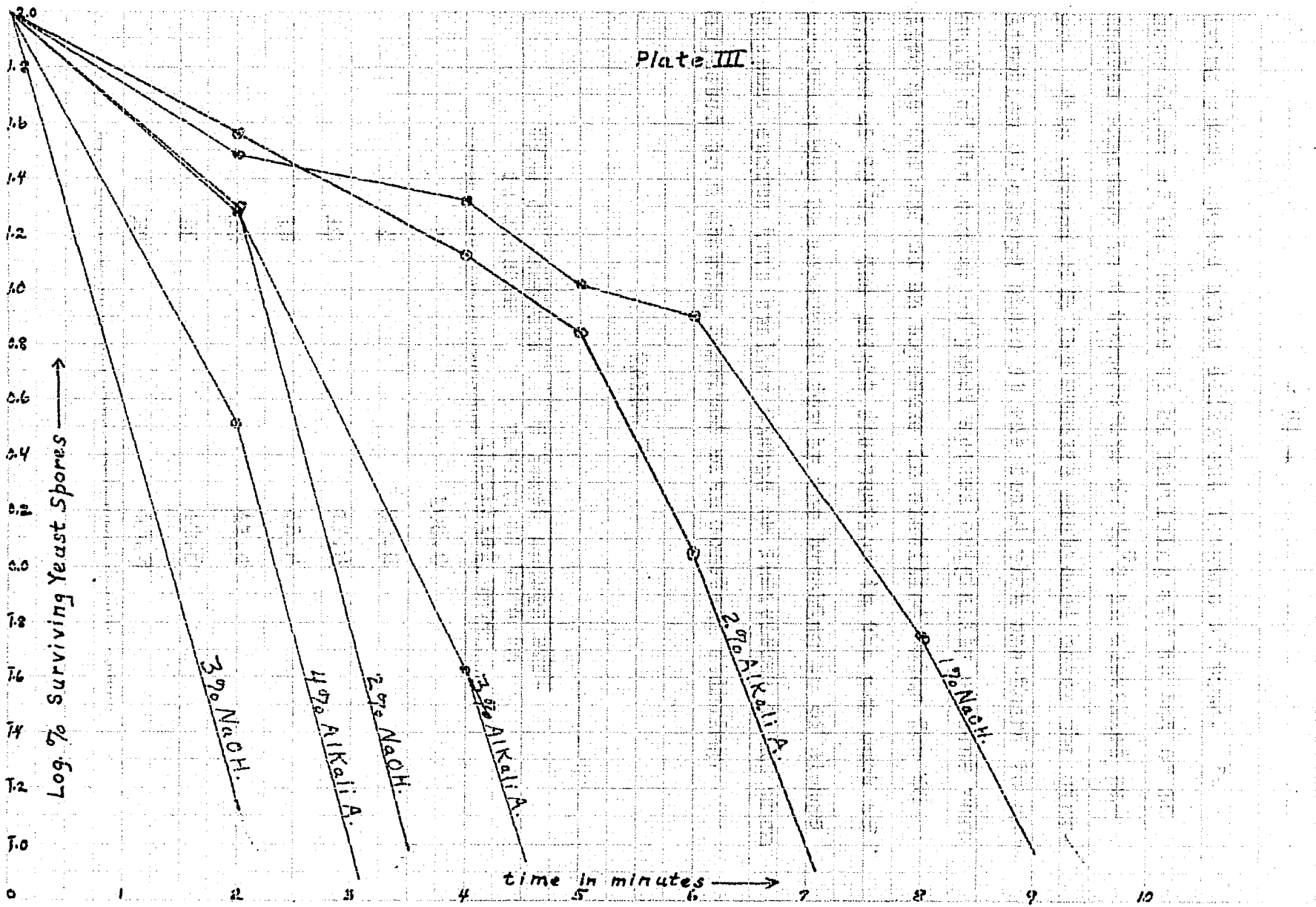


Plate III
Log. % Surviving Yeast Spores
time in minutes
3% NaOH.
4% Alkali A.
2.7% NaOH.
3.8% Alkali A.
2.7% Alkali A.
1% NaOH.
0
1
2
3
4
5
6
7
8
9
10
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
T.8
T.6
T.4
T.2
T.0

viving yeast spores after different lengths of time. It shows 46.2% of the spores to be surviving at the end of 30 minutes exposure to a temperature of 70°C in distilled water. This agrees with the results mentioned under the sterilization of sirups where it was found that one-third to one-fourth of the number of yeast spores originally present were alive at the end of an exposure in distilled water for 60 minutes at 70°C. The slight fluctuation in the per cent of survivors may be accounted for by the breaking up of clumps of yeast spores into smaller clumps or individual cells when subjected to a soaking combined with constant stirring.

TABLE V.

Time of Exposure in minutes	% Yeast spores surviving at 70°C in distilled water
0	100.0
5	50.5
10	47.3
15	45.6
20	41.4
25	49.5
30	46.2

D. Conclusions.

1. The time required for killing 99.9% of the yeast spores may be reduced either (a) by increasing the concentration of the alkali or (b) by raising the temperature.

2. Sodium hydroxide at a given concentration and temperature has a greater germicidal efficiency than a chemically equivalent solution of a mixture of sodium hydroxide and carbonate at the same temperature.

3. Exposure to a temperature of 70°C in distilled water for an hour is not sufficient to kill dried yeast spores.

4. The germicidal efficiency of a sodium carbonate-hydroxide mixture is greater than can be accounted for by the sodium hydroxide content alone.

5. There is little likelihood of bottles being the source of yeast contamination if a 2% sodium hydroxide or 3% Alkali A solution is used at a temperature of 60°C with a period of contact amounting to 5 minutes.

IV. THE HYDROLYSIS OF SUCROSE SIRUPS

A. Preparation of Reagents and Apparatus.

The sucrose used in this series of experiments was a highly refined grade of sugar with a moisture content of 0.028%. A solution of 26 grams of this sugar, when made up

to 100 cubic centimeters volume with distilled water, gave a reading of 99.9 on the saccharimeter (200 mm. tube).

The citric and tartaric acids used were of the C.P. grade. No further purification was made. A 7.074 normal citric acid solution and a 6.553 normal tartaric acid solution were made up and used since the addition of one cubic centimeter of these acid solutions to 100 cubic centimeters of sirup would maintain the same ratio of acid to sugar as the manufacturer of carbonated beverages uses in preparing his flavored sirups--(one ounce of 50% citric or tartaric acid solution per gallon of sirup).

A small constant temperature oil-bath was made from a hot-plate, asbestos boards, a six quart aluminum pan, and a DeKhotinsky bimetallic electric thermo-regulator. A fluctuation from the temperatures desired of $\pm 0.2^{\circ}C$ was observed as the maximum divergence. More frequently the temperature was maintained with a fluctuation of $\pm 0.1^{\circ}C$ or less.

B. Method of Procedure.

Sirups were prepared by weighing the proper amount of sugar required for the particular sirup desired, transferring this to a calibrated volumetric flask and making up to volume at $20^{\circ}C$ with distilled water. Heat was required for the com-

plete solution of the sugar in the more concentrated sirups.

Two hundred cubic centimeters of sirup were transferred to a 3-necked, 400 cc. round bottomed Pyrex flask which was fitted with a motor stirring device through the middle neck. One of the other necks was fitted with a thermometer and cork, while the other neck was closed with a cork. The flask was immersed in the bath until it had reached the desired temperature. Then the acid was added with a pipette through the third neck of the flask. The motor stirrer functioned continuously throughout each experiment. At stated intervals, a quantity of sirup was withdrawn from the reaction flask and transferred to calibrated 50 cubic centimeter volumetric flasks which contained enough sodium hydroxide solution to neutralize the acid which was contained in the sirup. The transfer pipette was rinsed each time with distilled water into the volumetric flask in order to eliminate the factor of varying amounts of drainage due to different densities of sirups. The transfer pipette which was used in all of the hydrolysis experiments was found to contain 9.933 cubic centimeters of liquid when calibrated at 100°C.

The 50 cubic centimeter flasks, which contained sufficient sodium hydroxide solution to neutralize the acid, were placed in a pan of cracked ice so that the temperature might

suddenly be reduced to further aid in stopping inversion. The contents of the flasks were made up to volume at 20°C and polarized in a 200 mm. tube using a Schmidt and Haensch single-wedge saccharimeter. The saccharimeter tube was a jacketed one, fitted with a thermometer so that the temperature of the solution under examination was known at all times. All saccharimeter readings were made at 20°C. The initial reading was obtained by withdrawing some sirup which had been brought up to temperature in a separate container in the same oil bath. This was transferred to a 50 cubic centimeter volumetric flask to which had previously been added equivalent quantities of sodium hydroxide and citric or tartaric acid in amounts corresponding with the other flasks of the experiment. This was to compensate for any effect which the sodium citrate or tartrate might have on the polarization.[31]

C. Experimental Results and Discussion.

The results of the experiments are given in the following tables numbers VI to XVII inclusive. Values of k were obtained by the use of equations based on Guldberg and Waage's law of mass action which states that the reaction velocity, or rate of change of concentration, must be propor-

31. Bowe, L. E., J. Phys. Chem. 31, 297 (1927).

tional to the concentration of each of the reacting substances. The reaction which takes place upon the hydrolysis of sucrose is the formation of one molecule of dextrose and one molecule of levulose for each molecule of sucrose decomposed. The equation

$$\underset{\text{sucrose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{dextrose}}{C_6H_{12}O_6} + \underset{\text{levulose}}{C_6H_{12}O_6}$$

empirically expresses the change which occurs. It will be seen that a molecule of water enters into the reaction for each molecule of sucrose decomposed. The reaction velocity then $= KC_s.C_w$ where C_s is the concentration of the sucrose and C_w is the concentration of water. Since the molecular weight of water is so small compared to that of the sucrose, the assumption is usually made that the concentration of water is constant (at least for dilute solutions). Thus $KC_w = k$ and the reaction velocity $= kC_s$.

Rates are usually expressed by differential equations so, if x represents the amount of sucrose transformed during time t where a is the original amount of sucrose, this may be expressed by the equation $dx/dt = k(a-x)$. The concentration of sucrose at any instant is represented by (a-x) for if $t = 0$ then $x = 0$. A more useful form of this equation is obtained by integration. This yields the equation

$$k = \frac{1}{t} \ln \frac{a}{(a-x)}.$$

If Briggsian logarithms are to be used, this becomes

$$k = \frac{2.3026}{t} \log \frac{a}{(a-x)}.$$

Very close agreement in the values of k at different times throughout the course of the reaction is not to be expected since the simplifying assumption, that the water does not appreciably change in concentration, is in error when the equation is applied to concentrated solutions.

The per cent of sucrose remaining unchanged at time t was calculated as follows:

$$\% \text{ sucrose} = \frac{\text{reading at time t--final reading}}{\text{total change in reading}} \times 100.$$

When numerical values of the saccharimeter readings at any time are substituted in the above equation, it yields a value for (a-x) or the per cent sucrose remaining unchanged. The initial concentration of sucrose, a, may be used by taking 100 as its numerical value. The equation for calculating k then becomes:

$$k = \frac{2.3026}{t} (2.000 - \log \% \text{ sucrose}).$$

Table VI for the hydrolysis of 900 grams of sucrose per liter at 100°C by citric acid in the concentration stated is given below. Samples of the calculations of k and the per

cent of sucrose remaining are given below.

TABLE VI.

Hydrolysis at 100°C.

900 g. sucrose per liter. 1 cc. 7.074 N citric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k	k'
0	0	67.3	100	2.0000	---	---
1	2	42.5	71.9	1.8567	0.1650	0.1650
2	4	23.3	50.1	1.6998	0.1728	0.1806
3	6	8.05	32.8	1.5159	0.1858	0.2117
4	8	-2.1	21.3	1.3284	0.1933	0.2159
5	10	-8.1	14.5	1.1614	0.1931	0.1923
6	13	-14.3	7.48	0.8739	0.1995	0.2207
7	16	-17.9	3.40	0.5315	0.2113	0.2628
8	19	-19.0	2.16	0.3345	0.2018	0.1512
9	22	-19.98	1.04	0.0170	0.2075	0.1828
10	26	-20.9	0	---	---	
11	30	-20.9	0	---	---	
					Ave. k = 0.1922	Ave. k' = 0.1981

Sp. Gr. at 20°C/20°C = 1.3343.

Calculations of per cent sucrose remaining and k are given for the 8 minute interval.

$$\% \text{ sucrose} = \frac{-2.1 - (-20.9)}{67.3 - (-20.9)} \times 100 = \frac{18.8}{88.2} \times 100 = 21.3\%.$$

$$k = \frac{2.3026}{8} (2.0000 - \log 21.3) = \frac{2.3026}{8} (2.0000 - 1.3284) = 0.1933.$$

TABLE VII.

Hydrolysis at 100°C.

800 g. sucrose per liter. 1 cc. 7.074 N citric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	59.55	100.0	2.0000	---
1	2	38.5	72.4	1.8597	0.1615
2	4	24.7	54.3	1.7348	0.1527
3	6	13.36	39.4	1.5955	0.1552
4	8	2.8	25.6	1.4082	0.1703
5	10	-3.97	16.7	1.2227	0.1790
6	13	-10.0	8.79	0.9440	0.1870
7	16	-13.1	4.72	0.6739	0.1908
8	20	-14.9	2.36	0.3729	0.1873
9	25	-16.56	0.18	$\bar{1}$.2553	0.2528
10	30	-16.7	0.0	---	---
					Ave. k = .1818

Sp. Gr. 20°C/20°C = 1.2982.

Values of k (in parenthesis) were not used in the evaluation of average k in any of the tables.

TABLE VIII.

Hydrolysis at 100°C.

700 g. sucrose per liter. 1 cc. 7.074 N citric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	53.6	100.0	2.0000	---
1	5	21.1	52.2	1.7177	(0.1300)
2	10	-1.3	19.4	1.2878	0.1678
3	15	-9.3	7.57	0.8791	0.1721
4	20	-12.9	2.28	0.3579	0.1890
5	25	-13.65	1.17	0.0682	0.1780
6	30	-14.1	0.51	$\bar{1}$.7076	0.1760
7	35	-14.4	0.07	$\bar{2}$.8451	0.2076
8	40	-14.45	0	---	
9	45	-14.45	--	---	

Ave. k = .1817

Sp. Gr. 20°C/20°C = 1.2653

TABLE IX.

Hydrolysis at 100°C.

600 g. sucrose per liter. 1 cc. 7.074 N citric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	44.1	100.0	2.0000	---
1	2	27.4	71.0	1.8513	0.1712
2	4	13.7	47.4	1.6758	0.1866
3	6	3.9	30.4	1.4829	0.1984
4	8	-2.25	19.8	1.2967	0.2024
5	10	-6.6	12.3	1.0899	0.2096
6	13	-10.0	6.4	0.8062	0.2115
7	16	-12.0	2.94	0.4683	0.2204
8	19	-12.9	1.38	0.1399	0.2254
9	22	-13.15	0.95	$\bar{1}$.9777	0.2117
10	26	-13.6	0.17	$\bar{1}$.2305	0.2452
11	30	-13.7	0.0	---	---

Ave. k = 0.2082

Sp. Gr. at 20°C/20°C = 1.2264

TABLE X.

Hydrolysis at 100°C.

400 g. sucrose per liter. 1 cc. 7074 N citric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	30.2	100.0	2.0000	---
1	5	6.4	39.3	1.5944	0.1872
2	10	-4.0	12.77	1.1062	0.2058
3	15	-7.2	4.60	0.6628	0.2053
4	20	-8.5	1.28	0.1072	0.2179
5	25	-8.6	1.02	0.0086	0.1834
6	30	-8.95	0.13	$\bar{1}$.1139	0.2215
7	35	-8.95	0.13	$\bar{1}$.1139	0.1899
8	40	-8.95	0.13	$\bar{1}$.1139	0.1661
9	45	-9.00	0.0	---	---

Ave. k = 0.1971

TABLE XI.

Hydrolysis at 100°C.

800 g. sucrose per liter. 2 cc. 7.074 N citric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	58.8	100.0	2.0000	---
1	2	26.7	58.3	1.7657	0.2698
2	4	4.7	29.7	1.4728	0.3035
3	6	-6.9	14.6	1.1644	0.3207
4	8	-12.9	6.77	0.8366	0.3366
5	10	-15.6	3.25	0.5119	0.3426
6	13	-17.6	0.65	$\bar{1}$.8129	0.3077
7	16	-18.1	0.0	---	---
8	20	-18.1	---	---	---

Ave. k = 0.3135

Sp. Gr. at 20°C/20°C = 1.3022

TABLE XII.

Hydrolysis at 100°C.

800 g. sucrose per liter. 1 cc. 6.553 N tartaric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	60.3	100.0	2.0000	---
1	2	35.0	67.2	1.8274	0.2068
2	4	13.1	38.7	1.5877	0.2373
3	6	0.9	22.8	1.3579	0.2464
4	8	-7.1	12.47	1.0959	0.2602
5	10	-11.3	7.02	0.8463	0.2657
6	13	-14.5	2.86	0.4564	0.2734
7	16	-15.9	1.04	0.0170	0.2854
8	20	-16.7	0.0	---	---
9	25	-16.7	0.0	---	---

Ave. k = 0.2536

Sp. Gr. at 20°C/20°C = 1.2992

TABLE XIII.

Hydrolysis at 100°C.

800 g. sucrose per liter. 2 cc. 6.553 N tartaric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	59.8	100.0	2.0000	---
1	2	20.4	48.8	1.6884	0.3588
2	4	-2.3	19.5	1.2900	0.4087
3	6	-11.7	7.27	0.8615	0.4369
4	8	-15.4	2.47	0.3927	0.4626
5	10	-16.5	1.04	0.0170	0.4566
6	13	-17.3	0.0	---	---
7	16	-17.3	0.0	---	

Ave. k = 0.4247

Sp. Gr. at 20°C/20°C = 1.3005

TABLE XIV.

Hydrolysis at 30°C.

800 g. sucrose per liter. 1 cc. 7.074 N citric acid per 100 cc. sirup.

Date	Time	Time of hydrolysis in days	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k ($\times 10^{-5}$)
Feb. 3, 1927	9:19 A.M.	0	60.3	100.0	2.0000	--
Feb. 5	9:54 A.M.	2.03	46.1	81.4	1.9106	7.05
Feb. 7	3:31 P.M.	4.25	36.0	68.3	1.8344	6.23
Feb. 10	10:23 A.M.	7.04	23.25	51.6	1.7126	6.49
Feb. 13	4:31 P.M.	10.30	13.0	38.2	1.5821	6.49
Feb. 17	2:12 P.M.	14.21	2.7	29.7	1.4728	5.94
Feb. 25	2:50 P.M.	22.23	-6.2	13.1	1.1173	6.35
Mar. 4	1:44 P.M.	29.19	-12.4	4.97	0.6964	7.15
Mar. 17	8:30 P.M.	41.97	-16.1	0.13	$\bar{1}$.1139	--
Mar. 26	3:13 P.M.	51.25	-16.2	0.0		--

Ave. k = 6.53 $\times 10^{-5}$

Sp. Gr. at 20°C/20°C = 1.3023

TABLE XV.

Hydrolysis at 30°C.

800 g. sucrose per liter. 2 cc. 7.074 N citric acid per 100 cc. sirup.

Date	Time	Time of hydrolysis in days	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k ($\times 10^{-5}$)
Feb. 3, 1927	9:26 A.M.	0	60.3	100.0	2.0000	--
Feb. 5	9:56 A.M.	2.03	41.4	76.1	1.8814	(9.35)
Feb. 7	3:32 P.M.	4.25	28.0	59.1	1.7716	8.59
Feb. 10	10:25 A.M.	7.04	13.1	40.3	1.6053	8.91
Feb. 13	4:33 P.M.	10.3	3.6	28.2	1.4502	8.54
Feb. 17	2:15 P.M.	14.2	-5.2	17.1	1.2330	8.64
Feb. 25	2:52 P.M.	22.23	-13.3	6.84	0.8351	8.40
Mar. 4	1:46 P.M.	29.19	-16.5	2.79	0.4456	8.52
Mar. 17	8:32 A.M.	41.97	-18.7	0.0	---	--
Mar. 26	3:15 P.M.	51.25	-18.7			--

Ave. k = 8.60×10^{-5}

Sp. Gr. at 20°C/20°C = 1.3023

TABLE XVI.

Hydrolysis at 90°C.

800 g. sucrose per liter. 1 cc. 7.074 N citric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	59.8	100.0	2.0000	---
1	5	37.7	71.5	1.8543	0.0671
2	10	20.4	49.2	1.6920	0.0709
3	15	7.8	33.0	1.5185	0.0738
4	20	-0.3	22.75	1.3570	0.0740
5	25	-6.4	14.7	1.1673	0.0767
6	30	-10.3	9.66	0.9850	0.0779
7	35	-12.9	6.32	0.8007	0.0789
8	40	-14.9	3.74	0.5729	0.0821
9	50	-16.9	1.16	0.0645	0.0891
10	60	-17.7	0.13	$\bar{1}$.1139	(0.1109)
11	70	-17.8	0.0	---	---

Ave. k = 0.0767

Sp. Gr. at 20°C/20°C = 1.3012

TABLE XVII.

Hydrolysis at 90°C.

800 g. sucrose per liter. 1 cc. 6.553 N tartaric acid per 100 cc. sirup.

Flask number	Time of hydrolysis in minutes	Saccharimeter readings	% Sucrose remaining	Log of % sucrose remaining	k
0	0	59.9	100.0	2.0000	---
1	5	31.3	63.6	1.8035	0.0905
2	10	11.3	38.1	1.5809	0.0965
3	15	-1.2	22.2	1.3463	0.1004
4	20	-8.5	12.85	1.1089	0.1026
5	25	-12.9	7.3	0.8633	0.1047
6	30	-15.7	3.7	0.5682	0.1099
7	35	-16.8	2.3	0.3617	0.1078
8	40	-17.6	1.28	0.1072	0.1090
9	50	-18.6	0.0	---	---
10	60	-18.6	0.0	---	---
11	70	-18.6	0.0	---	---

Ave. k = 0.1027

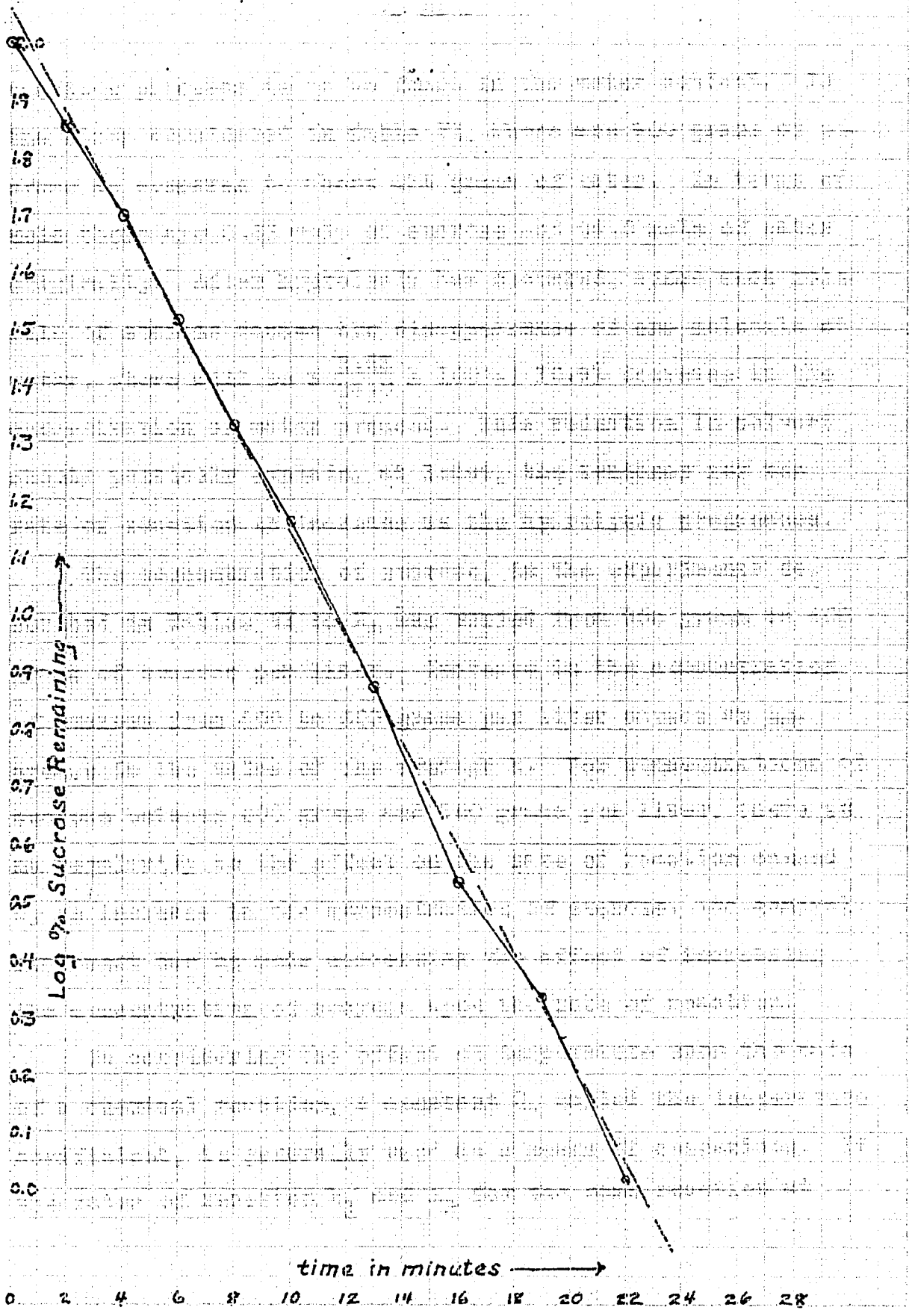
Sp. Gr. at 20°C/20°C = 1.3007

A comparison of the values of k in any of the tables will show that, in general, k increases as the reaction progresses. However, there seems to be little uniformity in the increase of value. Plate IV shows the curve obtained by plotting the logarithm of the per cent of sucrose remaining against time from data in Table VI. The broken line is the best straight line which can be drawn through these points. From the position of the points with reference to this line it appears that the reaction approaches that of a mono-molecular one as a limit. The slope of the broken line is equal to 0.2114 which is the value of k for the ideal mono-molecular reaction corresponding to these data.

If the value of k is calculated from point to point of the curve instead of from the initial concentration of sucrose each time, there seems to be more fluctuation in k. Values of k figured in this way are given in the column headed k' in Table VI. k', since it is calculated from point to point, is subject to wider fluctuation than the k calculated by the usual method since small experimental errors will cause a greater change from the average. If the k values are always based on the initial concentration, the fluctuations will not be as great.

A probable reason for the increasing value of k as the

Plate IX.


Log % Sucrose Remaining
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
time in minutes
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28

reaction proceeds is to be found in the water content. In the sirup considered in Table VI, there are 900 grams of sucrose as compared to about 434 grams of water. In terms of mols there are 2.63 mols of sucrose and 24.0 mols of water originally. After hydrolysis has occurred, since each molecule of sucrose causes the disappearance of one molecule of water, there will be a $\left(\frac{2.63}{24.0} \times 100 =\right)$ 10.9% decrease in the concentration of water present. This reduction in solvent should partially explain, at least, the tendency for the rate of reaction to increase as the hydrolysis progresses.

The concentration of sucrose, in the experiments described in Tables VI to X, was varied from 900 grams to 400 grams of sucrose per liter. Increase in the concentration of sucrose from 400 to 600 grams per liter causes an increase in the value of the average k. For concentrations of sucrose between 600 grams and 900 grams per liter, there is no regularity to the effect on the rate of reaction caused by an increase in the concentration of sucrose. No general statement can be made concerning the effect of increasing the concentration of sucrose upon the rate of reaction.

In considering the effect of temperature upon the rate of a chemical reaction, a constant Q, called the temperature coefficient, is generally used as a means of comparison. If the rates of reaction k_1 and k_2 for the same reaction at

temperatures 10°C apart are known, the temperature coefficient is then $Q_{10} = \frac{k_2}{k_1}$. If it is desired to express the temperature coefficient as Q_{10} (on the basis of k values 10° apart) but the k values are n degrees apart, this becomes $Q_{10} = \left(\sqrt[n]{k_2/k_1}\right)^{10}$.

Using the average k values obtained in Tables VII and XI to XVII and calculating Q_{10} in the manner indicated, the following values were obtained. Comparisons were made on solutions which had idential conditions except for differences of temperature.

TABLE XVIII.

Temperature Coefficients.

Temperature: range :	Q_{10}	:Tables from which k :values were obtained
100° to 90°	2.37	7 and 16
100° to 90°	2.47	12 and 17
100° to 30°	3.11	7 and 14
100° to 30°	3.23	11 and 15
90° to 30°	3.25	16 and 14

When a temperature range from 30°C to 90° or 100° is studied, the value of Q_{10} indicates that the rate of hydroly-

sis increases over 3 times for every 10 degrees rise in temperature. When the 90° to 100° interval is studied, the values of Q_{10} indicate an increase of slightly less than 2.5 times in the rate of hydrolysis. The values of Q_{10} in Table XVIII would indicate that the effect of an increase in temperature upon the rate of hydrolysis is independent of the acid used (citric or tartaric acids) or the concentration of those acids, and that the increase in rate per 10° rise in temperature is less between high temperatures than it is between two lower temperatures. From these data values of k and times for 99% hydrolysis, for intermediate temperatures in the ranges studied or for temperatures not far from this range, may be calculated by means of the van't Hoff isochore. For example, if k at 110°C was desired, it should be calculated from values of k at 90° and 100° rather than from values of k at 30° and 90° or 100°. The van't Hoff isochore is

$$Q_t = \frac{k_2}{k_1} = e^{\left(\frac{\mu}{2}\,\frac{T_2-T_1}{T_2T_1}\right)}$$

μ is a parameter since it depends on the temperature but may be considered as a constant for a small temperature range. When calculated from the values of k and T for 90° and 100°C, μ is found to equal 24,480. When k is calculated for 110°C it is found to equal 0.4181 for tartaric acid. In other

words, 5.5 minutes at 110°C should be a sufficient time in which to obtain 99% hydrolysis of an 800 gram per liter sucrose solution when one cubic centimeter of a 6.553 normal tartaric acid solution is added per 100 cubic centimeters of sirup. In comparison with this time of 5.5 minutes at 110° is the time of 15 minutes which is necessary at 100°C in order to obtain the same amount of hydrolysis.

Osaka[32] has shown that the inversion of sucrose by acids is incomplete in high concentrations although it may be regarded as complete in dilute solutions. For a sirup consisting of 800 grams of sucrose per liter of sirup, he says inversion is 98.9% complete when using a mineral acid for inversion at 25°C. Osaka considers that it is very probable that in a concentrated solution a reversion of sucrose from its hydrolytic products takes place. Determination of the amounts of invert sugar in equilibrium mixtures by the Munson-Walker gravimetric Fehling's method[33] before and after inversion by the Tucker[34] method showed inversion to be about 97.5% complete for sirups originally containing 800 grams of sucrose per liter. It is not surprising that the

32. Osaka, Y., J. Coll. Sci. Imp. Univ. Tokyo 25, 1-8 (1909).
33. Leach, Food Inspection and Analysis, 4th edition, 622 (1920).
34. ibid. p. 642.

equilibrium point is not the same as that reported by Osaka since he used mineral acids in his inversion process while citric and tartaric acids were used in obtaining these data. Spencer[35] reports that, for a given sugar solution, the equilibrium point for the inversion process depends on the H ion concentration and that less than 100% inversion takes place when citric and tartaric acids are employed.

Sale and Skinner[36] say that complete inversion was not obtained in a 30° Baume sirup when tartaric acid was used. They report an inversion of 96% when 0.021% by weight of tartaric acid crystals (99.5%), based on the weight of sugar and water taken, is added to a 30° Baume sirup which was subsequently boiled for 40 minutes. The addition of 0.013% of hydrochloric acid followed by boiling for 20 minutes is sufficient to cause practically complete inversion.

Jordan[37] gives directions for the preparation of commercial invert sugar sirups. On a 1000 pound producing basis, he advises the use of 750 pounds of sucrose, 250 pounds of water (about 30 gallons), and 10 ounces of tar-

35. Spencer, Grace G., M.S. Thesis, Dept. of Chemistry, Iowa State College Library, 1925. Unpublished.
36. Sale, J. W. and Skinner, W. W., Ind. Eng. Chem. 14, 522 (1922).
37. Jordan, S., Ind. Eng. Chem. 16, 307-10 (1924).

taric or 13 ounces of citric acid crystals. The whole mixture is heated at incipient boiling for 30 minutes. Jordan claims 98% completeness of inversion for this process.

Ruehe[38] advises the use of 50 grams (about 1.76 ounces) of tartaric acid crystals with 100 pounds of sucrose and 45 pounds of water. This should be boiled for 30 to 35 minutes. He makes the statement that invert sugar is sweeter than the sucrose from which it is prepared and advises the preparation of invert sugar as an economy measure for ice cream manufacturers. References have previously been cited to show that he was in error in his statement regarding the sweetness of invert sugar.

In the preparation of invert sugar sirups the time of boiling may be reduced by increasing the concentration of acid. If large quantities of the invert sugar sirup are to be prepared, steam jacketed kettles are generally used. Since it takes ten to fifteen minutes of heating in the average steam kettle to bring its sirup contents to the boiling temperature, much inversion would be accomplished in that time. Then by boiling for 5 minutes, the inversion would be completed or equilibrium attained. The boiling points of concentrated sirups may be as high as 115°C.

38. Ruehe, H. A., Cream and Milk Plant Monthly 8, No. 2, 45 (1919).

Calculations made with the aid of the van't Hoff isochore for 110°C showed 5.5 minutes as the time for 99% completeness of inversion.

In one of the previous sections, data have been given which show that sterilization of sirups, to which acid has been added, will have been effected in less than 5 minutes at the boiling temperature. Obviously, if the production of an invert sugar sirup is desired, it will also be sterilized simultaneously.

D. Conclusions.

1. Simultaneous inversion and sterilization of sucrose sirups may be advantageously accomplished by the addition of one cubic centimeter of 7.074 N citric or 6.553 N tartaric acids per 100 cubic centimeter quantities of sirup followed by heating the sirup to the boiling point and continuing the boiling process for 5 minutes.
2. Complete inversion is not reached when citric or tartaric acids are used to invert concentrated sucrose sirups. An equilibrium occurs when 97 to 98% of complete inversion has taken place in concentrated sirups.
3. The monomolecular reaction k is not a constant for the hydrolysis of sucrose sirups. In general, k increases as the hydrolysis progresses.

4. No general statement can be made concerning the effect of increasing the concentration of sucrose upon the rate of reaction for concentrations of sucrose greater than 600 grams of sucrose per liter.
5. The temperature coefficient for a ten degree rise in temperature is independent of the acid used (citric or tartaric acids) or the concentration of those acids. The increase in rate of hydrolysis per ten degree rise of temperature is less between high temperatures than it is between two lower temperatures.

V. SUMMARY

1. Yeast spores which have been exposed to a high temperature will not grow on wort agar which has been modified by the addition of 1.5 cubic centimeters of saturated citric acid solution per liter of media. Prior to exposure to the effect of high temperature, they would develop on this modified media, although not as readily as upon unmodified wort agar.
2. At any temperature, the greater the concentration of sucrose in the sirup, the longer was the time required to kill yeast spores.
3. At any definite temperature, the time for sterilization of sirups inoculated with yeast spores may be materially

reduced by the addition of acid to the sirup.

4. Simultaneous inversion and sterilization of sucrose sirups may be accomplished by the addition of one cubic centimeter of 7.074 N citric or 6.553 N tartaric acids per 100 cubic centimeter quantities of sirup followed by heating the sirup to the boiling point and continuing the boiling process for 5 minutes.
5. Dried yeast spores are not killed by 30 minutes exposure in distilled water to a temperature of 70°C.
6. The time required for sterilization of alkali solutions, which have been inoculated with yeast spores, may be reduced by increasing the concentration of the alkali or by raising the temperature.
7. Sodium hydroxide at a given concentration and temperature has a greater germicidal efficiency than a chemically equivalent solution of a mixture of sodium hydroxide and carbonate at the same temperature. The germicidal efficiency of a sodium hydroxide-sodium carbonate mixture is greater than can be accounted for by the sodium hydroxide content alone.
8. There is little likelihood of bottles being the source of yeast contamination if a 2% sodium hydroxide or 3% Alkali A solution is used in the bottle washing process

at a temperature of 60°C with a period of contact amounting to 5 minutes.

9. Complete hydrolysis of sucrose sirups is not obtained by the use of citric and tartaric acids. An equilibrium occurs when 97 to 98% of complete inversion has taken place in concentrated sucrose sirups.

10. The monomolecular reaction k is not a constant for the hydrolysis of sucrose sirups. In general, k increases as the hydrolysis progresses.

11. For concentrations of sucrose greater than 600 grams per liter, no general statement can be made concerning the effect of increasing the concentration of sucrose upon the rate of reaction.

12. The temperature coefficient for a ten degree rise in temperature is independent of the acid used (citric or tartaric acids) or the concentration of those acids. The increase in reaction rate per ten degree rise of temperature is less between high temperatures than between two lower temperatures.