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THE ACTION OF CITRIC ACID AND ITS SALTS IN SUGAR SOLUTIONS

by

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THE ACTION OF CITRIC ACID AND ITS SALTS IN SUGAR SOLUTIONS

INTRODUCTION

One of the important agencies causing loss in the carbonated beverage industry has been that of spoilage in the finished product. The source of much of this spoilage has been shown to be due to fermentation, principally by yeast. The chief concern of the beverage manufacturer today has become, therefore, one of producing a product free from yeast, or other micro-organisms.

In order to produce this sterile product the manufacturer has but three alternatives. He may take the raw materials of his trade, pass it thru some sterilizing procedure in the course of the processing, and take care that it is thereafter handled aseptically. He may sterilize the finished product after it has been sealed in the bottle. Finally, he may so regulate conditions in the bottle that the product will become sterile after it has been bottled. Conditions governing acidity, taste, gas pressure, etc., present certain difficulties in every case.

The chief raw material, other than water, is sugar. The industry uses 300,000 tons of sugar yearly. Each six ounce bottle of finished beverage contains about three-quarters of an ounce of sugar, giving an average content of about twelve

percent (1). As will be indicated below, the organisms frequently found in sugar are the chief sources of the bottlers difficulty.

The most common type of spoilage encountered is fermentation due to yeast. This spoilage has not been proven to be deleterious to health, but it is unsightly because of the sedement of yeast that is deposited, and it indicates careless methods of manufacture. These organisms are able to multiply in the bottle under most conditions of acidity and gas pressure. Taste, as a rule, is not greatly affected other than by these changes due to the formation of alcohol and other fermentation products.

Other types of spoilage are those caused by bacteria and by mold. The former is most often found in the non-acid beverages such as cream soda: the later chiefly in non-carbonated (still) beverages. The remedy for the first is easily found in the addition of a very slight amount of acid, and for the second either in the elimination of still drinks, since carbon dioxide inhibits mold growth, or by sterilization, either in the bottle, or in the course of a process insuring the absence of recontamination. For this reason the present study will not consider other organisms than yeast in so far as the experimental work is concerned.

That sugar can and does contain yeast, bacteria, and mold is abundantly shown by the work of Owen (2)(3)(4), Kopeloff (5)(6) and others. Working on Cuban and Louisiana raw and

commercial sugars the reports cited above indicate the presence of all three groups of micro-organisms. From the Fellowship laboratory of the American Bottlers of Carbonated Beverages, under whose direction the present work has been carried out, McKelvey (7) reported that 62 out of 132 samples of sugar obtained in 1925 contained viable yeast in five gram samples. At the same time yeast were seldom found in samples of water from bottling plants encountering fermentation troubles, and were almost always found in samples of simple syrup and in beverages. The fellowship has found yeast in 1464 out of 1719 bottles of spoiled beverages examined, or an average of 85.17%. Turner (8) reported cultural characteristics of 51 yeasts isolated from beverages.

It is well established, therefore, that sugar as a raw material is one primary source of the organism causing most of the spoilage in carbonated beverages. The processes by which sugar is fabricated into the finished beverage determine the fate of these organisms.

The processes under which sugar is dissolved to form syrup fall into three classes: the dry mix, the cold process, and the hot process.

The dry mix is that process by which the sugar is first mixed with a very small amount of water, together with acid (usually citric), flavor, and color. The mass is stirred until a fairly uniform mixture is obtained, and it is

then allowed to stand for several hours to "blend". Not enough water is added to dissolve the sugar, but simply to make a thick paste. In some cases the sugar is no more than moistened. After the proper blending time the requisite amount of water is added and the final syrup made. The dry mix process has a basis of merit, which will be shown in the experimental part.

The cold process is simply the addition of enough water to a given amount of sugar to make a syrup of definite strength. The mixture is agitated until complete solution is obtained after which the syrup is ready for use.

Percolation is a modification of the cold process, in which water is allowed to percolate slowly thru a large mass of crystalline sugar into a receiving vessal. The rate of flow of the water determines the strength of the syrup, which often is obtained in rather high densities.

It will be noted that the above procedures do not furnish any means of sterilizing the syrup as such.

The hot process is simply a means of making syrup with, or followed by, the application of heat. It can be depended upon to give satisfactory results if certain limits of heating, etc. (9)(10) are adhered to. Acid may be added during the heating process in order to facilitate sterilization. This method has certain advantages over the two procedures just described:-

1. Complete sterilization is assured especially in the presence of a small amount of acid.

2. Inversion will take place if acid is added, thus giving a more uniform product for the market. Slow inversion in the bottle is apt to cause a noticable change in the sweetness of the product.

3. If the hot process is a part of a sterilized closed system, in which no further exposure of the syrup is permitted, and if all subsequent additions are made aseptically, and with sterile materials, the final product will be sterile.

Disadvantages may be as follows:-

1. A relatively non-corrosive container must be used. It should contain no material capable of introducing lead, copper, zinc or other injurious materials into the syrup.

2. Steam should be available, or if absent, an adequate source of heat must be provided. Many of the so-called "failures" of the hot process may be traced to slow, non-uniform, or insufficient heating.

3. Equipment is likely to be rather expensive from the standpoint of the small installation.

4. The method of handling the sterilized syrup must be well understood by the plant operator, since such syrup is easily recontaminated.

The Fellowship has had the opportunity of examining a large number of samples of bottlers syrups. From its records

one fact stands out. Over a period of six years, and from the analytical work of four Fellows, the assembled data indicated that simple syrup (made from sugar and water alone) may be found to be highly contaminated at the end of seven to ten days, while the same syrup with added flavor, acid, and color, shows a marked decrease in count. The tables which follow, taken from these data, show this fact very clearly.

In Tables I, II, and III, the results for the flavored syrup are on the same line with the results for the corresponding simple syrup made from the same sugar. The samples of both kinds of syrup were examined after seven to ten days following their manufacture in commercial bottling plants.

TABLE I

Yeast Content of Simple and Flavored Syrups compared with their acidity (p H) and specific gravity

Simple Syrup			Flavored Syrup		
Living Yeast per c.c.	Sp. Gr.	p.H	Living Yeast per C.C.	Sp. Gr.	p.H
15,000	1.276	5.6	18	1.262	2.0
12,000	1.252	6.1	0	1.248	2.11
12,000	1.252	6.1	6	1.2414	2.15
10,000	1.287	- -	0	1.1737	1.93
40,000	1.226	5.9	4	1.2081	2.18
100,000	- ---	- -	0	- ---	1.79
25,000	1.283	- -	44	1.280	2.12

The group in table II includes a number of samples of syrup in which the p.H of the flavored syrup was not determined. The acidity of beverages made from these syrups is given as an index of the acidity of the syrups. If allowance is made for the fact that a dilution of from one part in four to one part in six is made in adding Carbonated water to the syrup, the p.H of the syrup can be estimated to be not more than 0.5 p.H less than that of the beverage.

TABLE II

Yeast Content of Simple and Flavored Syrups compared with the Acidity of the Beverages made from them

Simple Syrup		Flavored Syrup		Beverage
Living Yeast per c.c.	Sp. Gr.	Living Yeast per c.c.	Sp. Gr.	p.H
>100,000	1.285	0	1.255	3.00
46,120	1.263	0	1.255	2.92
46,120	1.263	0	1.221	2.75
46,120	1.263	0	1.209	3.25
46,120	1.263	0	1.197	2.75
36,260	1.289	0	1.285	2.38
>100,000	1.284	0	1.281	2.83
>200,000	1.281	1000	1.267	3.17
60,000	1.246	0	1.232	2.80

Table III gives results which at first thought might seem to be exceptions to the foregoing results. However, an

inspection of the figures for the acidity of the finished beverages show that the flavored syrup has been made with little or no acid.

TABLE III

Yeast Content of Simple and Flavored Syrups conpared with the Acidity of the Beverages made from them

Simple Syrup		Flavored Syrup		Beverage	
Living Yeast per c.c.	Sp. Gr.	Living Yeast per c.c.	Sp. Gr.	p.H	Flavor
180,000	----	60,000	----	4.56	Vanilla
1,000	1.303	100,000	1.30	4.54	Peach
1,000	1.303	740	1.277	3.62	Strawberry
>200,000	1.281	2,400	1.276	4.44	Root Beer
70,000	1.318	27,000	1.272	4.12	Strawberry
100,000	1.282	100,000	1.190	4.50	Grape

The above Tables point out the possibility of a "cold process" based upon definite conditions of time and of acid concentration. Since citric acid is used to acidify almost all beverages its use under carefully determined procedures can be a definite part of the process of manufacture. The tables also indicate possible explanations for the fact that the "dry mix", as described earlier, would often give satisfactory results under certain conditions. The quality of the finished product was no doubt a function of the time during which the sugar mixture was in contact with the high concentration of acid.

It is also evident that yeast may grow in heavy sugar syrups, even as high as 30° Be. (55.7% sugar)

These observations led to the thought that a careful study of the factors influencing the rate of death of yeasts in heavy sugar solutions might throw considerable light on the mechanism of sterilization of syrups. With this in view the experiments following were carried out.

The plan of the investigation was:-

1. To observe conditions of time, concentration of sugar and strength of citric acid in the killing of yeast (both vegatative and spore forms) in heavy sugar syrups.

2. To observe the effect of various salts of citric acid upon the killing time.

3. To observe in particular the effect of small amounts of sodium di-hydrogen citrate on the death time of yeasts in sugar-citric acid-water media. This combination of citric acid might readily be encountered in syrups made from highly alkaline waters.

4. To observe changes in p.H, conductivity and inversion when citrates are added to sugar solutions acidified with citric acid.

HISTORICAL

In summing up such work as has been reported having bearing on this subject, we should keep two things in mind. The first has to do with known effects of acids, or acid salt systems, upon micro-organisms in general, and upon yeast in particular. The other takes in the properties of acid and acid salt systems in water alone and in sugar solutions. From either source some light might be expected which would have bearing upon the subject.

A. The effect of acid and of acids and their salts on the growth or death of yeast.

Many authors could be cited with reference to a difference in the acid tolerance between yeast and bacteria. In order to establish the growth ranges of each, some reference to the limiting reaction for certain bacteria should be made.

The one case of an acid-salt system in connection with a growth phase of a micro-organism, was found in the work of van Dom (11). He studied the influence of lactic acid and sodium lactate on lactic acid fermentation. Two limits were found: one t he acidity of the medium, and the other the amount of undissociated lactic acid. His method was to add certain amounts of lactic acid, sodium lactate, or combinations of both, to a lactose medium, which was then inoculated with lactic acid bacteria and allowed to remain until growth had ceased. He then measured the reaction and total

lactate, etc., of the end products. Two factors causing death of the organism were found, each independent of the other. If enough lactic acid was present to give an end reaction pH 4, growth ceased. If enough sodium lactate were present to cause the amount of undissociated lactic acid to reach 0.01 N, growth also ceased.

Grove (12) also established limits, in terms of percent acid, for the growth of the "cider sickness bacillus". Recalculating from his figures the p.H of those concentrations of acid preventing growth, we obtain the following:--

Limiting concentration of acid		p H
All acids	> .5%	
Sulfuric	0.05%	2.07
Salicylic	0.07%	2.72
Tartaric	0.3%	3.00

These different values for the reaction would indicate that some factor other than concentration of the hydrogen ion must be involved.

Kolthoff also points out that the undissociated molecule has a bearing on the growth of bacteria. He states that "growth in cultures is brought to a stand still, not by the hydrogen ion concentration but by the undissociated acid formed by the growing bacteria".

In a study of yeasts isolated from carbonated beverages Weldin (14) found the limiting reaction to be from p.H 3.3 to 2.6 in a medium consisting of 0.1% peptone and 10%

sugar, made solid with 2% agar agar. In a similar medium without agar a p.H of from 2.7 to 2.3 was required to inhibit growth. The temperature was 25° C.

Turner (8) reported upon a series of 14 groups of yeasts isolated from carbonated beverages. These groups ranged in their growth characteristics, but growth was reported in some groups in a medium with a reaction as acid as p.H 2.3 and as alkaline as 12.0. There were some organisms that could not stand an acidity of more than 3.3, and others that could not grow in a medium more alkaline than 7.2. Others showed some degree of growth over the whole range over which experiment was carried out. The optimum p.H range in most cases was between 4.3 and 5.9. Peterson (9) (10) also reports some work with acid and high heat, but no values for the reaction of the medium is given. The time required to sterilize syrup of 36° Be. density with the addition of one cubic centimeter of 7.074 N citric acid to 100 cubic centimeters of this syrup was less than two minutes. The same syrup without acid took 28 minutes to become sterile. The temperature of both experiments was 100° C.

In work of a nature similar to that of Weldin and Turner, Svanberg (15) finds the optimum range for bottom yeast to fall between p H 4 and 6, the optimum range for top yeast between p H 3 and 6, and for torulae between 2.5 and 6.

Somagye (16) also noted the effect of acids on yeast fermentation in 10% syrup. He decided that the hydrogen ion

concentration was not the only factor but that other characteristics should be considered. He lists swelling, surface tension, and flocculating powers as factors having influence. Clark (43) also points out that inhibition of yeast growth is not a function of the H^+ ion concentration alone.

That yeast can tolerate a high degree of acidity is shown by the work of Kataguri (17). Fermentation was found to take place in acetic acid solutions with a concentration as high as 0.5 M. The p.H of this strength of acetic acid is 2.55 according to tables quoted in Van Nostrand's Chemical Annual (44). The author finds that at constant concentration of acid, the rate of fermentation is almost independent of total acetate concentration.

In a study of the effect of certain acids on alcoholic fermentation, Rosenblatt (18) reports a list of acids in the concentrations which exercise retarding effect. The reaction of these acids are given as estimated from the source mentioned in the perceding paragraph (44).

Hydrochloric acid	M/6000	p.H	3.8
Carbonic acid	M/5000	- -	- -
Acetic acid	M/300		3.6
Sulfuric acid	M/6000		3.6
Tartaric acid	M/1000		3.15
Phosphoric acid	M/5000		3.5
Citric acid	M/3000		3.5
Potassium acid sulfate	M/2000		

Some salts are listed as exercising some effect, either accelerating or retarding, but no combination of acid and salt is mentioned by Rosenblatt. No work seems to have been reported by any author relative to the effect of salts on acids in strength capable of killing or inhibiting yeast growth. Except for the work on lactose fermentation already referred to, no experimental work of this nature was found in the field of bacteriology.

In work of another nature, Wagner (19) found that carbohydrates had a marked effect on the sporulation of yeast, in that non-fermentable carbohydrates did not stimulate the production of spores nearly as greatly as did fermentable carbohydrates. It was also stated that, "the richer the medium was in nutrient the higher acidity the yeast could withstand."

In summorizing the foregoing one thing should be especially noted:

Most of the authors cited brought out in one way or another the fact that p.H was not the sole limiting factor in the growth of yeast or bacteria. The anion and the undissociated acid have very important parts. That the anion is not always a factor of definite germicidal value can be established from the fact that the salts of an acid in question, even in high concentrations are not germicidal until some slight acidity is formed by the organism. In van Doms, work on the part of the undissociated acid, it was brought out that at different salt concentrations, giving different amount of the (lactic)

anion, the product of the H ion concentration and the lactate ion was the same in all solutions after fermentation had ceased. This would indicate that both reaction and concentration of undissociated acid had a part to play, and were mutually interdependent. This interpretation modifies his statement that the limite first to be reached, whether pH or concentration of undissociated acid, was the controlling factor.

An analogy to the action of acid in killing yeast is found in the work of Buchanan, Levine and associates (20) (21) (22) (23) on the killing power of solutions of sodium hydroxide and its salts. It was reported that altho sodium hydroxide was found to have very good germicidal powers, these properties were greatly incfeased when sodium carbonate, sodium phosphate, or sodium chloride were added. These salts were in themselves almost inert insofar as their killing power for yeast was concerned. In a series of experiments in which sodium hydroxide was the first member, and in which sodium hydroxide with the addition of increasing amounts of different salts made up the remainder, the phenomena of increasing germicidal value with no determinable change in hydrogen ion concentration was observed. When the increase in the undissociated sodium hydroxide wss calculated from the mass law, there seemed to be a constant relation between the undissociated portion so calculated and the time necessary to kill 99.9% of the yeast in the initial inoculation. In a series in which the same reaction was established, there was a wide range in the killing time.

B. The effect of added salts on the properties of acids with and without the addition of sugar.

The effect of added salts on the properties of an acid has been studied for sometime. There has been a great deal of discussion as to the value of potential and conductimetric measurements as indices of activity, of hydrogen ion concentration and of dissociation. The term activity has been applied to the values obtained by potential measurements, instead of considering these as a measure of hydrogen ion concentration. The evidence presented here is given to advance possible reasons for change in yeast killing powers of acids with the addition of salts.

In 1899 Arrhenius added to his dissociation theory the concept that salts increase the dissociation constants of weak acids. Later McBain and Coleman (25) recalculated his results and found this effect to be lacking and the original concept to be in error.

Later McBain and Kam (26) offered further evidence toward the solving of this question. In the case of acetic acid the presence of NaCl gave higher potential readings than for acetic acid alone. This had been interpreted to mean that a greater dissociation had taken place. In the light of vapor pressure determinations, based upon the amount of acetic acid in various fractions from the distillate of a solution of acetic acid with and without the addition of salt, these authors find that there is an increase in the amount of

undissociated acetic acid, rather than a decrease. They find an increase in the undissociated acetic acid activity almost equal in value to the increase in H^+ ion concentration as measured by the hydrogen electode potential measurements.

Brönsted (27) states that the addition of salts to a solution of weak acid decreases the activity coefficient but increases the activity of the undissociated molecules. The net result, if the acid be weak enough, is that the total activity will be increased. In the case of stronger acids (hydrochloric and sulfuric in chrom tanning liquors), Thomas and Baldwin (28) find chlorides increase the H^+ ion concentration, while sulfates cause a decrease. In a system composed of acetic acid, sodium acetate, and water, Schneiner (24) found good agreement between potential and conductivity measurements.

When sucrose is added to a system of acid, salt, and water, there is often shown an increase in the activity of the solution. This is shown in some of the references cited below. Some of these authorities prefer to call the phenomena an increase in the activity of sucrose. Others explain it on a basis of the decreased solvent material present when sucrose has been substituted for solvent. Calculations are cited below to show "hydration" of sucrose with six to eight molecules of water.

Some very definite values for the hydration of the added salts are given by Whymper (29). He finds that the presence of salts increases the velocity constant of inversion

of sucrose by nitric acid, but that the effect is balanced by the addition of a certain amount of water. If one mol of HNO_3 and 0.5 mol cane sugar are dissolved in 1000 grams of water, the velosity constant is 465. If 1 mol $AgNO_3$ is added to this system the velocity constant becomes 531. But if 1 mol $AgNO_3$ and 5 mols of water are added to the system the velocity constant remains at the original value. From other determinations of this kind he finds the following "hydration value", which are simply the mols of water which when added along with the salt, produced no change in the velocity coefficient:-

Average Hydration Values per Mol of Added Salt

Sugar plus	Ag	NH_4	K	Na	Ba	Ca	Sr
HCl		10	10	13	19	22	
HNO_3	5	7	8	1			18

This offers an explanation of the added effect solely on a basis of concentration change. It will be noted that the above experimental work is with strong acids.

Kolthoff (30) found that sucrose increased the pH of an HCl solution, and that the hydration of the sucrose per mol could be expressed by $8 \pm$ 2 mols of water. Scatchard (31) (32) also concludes that sucrose becomes hydrated with six or seven mols of water. Other authors point to the apparent increased activity of the solution upon the addition of neutral salts as a case of displacement of solvent. (33) (34) (35) (36). Jones and Lewis (37) offered a complete explanation

based upon the stoichiometric loss of water for the increased H^+ ion activity in solutions containing added sucrose, as though the sucrose were a chemically inert diluting material. They question the presence of hydrated sucrose.

The undissociated ion is considered important by Taylor (38) and by Brönsted (27). The latter states that in sugar inversion the product of the velocity and activity coefficient is a constant. He considers the activity coefficient to be that of the entire molecule, the algebraic sum of that of the undissociated and dissociated portions. This view is also expressed by Colin and Chaudin (39).

A distinction should be made with respect to strong and weak acids. While a definite increase in the rate of hydrolysis of sucrose has been demonstrated in the case of strong acids in the presence of their salts, the reverse is true in the case of weak acids. Salts of weak acids decrease the rate of inversion even in the presence of strong acids, because of the double decomposition taking place, ending with the formation of the weak acid and salt of the strong acid (40). Statements are made that the addition of salts with a common ion (41) increases the rate of hydrolysis, but this is found to be true only in the case of strong acids.

In the cases of commercial sugars, allowance must be made for the presence of salts (42). The poorer grades are usually quite well buffered, and they need a correspondingly greater amount of acid in order to reach a definite H^+ ion

concentration.

The references that have been cited have been those dealing with the effect of acids with and without the addition of their salts, on sugar solutions and on micro-organisms found in these solutions. It has been shown that the germicidal value of acids are affected by salts of these acids in a very definite way. Sugar also affects the activity of an acid or an acid-salt system. There is a change in reaction, in inversion, in activity and in germicidal value when salts are added. Some analogy from other systems must be brought to bear on the effect of acids and their salts upon yeast. Bacteria, being less acid tolerant, furnish one field for comparison. The effect of alkalies and their salts furnish another. The effect of acids and salts on sugar furnish a third. Back beyond all of this, the fact that H^+ ion concentration alone does not satisfactorily explain either the inversion of sugar or the killing of yeast, might indicate that the anion or the undissociated part may be equally responsible for such varying results as have been recorded.

With this in view, the experimental work which follows was begun, with the plan of recording those changes in the rate of death of the yeast, in sugar inversion, reaction and conductivity, as might give some light upon the subject.

C. Studies or yeast obtained from Carbonated Beverages.

It has been pointed out very briefly in the introduction that yeasts have been considered responsible for much of the spoilage in bottled carbonated beverages. A more detailed explanation should be given at this point.

Upson (45) pointed out that many of the yeasts present in bottled carbonated beverages are present in the sugars from which they were made. Owen and Kopeloff (loq. cit.) also found yeasts in sugar, and the former developed a method of treating raw sugar based upon inoculation with torulae in order to supply a small amount of carbon dioxide. De Grotte (46) stated that fruit juices used in the manufacture of carbonated beverages "contain yeasts which are common to all fruit juices" and were in a dormant or spore form.

Levine, Buchanan and McKelvey (47) reported that over 90% of the spoilage encountered in examination of beverages examined by them was due to yeast. In earlier work Levine (48) (49) discusses the manufacturing procedure in beverage production with emphasis on the sanitary control of various prices of equipment.

McKelvey (7) isolated over one hundred strains of yeast from beverages examined at the Fellowship established by the American Bottlers of Carbonated Beverages. He studied their spore production on Malt extract agar, carrots, and carrot agar.

Turner (8) studied fifty-one of the yeast isolated

by McKelvey, and divided them into fourteen groups on the basis of their cultural characteristics. Twenty-eight of these were found to produce spores, and eighteen of the spore formers were separated into one group on the basis of cultural characteristics. Since this group represented many different sources and contained over one third of all the yeasts studied by Turner, it might be termed the most characteristic group as regards yeast in carbonated beverages.

The work of Weldin (14) has already been mentioned. His study was in more detail, but on a fewer number of organisms, than that of Turner. The work of Peterson has also been mentioned earlier in this paper (9) (10).

EXPERIMENTAL

A. Purpose

The results of observations on the sterility of simple and flavored syrups showed that badly contaminated syrups would become sterile after a short storage time if the proper amount of acid (usually in the form of citric acid) were added. It is a common procedure in bottling practice to make syrups by mixing 10 pounds of sugar with each gallon of water. The resulting syrup has a Baume strength of about 29.5°, or nearly 55% sugar. Most of the beverages based upon this syrup are made by adding one fluid ounce of fifty percent citric acid to one gallon of simple syrup, together with the proper amount of flavor and color. Such flavored syrups may eventually become sterile as is shown by Table I and II.

The plan of the present work is to study the rates of death or the death time in syrup having the above composition, as well as syrups of other strengths, under the influence of varying amounts of citric acid and sodium citrate. With this information, and that concerned with the relations of density, reaction, conductivity and rate of inversion, some mechanism explaining the death of yeast might be arrived at. The experimental work will be arranged in this manner:-

1. Rates of death of yeast, and death time.
   - a. 24° Be syrup
   - b. 27° Be syrup

c. 30° Be syrup

d. 33° Be syrup

e. 36° Be syrup

2. Reaction in syrup as above, in terms of pH.

3. Conductivity in syrups as above.

4. Rate of inversion of sucrose in syrups as above.

B. Source of Materials

a. The yeast

The yeast chosen was selected from those organisms studied by Turner (8). It was a strain that readily formed spores, and appeared to be characteristic of the group. It was chosen because of its ready spore formation and because of the well defined colonies it formed on Wort agar (Bacto). Turner had designated it as No. 11 in his discussion of yeasts from carbonated beverages. Some of its history, as given by Turner, follows.

Yeast No. 11 was isolated from a spoiled cherry soda. It easily demonstrated the spore stage, since it formed spores readily on gypsum blocks, malt agar, and carrots at 22° C. It formed spores on carrots only, at 28° C.

The optimum temperature for its growth appeared to be 25° C, growing better in the presence of air than in the absence, but having fair growth in either condition. Turner classified it in his group N, which comprised 18 out of 51 yeasts studied. The basis of his separation of this group was:-

A. Spore production on gypsum blocks.

B. No growth on surface of liquid media.

C. Poor growth at 37° C, producing acid and gas.

D. Production of acid and gas from maltose and raffinose.

The following data are from p. 23-24 of Turner's

thesis and describe the group to which no. 11 was assigned.

1.	Surface growth,	none
2.	Shape,	oval
3.	Size	4.2 u by 2.5 u
4.	Optimum temperature,	25° C
5.	Anaerobic growth,	yes
6.	Milk,	no change
7.	Glucose, levulose, galactose, maltose, sucrose, raffinose	acid and gas
8.	Glycerol, aesculin, salacin	no growth
9.	Growth in old culture	brownish

The colonies on Wort agar (Bacto) were very characteristic. As observed by the author they were glistening, smooth and opaque. Old colonies often developed a brown raised spot in the center of the colony. Surface colonies were circular in shape and symetrical. Sub-surface colonies were often wedge, triangular or star-shaped, typical of rapidly growing organisms producing gas and splitting agar medium. Colonies could often be observed ten to twelve hours after plating by reason of the glistening point of light reflected by them.

Optimum growth was obtained for the group to which this strain was assigned, at a reaction varying from 4.3 to 5.9. The highest acidity showing growth after a period of four to seven days was a pH 2.4. The alkaline limits vary from 8.6 to 10.6.

b. The innoculating material.

The yeast used was passed thru several platings, each time selecting one colony for suspension and replating. This procedure, together with the fact that no observable change in the organism took place over the time of the experimental work, is presented in proof that a pure culture was used in the experimental work.

For inoculation a two day malt extract broth culture was used. At the end of this time the yeast were well developed and there were always between six and ten million yeast per cubic centimeter. A heavy dark brown deposit of yeast would form, and a great many yeasts in spore form could be demonstrated, even in three day broth cultures. The same is true of malt agar and carrot agar slant cultures. For this reason the inoculating solution always contained a very large amount of spores, as well as vegetative forms.

c. The sugar, acid and salts.

The sugar used was a high grade commercial cane sugar, whose normal solution gave a reading of 99.9 when polarized in a 200 mm tube in a saccharimeter at 20° C. The moisture content was 0.034%. The syrups were made up to densities of 24, 27, 30, 33, and 36° Baume with distilled water. These are equivalent to specific gravities at 20°/20° of 1.1983, 1.2288, 1.2609. 1:2946, and 1.3303 respectively.

The citric acid and sodium citrate were both of the C. P. grade and were used from a fresh, previously unopened bottle. Stock solutions were made, sterilized because of the ease of mold growth on even fairly concentrated solutions, and made up to volume after sterilization with sterile distilled water. In all solutions of acid, salt or syrup in which conductivity was to be taken, conductivity water was used.

d. Plating medium.

Wort agar (Bacto) was used for all plating work. The formula (claimed by label) was:-

Maltose	12.75 parts	K_2HPO_4	1.00 parts
Malt Extract	15.00 "	NH_4Cl	1.00 "
Dextrin	2.75 "	Peptone	.78 "
Glycerine	2.35 "	Agar	15.00 "

Of this agar preparation 50.63 parts were added to 1000 c.c. of distilled water. Since the mixture did not "set up" well, 5 grams of agar were added in addition to the above material in every 1000 c.c. of water.

C. Methods and Technique.

In preparing syrups, the proper amount of sugar and water were added, with allowance for the salt and acid solution to be added after sterilizing. Sterilizing losses were found to be almost nil, due to heavy plugging, to the short time of sterilizing, and to the lowering of vapor pressure in heavy syrups. After sterilizing the sugar solutions, the acid and salt solutions were added and well mixed with the syrup. Then one c.c. of a broth culture of yeast was added to each sugar solution, which averaged 125 cubic centimeters total volume.

Syrup solutions after inoculation were stored at twenty-five degrees centigrade when not being plated out. Initial counts were taken and the count by plating methods was repeated at definite intervals. At these times, the flask was well shaken and one c.c. removed for dilution purposes. The time intervals ranged from two hours to three or four days on various solutions used.

Counts were made at the end of 72 hours. Colonies were fished from plates representing platings at the beginning of certain runs, and again at the end. Examination of these colonies verified the maintenance of a pure culture.

Determinations of hydrogen ion concentrations were made by both a hydrogen train, and the quinhydrone electrode, Both methods were used with t he same normal calomel cell.

The instruments used were a Leeds and Northrup Students Potentiometer and a pointer type galvanometer. A standard Weston cell whose potential had been checked against a certified standard was used frequently as a reference. A determination against a standard solution of potassium acid phthalate showed an error of less than 0.02 pH.

Conductivity was measured by means of a Leeds and Northrup type K bridge and a microphone hummer.

D. Results

The first series of runs was made using 24, 30, and 36° Be syrups. The concentrations of citric acid used were 0.02, 0.04, and 0.08 M. For each acid concentration, three solutions containing added salts were studied, in addition to one with acid alone. This gave four different combinations of acid and salt for each acid level of each sugar density used:-

1. Citric acid.
2. 1 mol citric acid plus 0.5 M sodium citrate equivalent to 1.5 M NaH_2 citrate.
3. 1 mol citric acid plus 1 mol sodium citrate, equivalent to 1 mol NaH_2 citrate plus 1 mol Na_2H citrate.
4. 1 mol citric acid plus 2 mol sodium citrate, equivalent to 3 mols Na_2H citrate.

In bottling practice the amount of added acid is expressed in ounces per gallon. One ounce of a 50% solution of citric acid, when diluted to one gallon is practically 0.02 molar. Hence, 0.01 M, 0.02 M, 0.04 M, and 0.08 M can be translated directly to the commercial terms, one-half, one, two, and four ounces per gallon respectively.

The following tables give the results of different concentrations of citric acid and various citrates on the rate of death of yeast in several densities of syrup.

TABLE IV

24° Be. Syrup, with 0.02, 0.04, and 0.08 Molar Citric acid.

Solution number	1	2	3
Molarity of acid	0.02 M	0.04 M	0.08 M
Original count	188,000	188,000	188,000
Count at end of 12 hours	53,000	36,200	17,600
21 "	24,800	12,100	5,260
32 "	11,800	3,380	1,700
45 "	4,860	1,200	420
Percent survivors			
12 hours	28.2	19.25	9.35
21 "	13.2	6.43	2.80
32 "	6.26	1.80	.90
45 "	2.58	.64	.22

TABLE V

24° Be. Syrup with Citric acid and sodium citrate equivalent to NaH_2 Citrate.

Solution number	4	5	6
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.01	0.02	0.04
Original count	188,000	188,000	188,000
Count at end of			
3 days	642,000	492,000	481,000
12 "	2,040,000	1,600,000	632,000
18 "	5,260,000	5,420,000	2,462,000
Percent survivors			
3 days	343	263	257
12 "	1090	855	338
18 "	2810	2890	1314

TABLE VI

24° Be. Syrup with citric acid and sodium citrate in equivalent molar quantities.

Solution number	7	8	9
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.02	0.04	0.08
Original count	188,000	188,000	188,000
Count after 3 days	492,000	378,000	326,000
12 "	714,000	-------	432,000
18 "	1,242,900	892,000	704,000
Percent survivors			
3 days	262	201	174
12 "	382	---	235
18 "	660	476	376

TABLE V11

24^{o} Be. Syrup with citric acid and sodium citrate equivalent to Na_2H Citrate.

Solution number	10	11	12
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.04	0.08	0.16
Original count	188,000	188,000	188,000
Count after			
3 days	222.000	123,000	78,500
12 "	148,000	32,000	4,750
18 "	123,000	12,000	520
Percent survivors			
3 days	108	65.4	41.7
12 "	78.7	17.0	2.53
18 "	65.4	6.38	.276

TABLE VIII

30° Be. Syrup with 0.02, 0.04, and 0.08 molar citric acid.

Solution number	13	14	15
Molarity of citric acid	0.02	0.04	0.08
Original count	62,000	62,000	62,000
Count after			
3 hours	34,000	21,550	18,300
6 "	14,960	9,200	7,400
9 "	9,120	3,600	3,160
12 "	4,640	1,260	466
15 "	2,100	486	10
Percent survivors			
3 hours	51.6	34.8	29.5
6 "	24.1	14.96	11.92
9 "	14.7	5.81	5.10
12 "	7.48	2.0	.752
15 "	3.39	.784	.161

TABLE IX

30° Be. Syrup with citric acid and sodium citrate equivalent to NaH_2 citrate.

Solution number	16	17	18
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.01	0.02	0.04
Original count	62,000	62,000	62,000
Count after			
3 days	512,000	320,000	383,000
12 "	816,000	401,000	656,000
18 "	942,000	796,000	582,000
Percent survivors			
3 days	827	517	618
12 "	1320	647	1060
18 "	1520	1285	940

TABLE X

30° Be. Syrup with citric acid and sodium citrate in equivalent molar quantities.

Solution number	19	20	21
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.02	0.04	0.08
Original count	62,000	62,000	62,000
Count after			
3 days	274,000	263,000	288,000
12 "	188,000	179,000	156,000
18 "	147,000	164,000	119,000
Percent survivors			
3 days	442	424	465
12 "	332	289	252
18 "	237	264	192

TABLE XI

30° Be. Syrup with citric acid and sodium citrate equivalent to Na_2H Citrate

Solution number	22	23	24
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.04	0.08	0.16
Original count	62,000	62,000	62,000
Count after			
3 days	202,000	123,000	78,500
9 "	70,000	16,240	6,100
12 "	31,000	7.200	1,200
Percent survivors			
3 days	326	198	127
9 "	113	26.2	9.85
12 "	50	11.6	1.94

TABLE XII

36° Be. Syrup with 0.02, 0.04, and 0.08 molar citric acid.

Solution number	25	26	27
Molarity of acid	0.02	0.04	0.08
Original count	68,000	68,000	68,000
Count after			
4 hours	-	24,000	16,240
8 "	4,000	5,100	2,100
12 "	-	1,200	300
16 "	300	100	20
24 "	30	20	-
34 "	5	-	
Percent survivors			
4 hours	-	35.3	23.8
8 "	7.06	7.5	3.1
12 "	-	1.76	.44
16 "	.44	.15	.029
24 "	.04	.029	
34 "	.007		

TABLE XIII

36^{0} Be. Syrup with citric acid and sodium citrate equivalent to NaH_2 Citrate.

Solution number	28	29	30
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.01	0.02	0.08
Original count	68,000	68,000	68,000
Count after			
4 hours	-	-	19,600
8 "	12,300	8,200	2,500
12 "	-	-	640
16 "	550	200	-
24 "	80	20	22
34 "	25		
Percent survivors			
4 hours	-	-	28.8
8 "	18.2	12.1	3.68
12 "	-	-	.94
16 "	.81	.294	-
24 "	.117	.029	.032
34 "	.037		

TABLE XIV

36^{o} Be. Syrup with citric acid and sodium citrate in equivalent molar quantities.

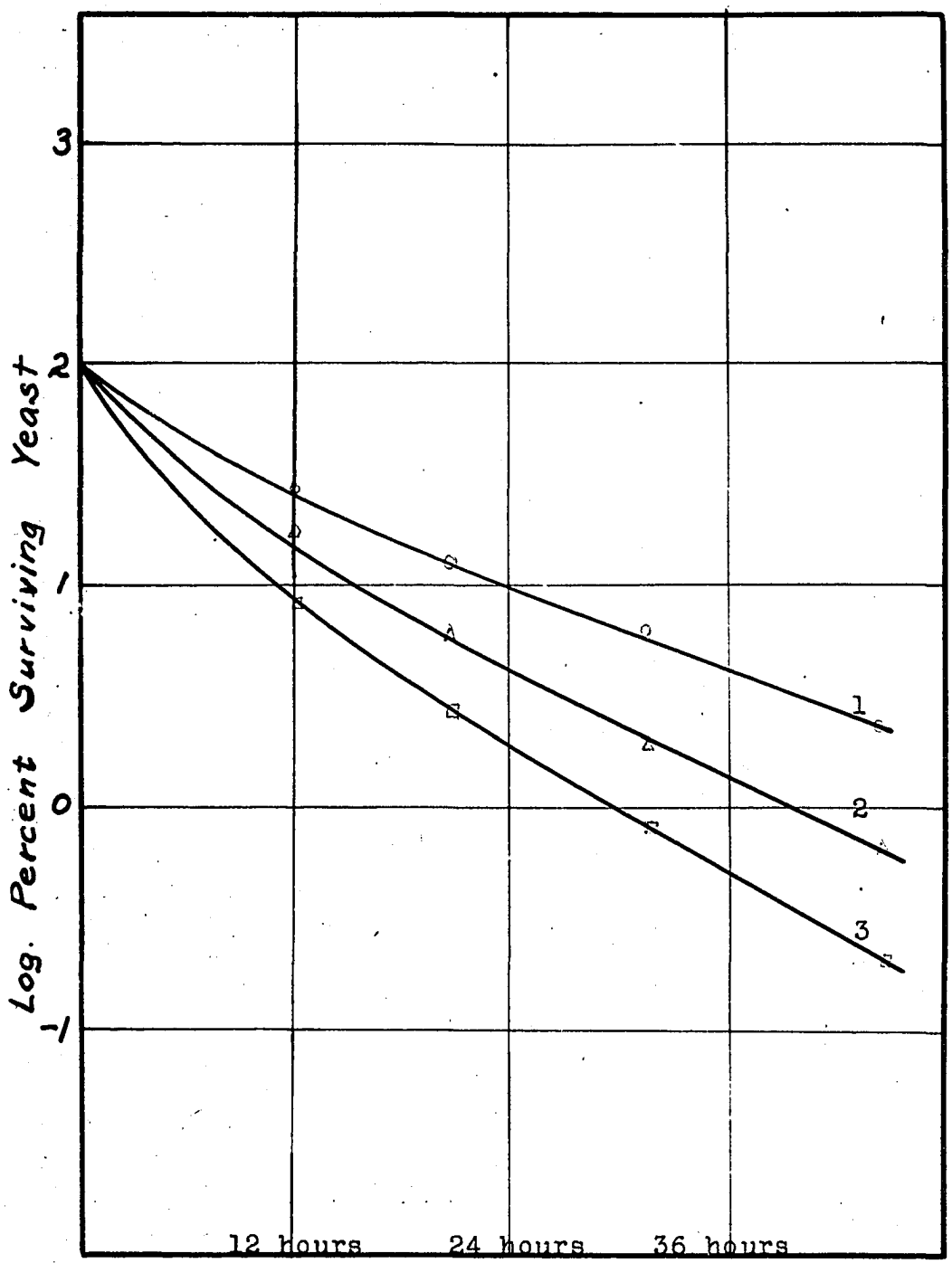
Solution number	31	32	33
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.02	0.04	0.08
Original count	68,000	68,000	68,000
Count after			
8 hours	12,400	8,000	2,800
16 "	-	-	200
24 "	70	120	60
34 "	25		
Percent survivors			
8 hours	18.2	11.8	3.98
16 "	-	-	.294
24 "	1.03	.174	.088
34 "	.037		

TABLE XV

36° Be. Syrup with citric acid and sodium citrate equivalent to Na_2H Citrate.

Solution number	34	35	36
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.04	0.08	0.16
Original count	68,000	68,000	68,000
Count after			
8 hours	5,500	7,600	2,600
16 "	-	542	168
24 "	52	60	12
34 "	12	10	
Percent survivors			
8 hours	8.09	11.18	3.82
16 "	-	.79	.247
24 "	.076	.088	.017
34 "	.018		

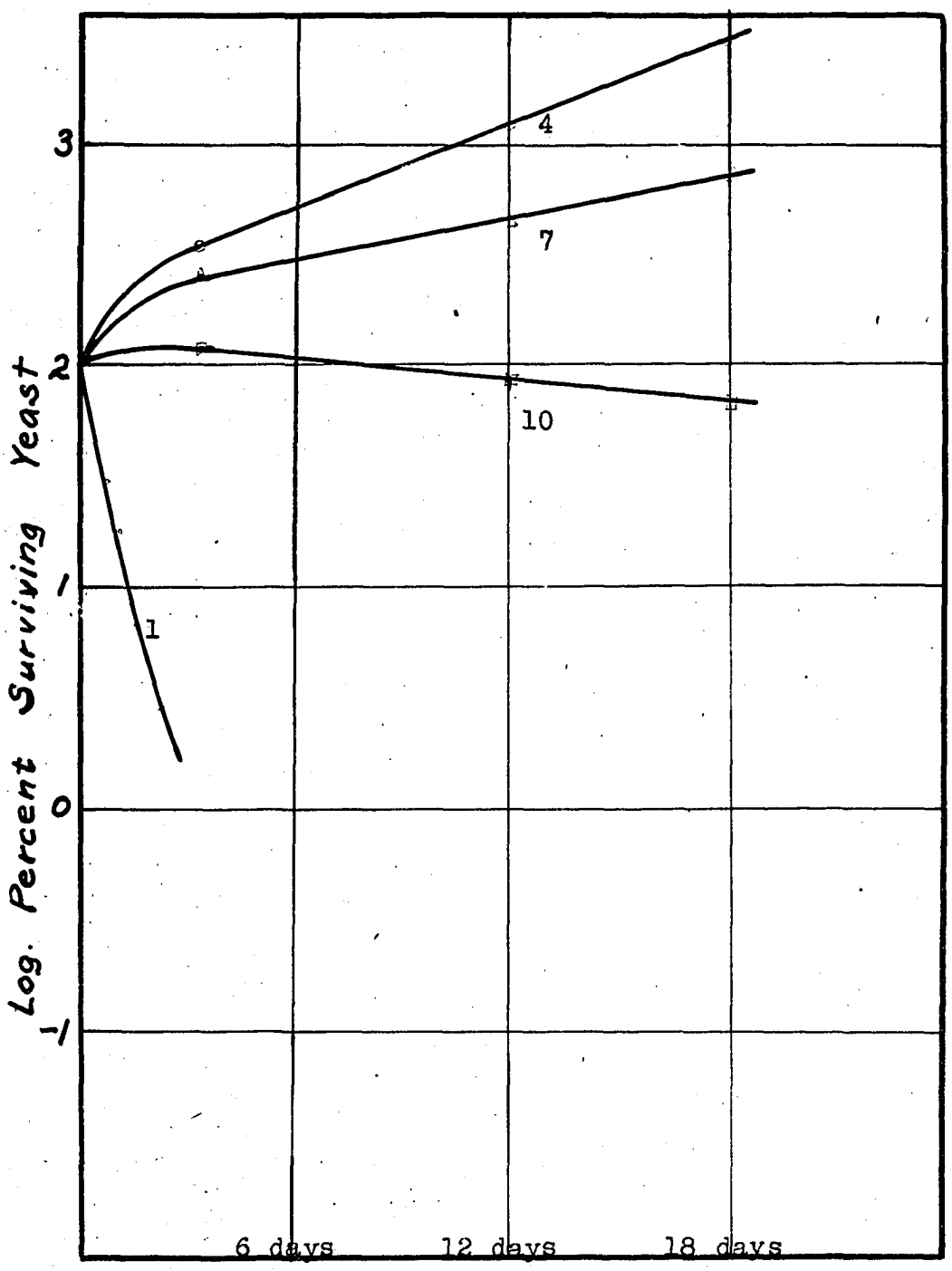
Plate 1.



The effect of increasing acid concentration on the rate of death of yeast in 24° Be. syrup.

Solutions 1, 2, and 3.

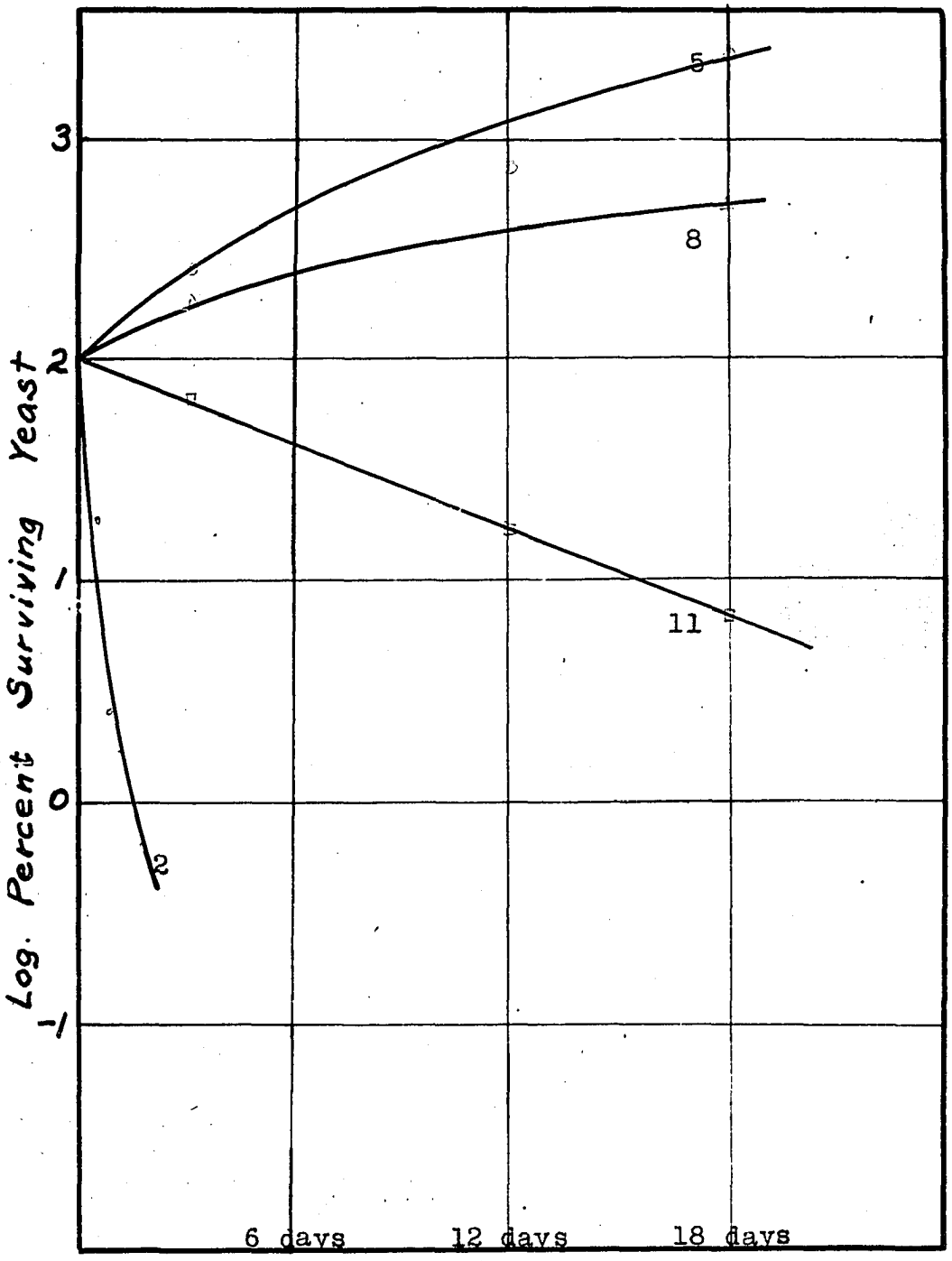
Plate 2.



The effect of added sodium citrate upon 0.02 M citric acid in the killing of yeast in 24° Be. syrup.

Solutions number 1, 4, 7, and 10.

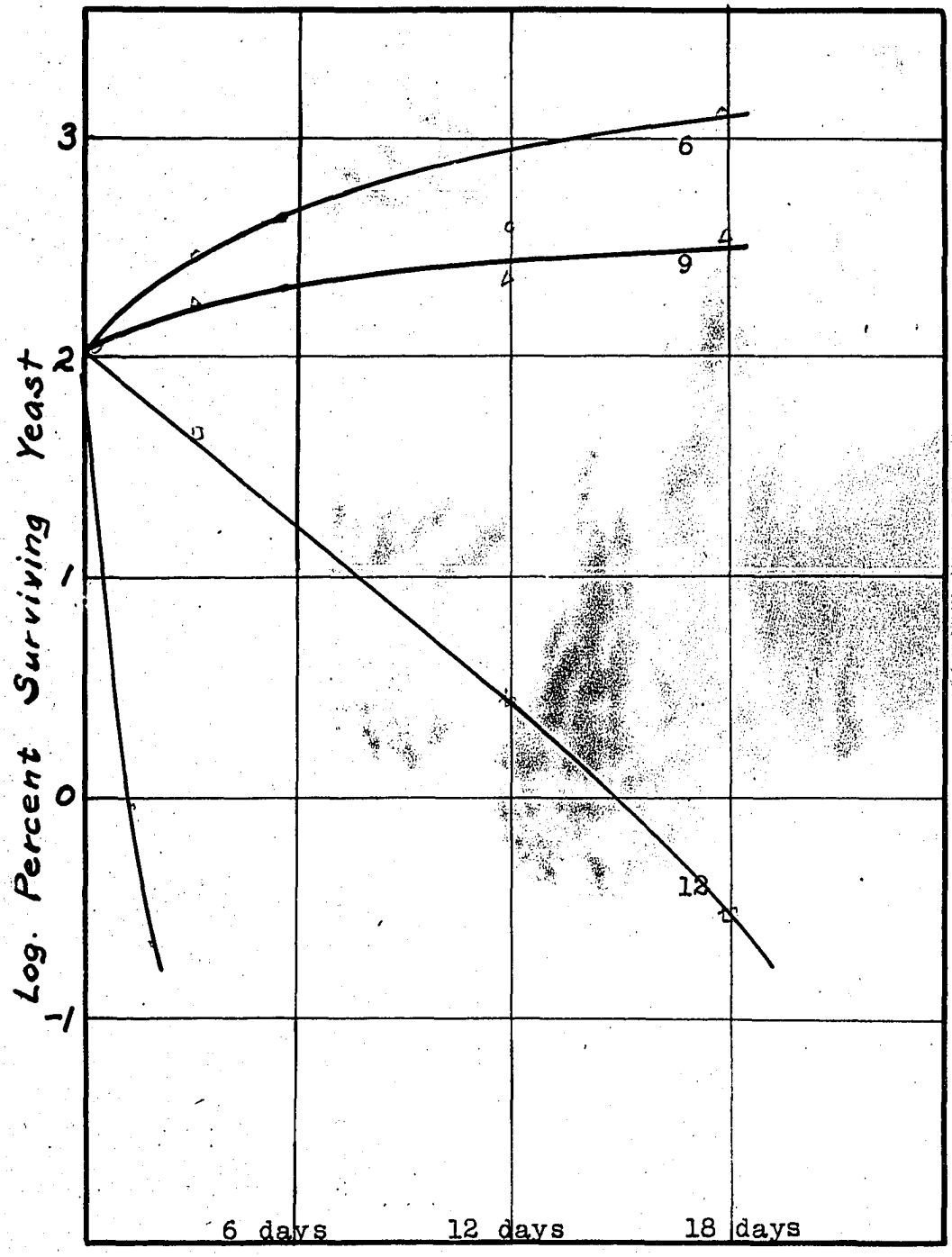
Plate 3.



The effect of added sodium citrate upon 0.04 M citric acid in the killing of yeast in 24° Baume syrup.

Solutions number 2, 5, 8, and 11.

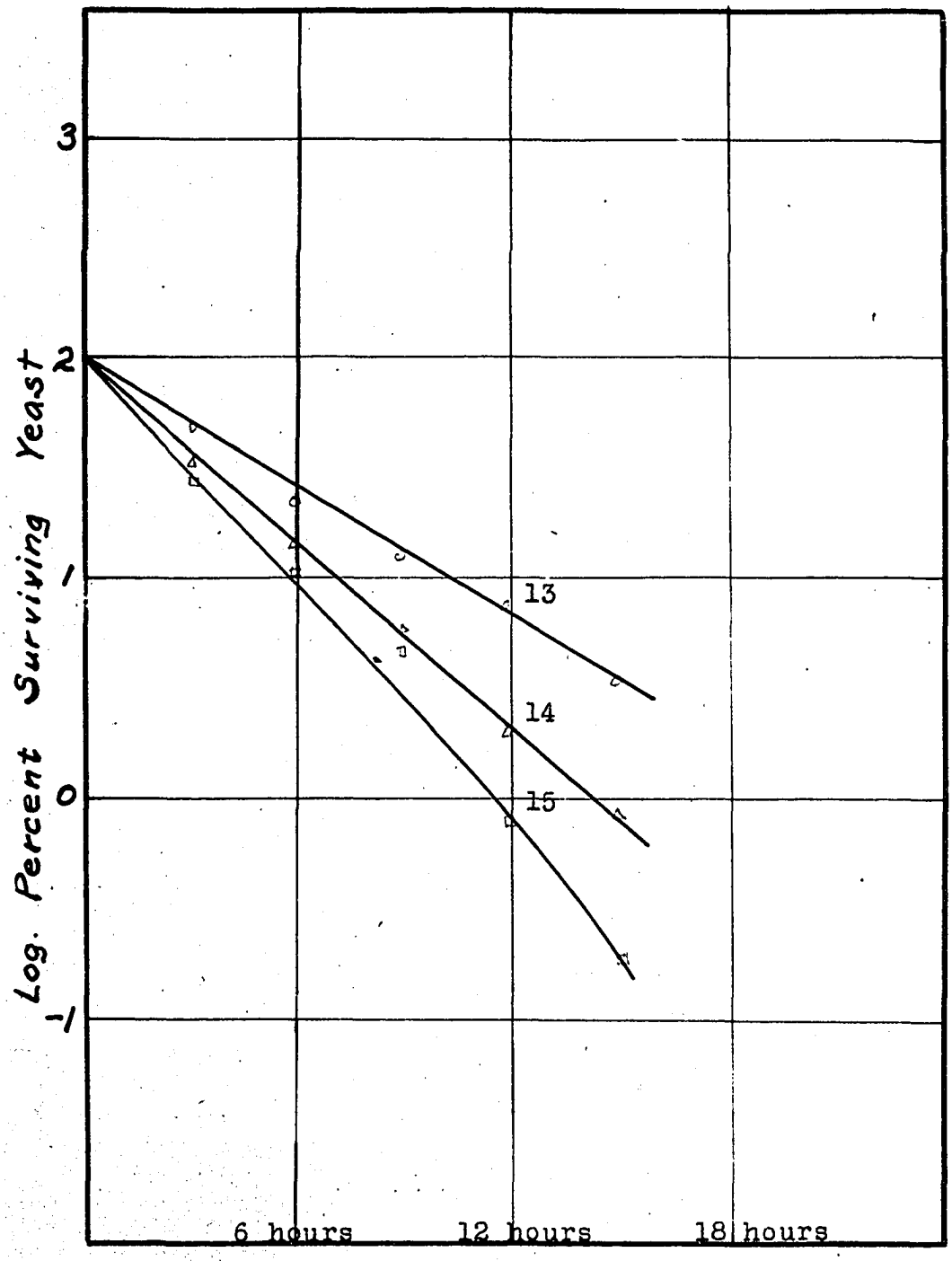
Plate 4.



The effect of added sodium citrate upon 0.08 M citric acid in the killing of yeast in 24° Be. syrup.

Solutions number 3, 6, 9, and 12.

Plate 5.

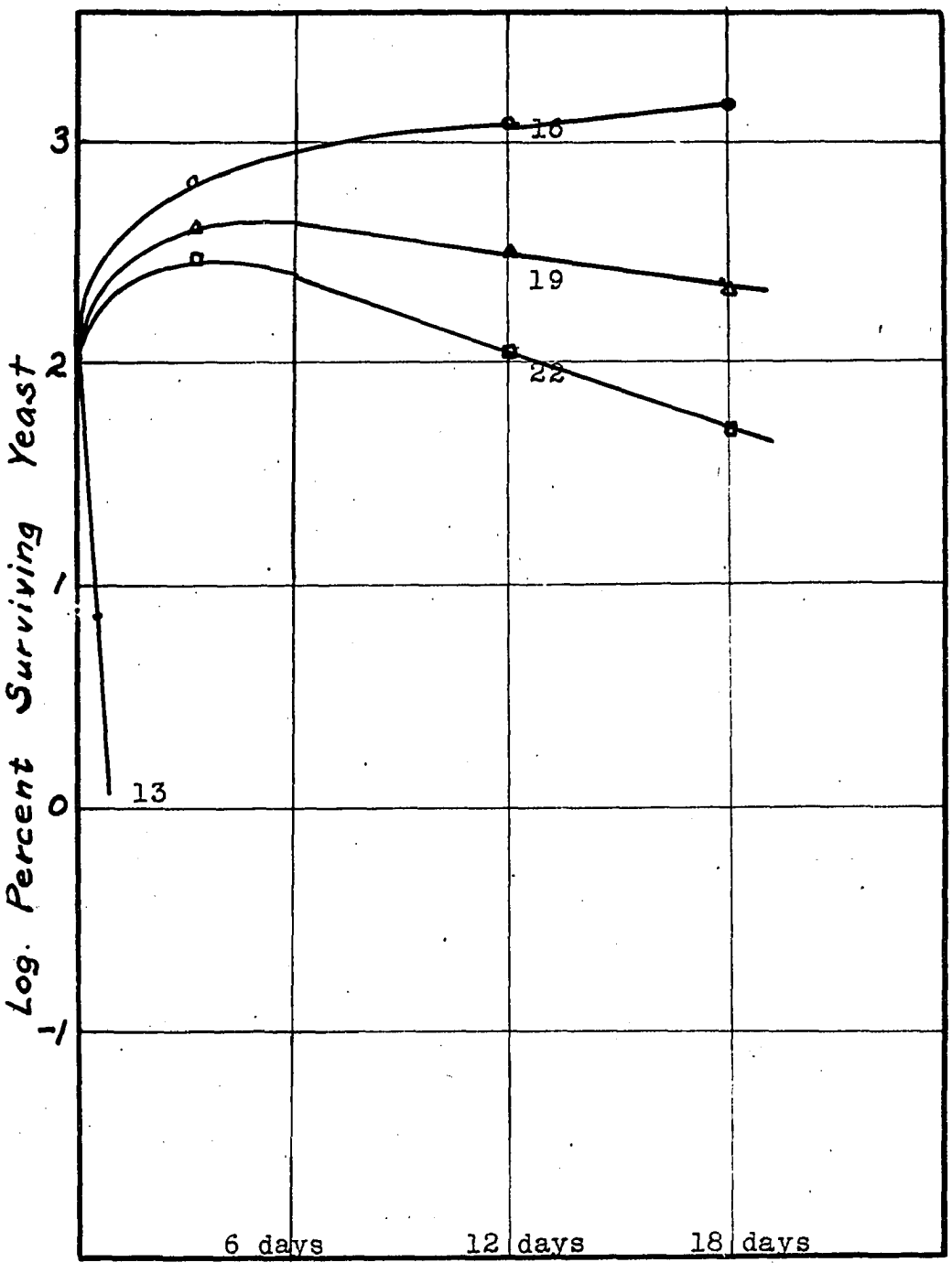


The effect of increasing acid concentration on the rate of death of yeast in 30° Baume syrup.

Solutions number 13, 14, and 15.

53

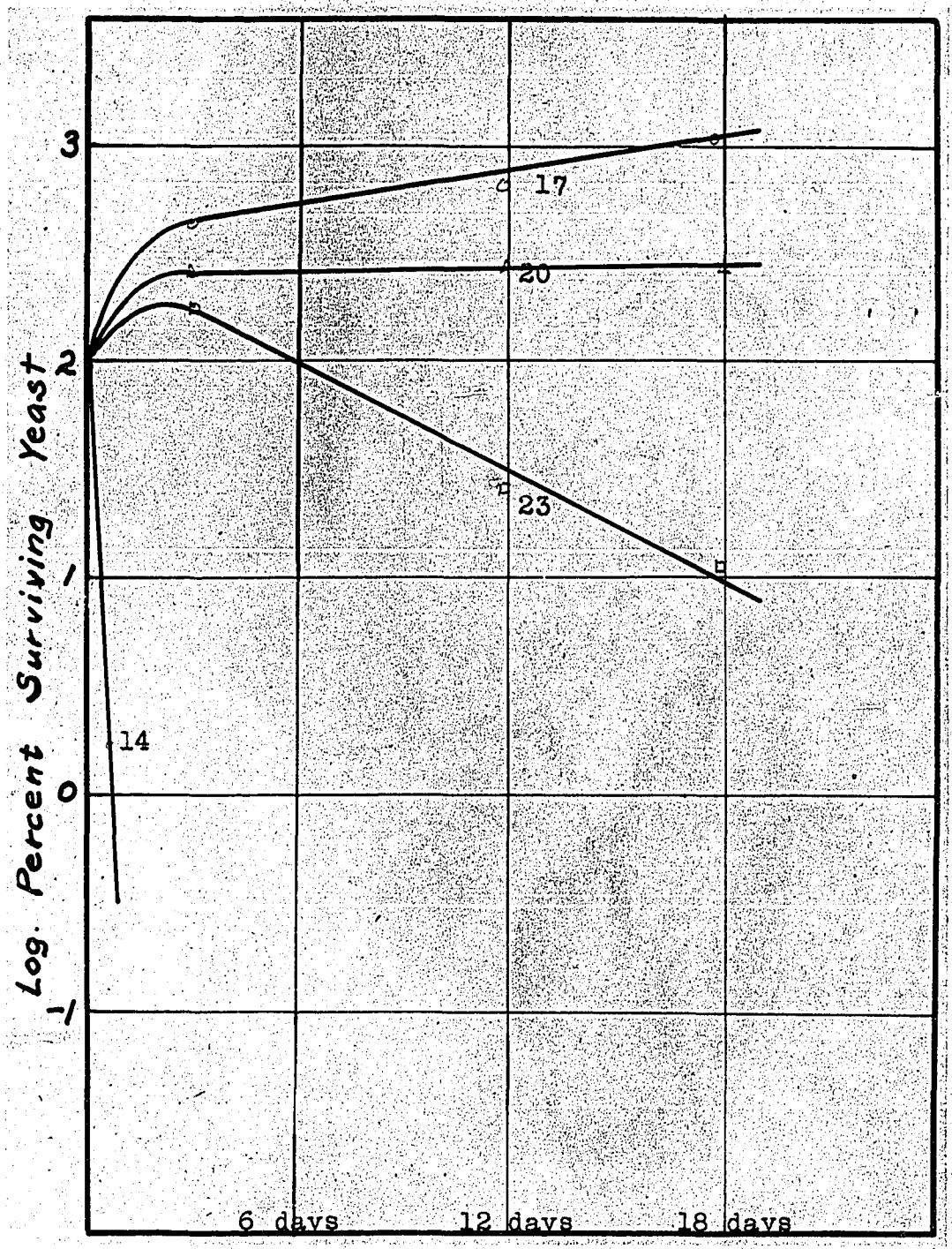
Plate 6.



The effect of added sodium citrate upon 0.02 M citric acid in the killing of yeast in 30° Be. syrup.

Solutions number 13, 16, 19, and 22.

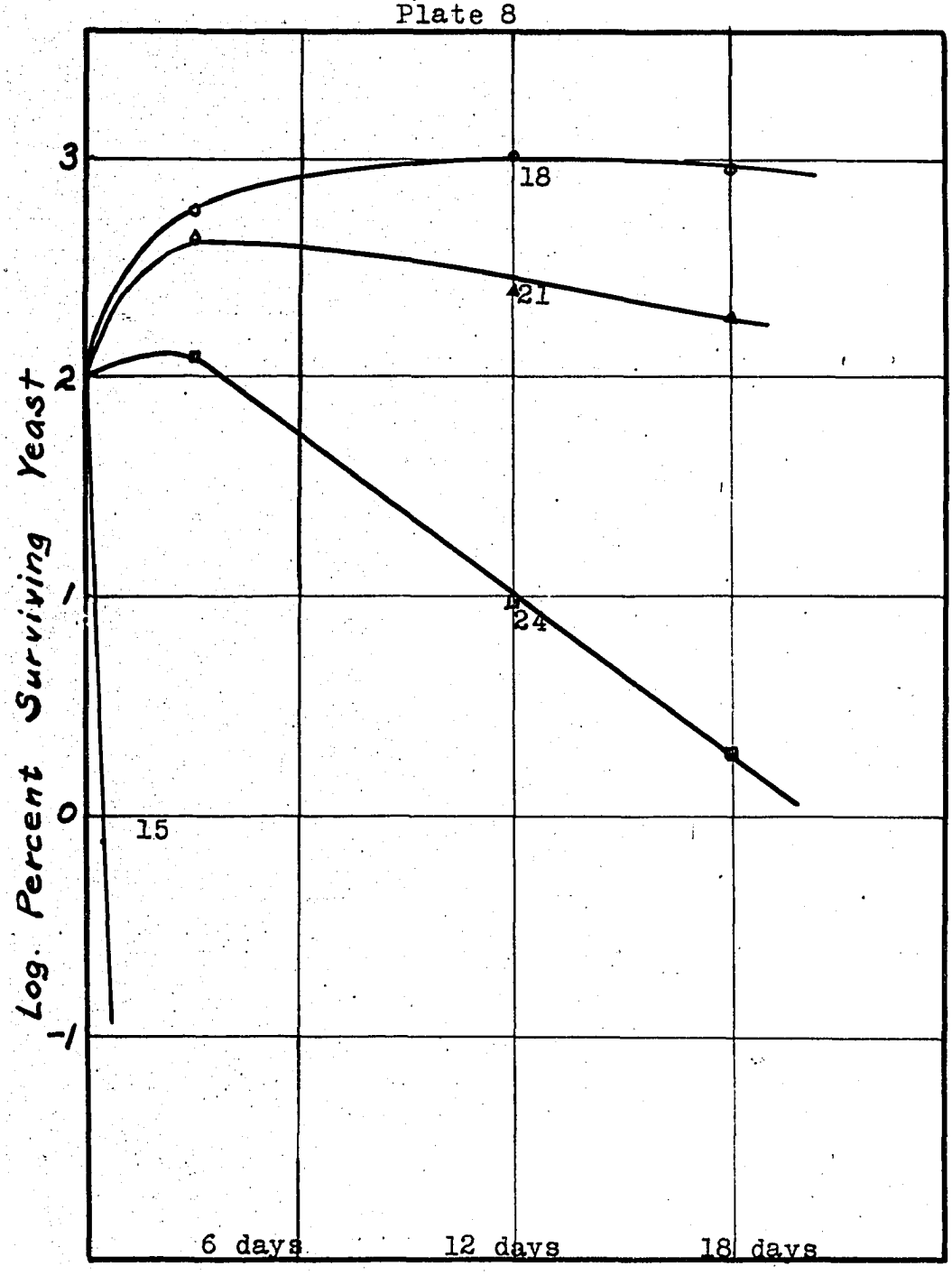
Plate 7.



The effect of added sodium citrate upon 0.04 M citric acid in the killing of yeast in 30° Be. syrup.

Solutions number 14, 17, 20, and 23.

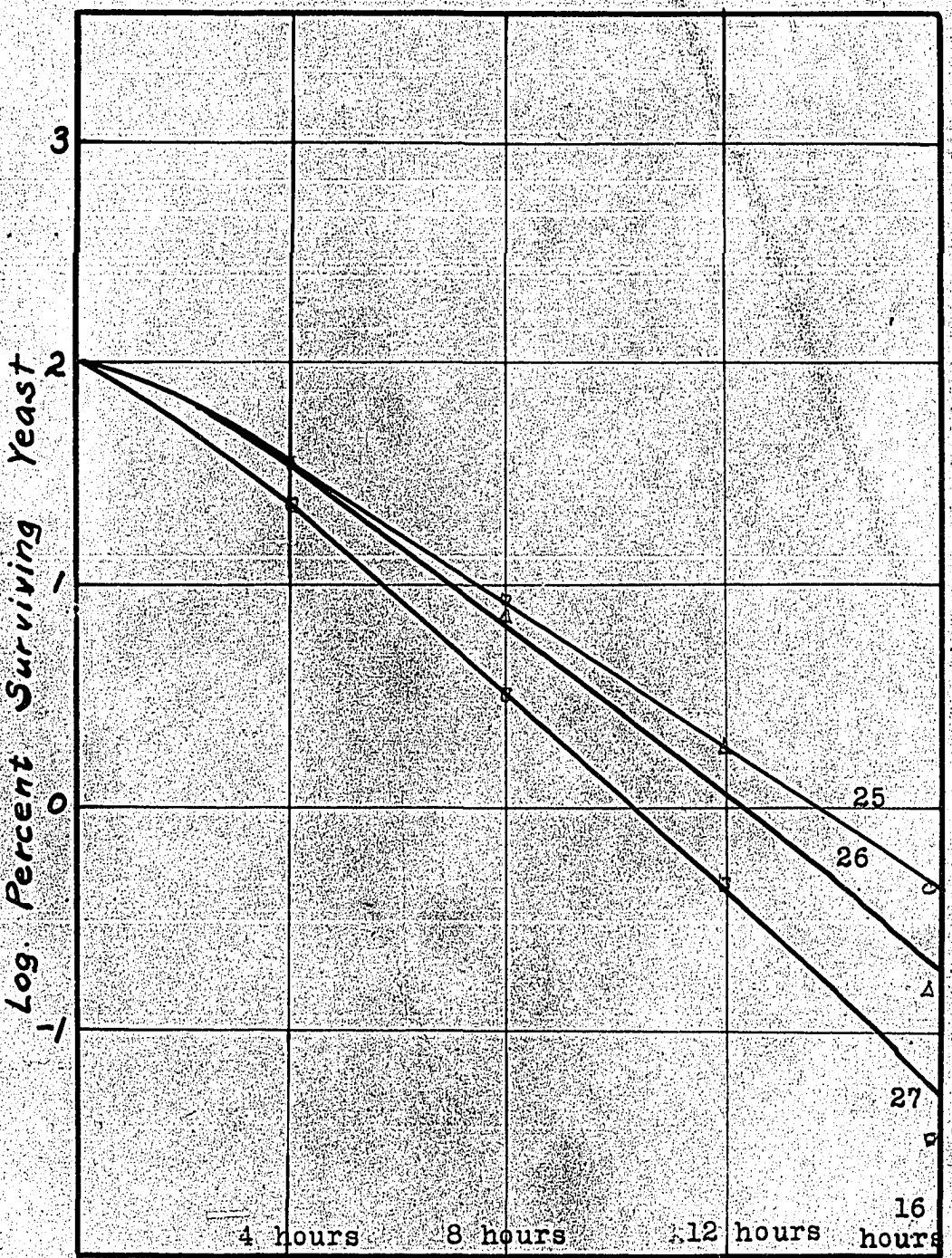
Plate 8



The effect of added sodium citrate upon 0.08 M citric acid in the killing of yeast in 30° Be. syrup.

Solutions number 15, 18, 21, and 24.

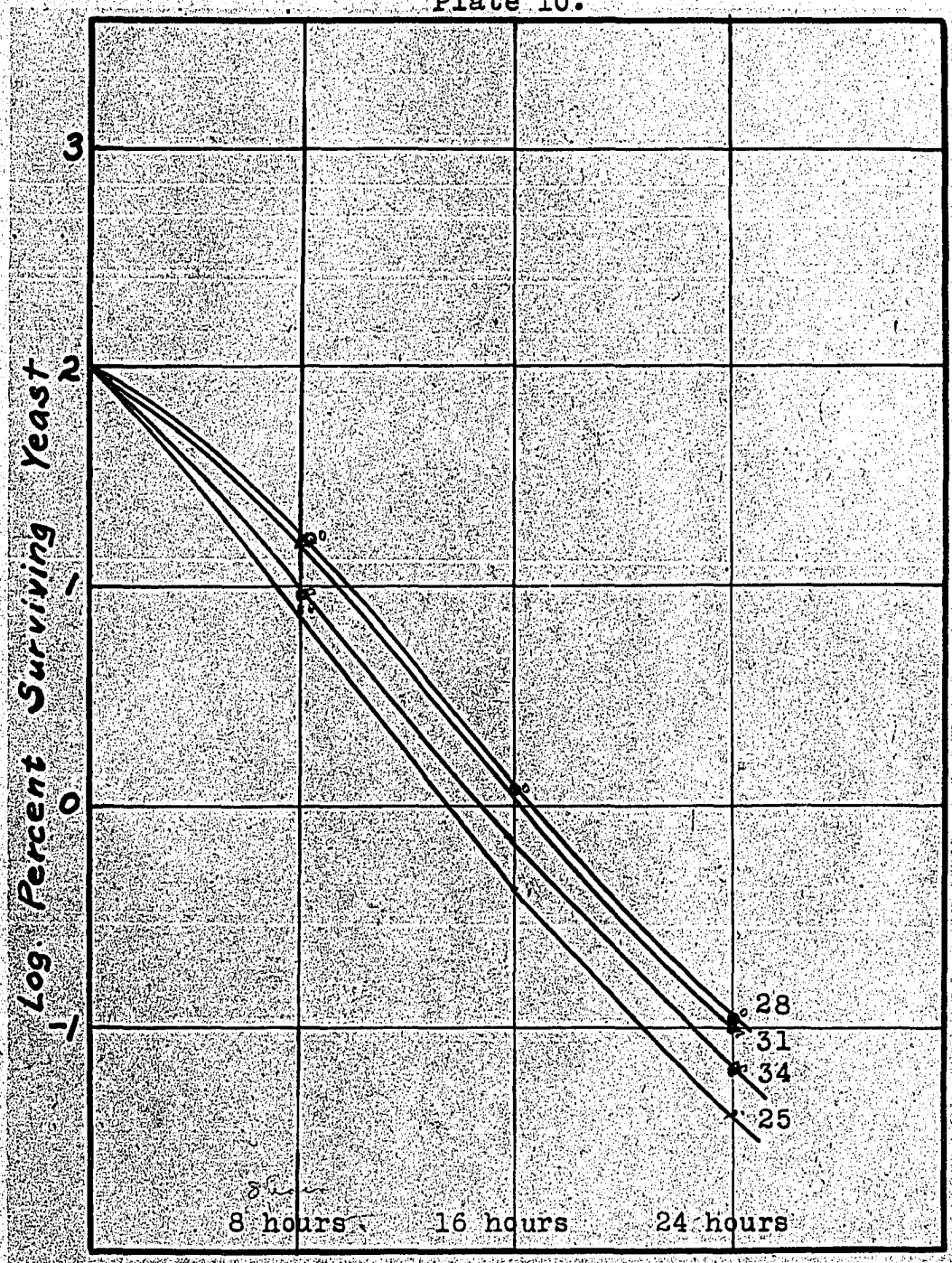
Plate 9.



The effect of increasing acid concentrations on the rate of death of yeast in 36°Baume syrup.

Solutions number 25, 26, and 27.

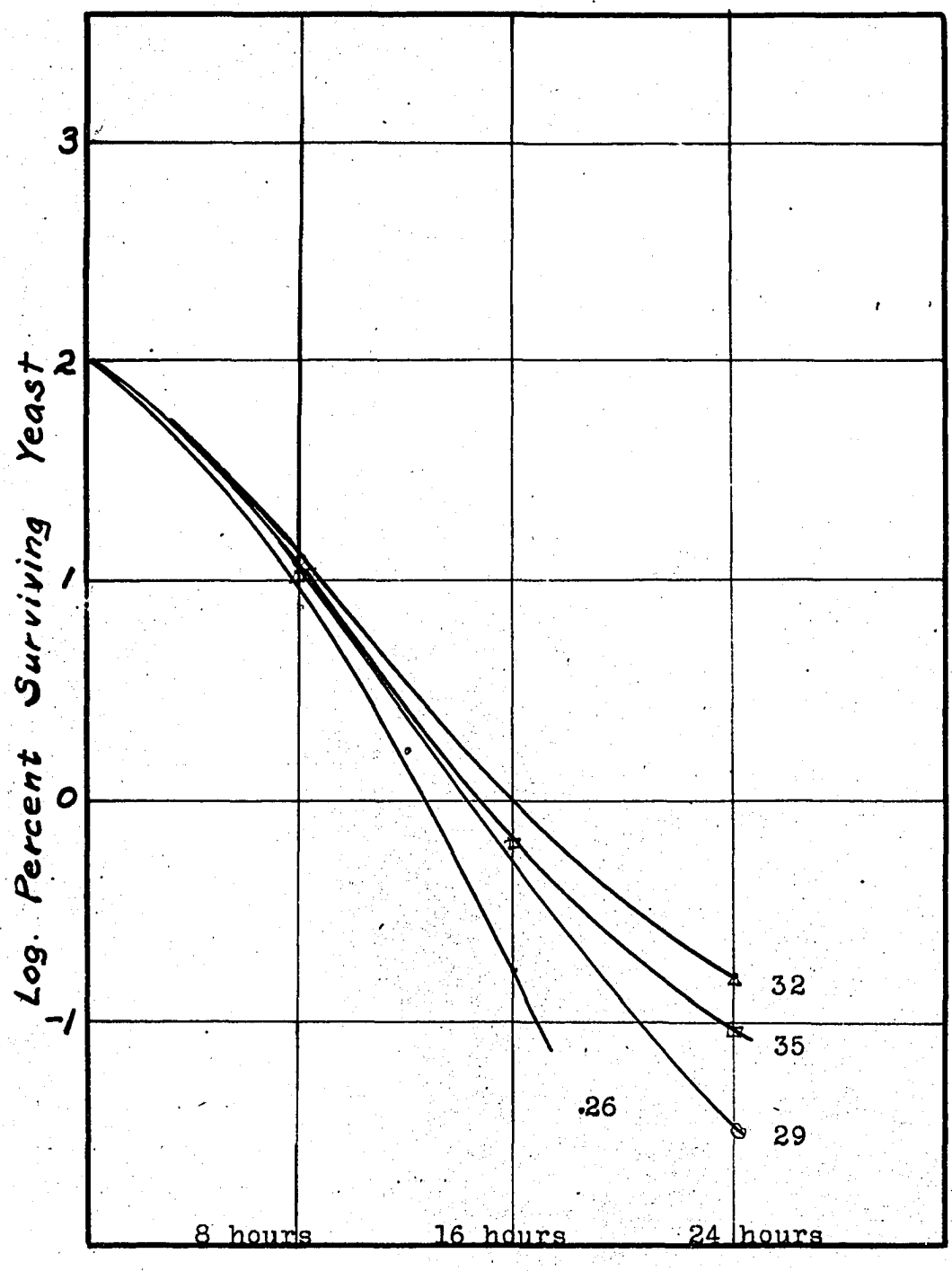
Plate 10.



The effect of added sodium citrate upon 0.02 M citric acid in the killing of yeast in 36° Be. syrup.

Solutions number 25, 28, 31, and 34.

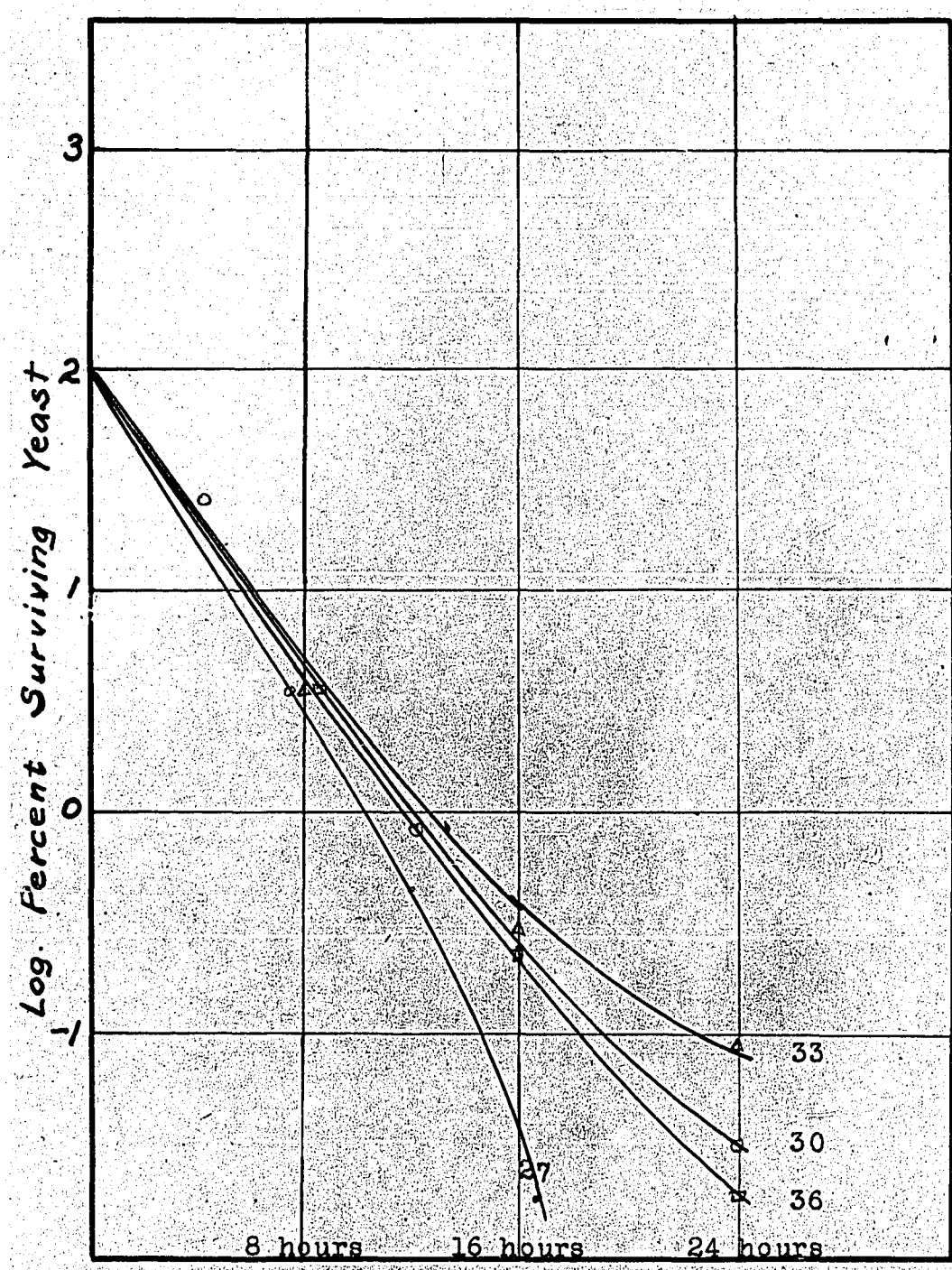
Plate 11.



The effect of added sodium citrate upon 0.04 M citric acid in the killing of yeast in 36°Be. syrup.

Solutions number 26, 29, 32, and 35.

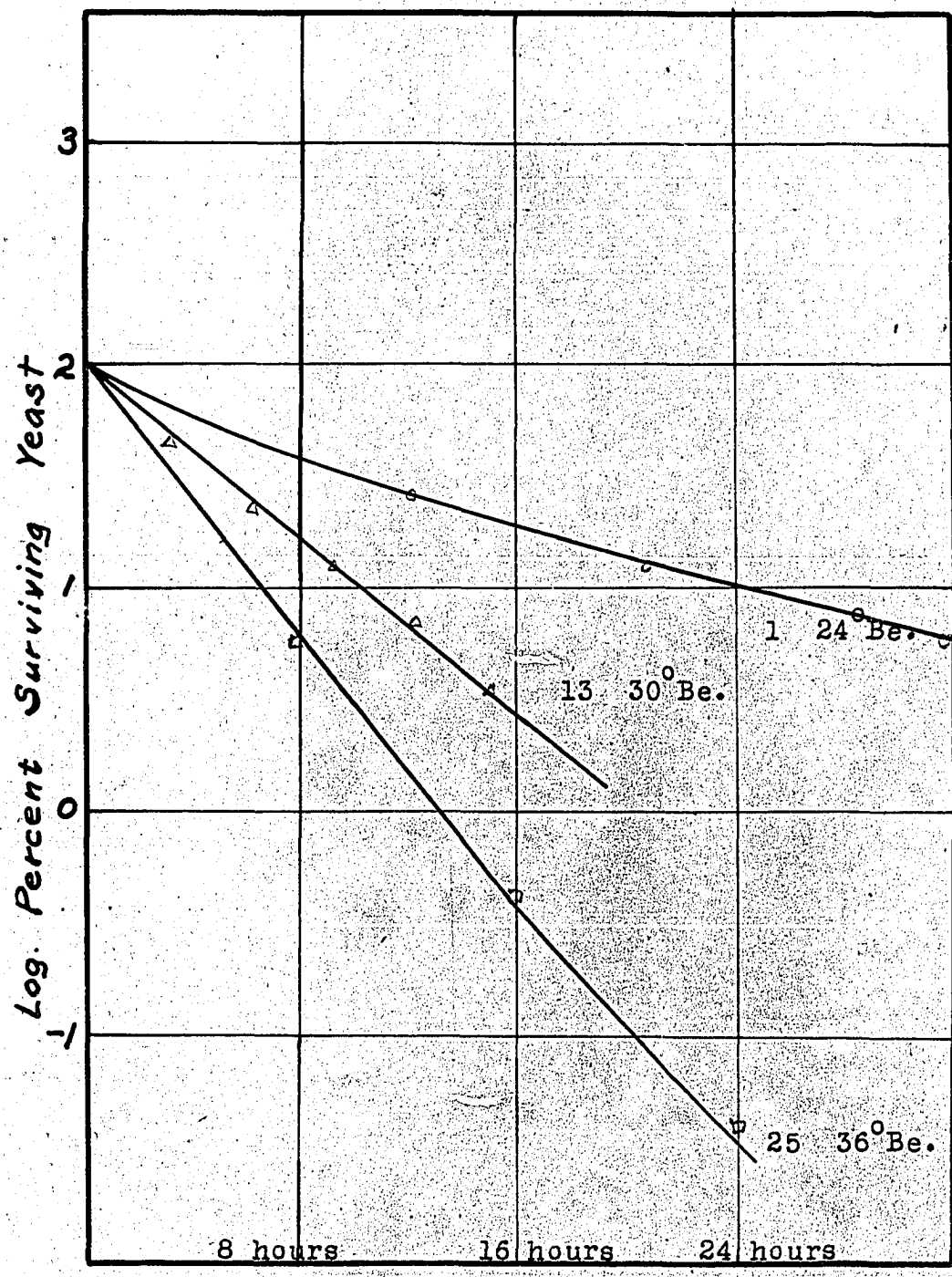
Plate 12.



The effect of added sodium citrate upon 0.08 M citric acid in the killing of yeast in 36° Be. syrup.

Solutions number 27, 30, 33, and 36.

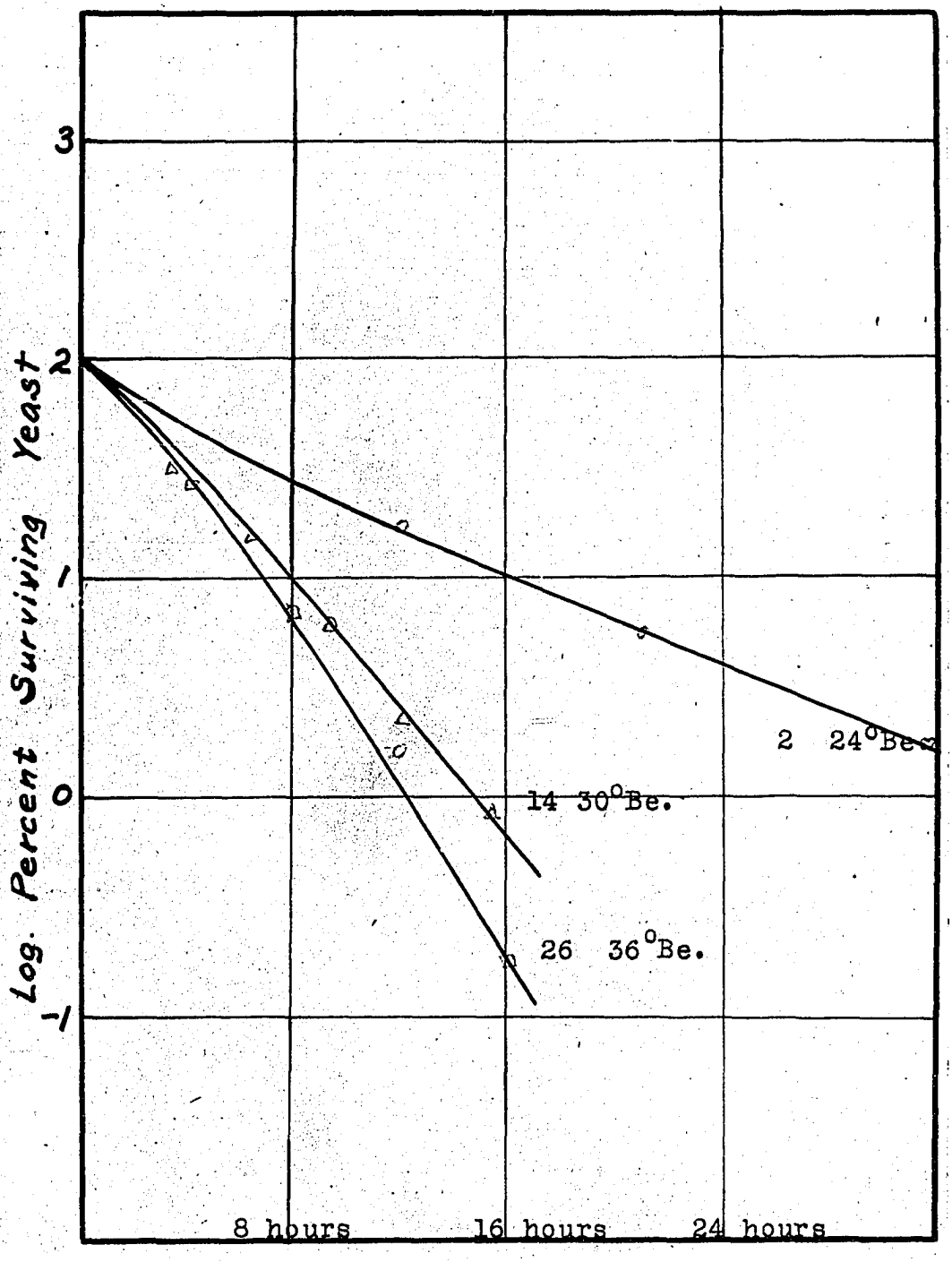
Plate 13,



The effect of increasing syrup concentration on the rate of death of yeast in 0.0à M citric acid.

Solutions number 1, 13, and 25.

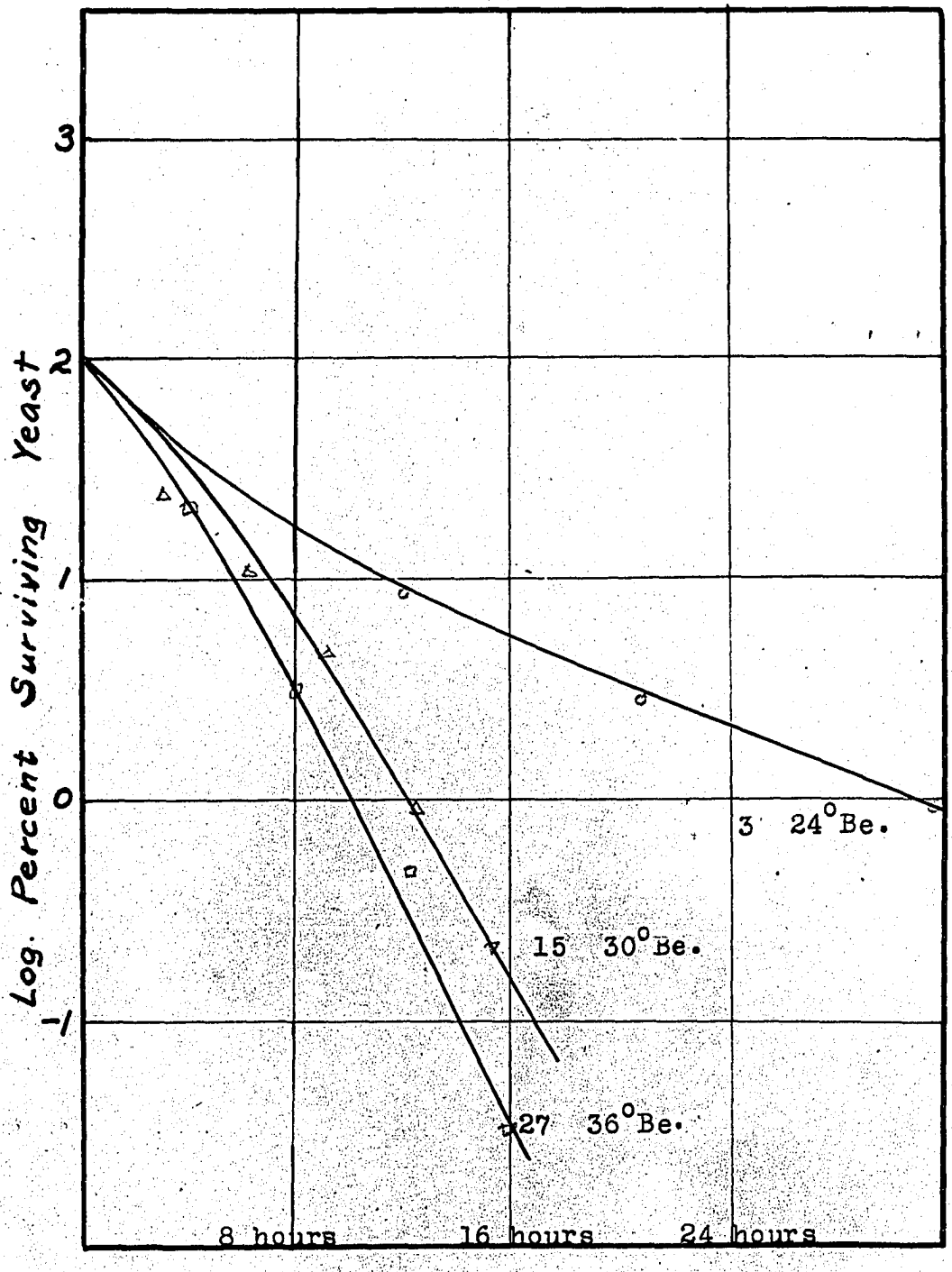
Plate 14.



The effect of increasing syrup concentration on the rate of death of yeast in 0.04 M citric acid.

Solutions number 2, 14, and 26.

Plate 15.



The effect of increasing syrup concentration on the rate of death of yeast in 0.08 M citric acid.

Solutions number 3, 15, and 27.

The next series was with 30° Be. syrup alone. The work of the first series for syrup of this strength was repeated in part, but with the addition of one other acid concentration, several more salt concentrations, and a run for sodium citrate alone in a number concentrations. At the same time controls with simple syrups alone were run.

TABLE XVI

30° Be. Syrup with citric acid of various strengths.

Solution number	37	38	39	40
Molarity of acid	0.01	0.02	0.04	0.08
Original count	63,000	63,000	63,000	63,000
Count after				
8 hours	22,300	10,300	8,000	3,200
16 "	-	3,600	1,600	300
24 "	8,640	1,490	520	35
34 "	1,680	684	137	0
45 "	317	30	0	
Percent survivors				
8 hours	35.41	16.4	12.7	5.08
16 "	-	5.72	2.54	.477
24 "	13.7	2.37	.826	.0556
34 "	2.67	1.09	.218	
45 "	.504	.05		

TABLE XVII

30° Be. Syrup with citric acid and sodium citrate in the molecular ratio 4:1. On this basis there are 2 mols of citric acid to 3 mols of NaH_2Citrate.

Solution number	41	42	43	44
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of salt	0.00250	0.0050	0.01	0.02
Original count	63,000	210,000	210,000	210,000
Count after				
2 days	-	389,000	366,000	148,000
4 "	1,760,000	694,000	492,000	93,800
10 "	2,160,000	757,000	619,000	49,400
16 "	2,820,000	850,000	520,000	33,800
21 "	-	800,000	480,000	24,200
Percent survivors				
2 days	-	185	174	70.4
4 "	2790	330	234	44.5
10 "	3430	360	295	23.5
16 "	4480	404	247	16.1
21 "	-	381	228	11.5

TABLE XVIII

30^{0} Be. Syrup with citric acid and sodium citrate equivalent to NaH_2 Citrate.

Solution number	45	46	47	48
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of salt	0.005	0.01	0.02	0.04
Original count	186,000	186,000	210,000	210,000
Count after				
4 days	2,540,000	1,125,000	1,690,000	1,176,000
10 "	3,507,000	2,440,000	4,740,000	3,980,000
16 "	2,600,000	3,195,000	2,140,000	1,720,000
21 "	3,660,000	2,680,000	1,480,000	480,000
Percent survivors				
4 days	1,360	605	804	560
10 "	1,884	1,312	2,260	1,895
16 "	1,398	1,720	1,020	819
21 "	1,968	1,440	705	221

TABLE XIX

30° Be. Syrup with citric acid and sodium citrate in equal molar quantities.

Solution number	49	50	51	52
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of salt	0.01	0.02	0.04	0.08
Original count	210,000	210,000	186,000	186,000
Count after				
4 days	1,342,000	1,190,000	406,000	250,000
10 "	3,045,000	3,695,000	580,000	192,000
16 "	1,940,000	1,720,000	726,000	48,600
21 "	1,690,000	1,040,000	682,000	23,480
Percent survivors				
4 days	638	563	218	134
10 "	1,450	1,760	319	103
16 "	923	818	390	26.10
21 "	805	495	366	12.6

TABLE XX

30° Be. Syrup with citric acid and sodium citrate equivalent to Na_2H Citrate.

Solution number	53	54	55	56
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of salt	0.02	0.04	0.08	0.16
Original count	186,000	186,000	186,000	186,000
Count after				
1 day	396,000	251,000	-	96,600
2 "	2,760,000	1,742,000	191,000	55,000
6 "	5,115,000	5,310,000	246,000	28,200
10 "	6,060,000	5,370,000	272,000	6,900
16 "	4,070,000	2,960,000	38,200	2,246
21 "	1,180,000	2,900,000	26,000	492
Percent survivors				
1 day	213	135	-	51.8
2 "	1,484	936	102	29.5
6 "	2,745	2,850	132	15.16
10 "	3,357	2,883	146	3.71
16 "	2,188	1,591	20.6	1.21
21 "	634	1,558	13.97	.264

TABLE XXI

30° Be. Syrup with citric acid and sodium citrate in the molar ratio 1:3. On this basis there are 3 mols of Na_2H Citrate to 1 mol of Na_3 Citrate.

Solution number	57	58	59	60
Molarity of acid	0.01	0.02	0.04	0.08
Molarity of salt	0.03	0.06	0.12	0.24
Original count	232,000	63,000	232,000	232,000
Count after				
1 day	417,000	-	-	-
2 "	620,000	-	-	39,600
4 "	839,000	45,000	51,300	-
6 "	1,600,000	32,600	14,500	7,750
10 "	-	17,200	3,120	1,700
16"	2,560,000	4,964	400	129
21 "	3,200,000	453	22	0
Percent survivors				
1 day	179	-	-	-
2 "	267	-	-	16.9
4 "	362	71.4	21.9	-
6 "	690	51.8	6.19	3.31
10 "	-	27.3	1.33	.726
16 "	1,103	7.88	.171	.055
21 "	1,379	.72	.009	-

TABLE XXII

30° Be. Syrup with sodium citrate.

Solution number 61	62	63	64	65	66
Molarity of salt 0.0025	0.010	0.02	0.04	0.08	0.16
Original count 226,000	226,000	226,000	226,000	226,000	226,000
Count after 4 days 2,230,000	1,510,000	1,216,000	410,000	58,000	34,500
Count after 10 days 4,160,000	3,400,000	3,980,000	746,000	14,400	5,860
Count after 16 days 4,300,000	4,446,000	2,860,000	1,500,000	3,641	533
Count after 21 days 1,660,000	4,082,000	2,800,000	1,220,000	260	12
Percent survivors					
After 4 days 987	668	538	181.4	25.7	15.3
After 10 days 1,840	1,506	1,762	330	6.37	2.69
After 16 days 1,904	1,966	1,266	663	1.61	.236
After 21 days 734	1,806	1,239	540	.115	.005

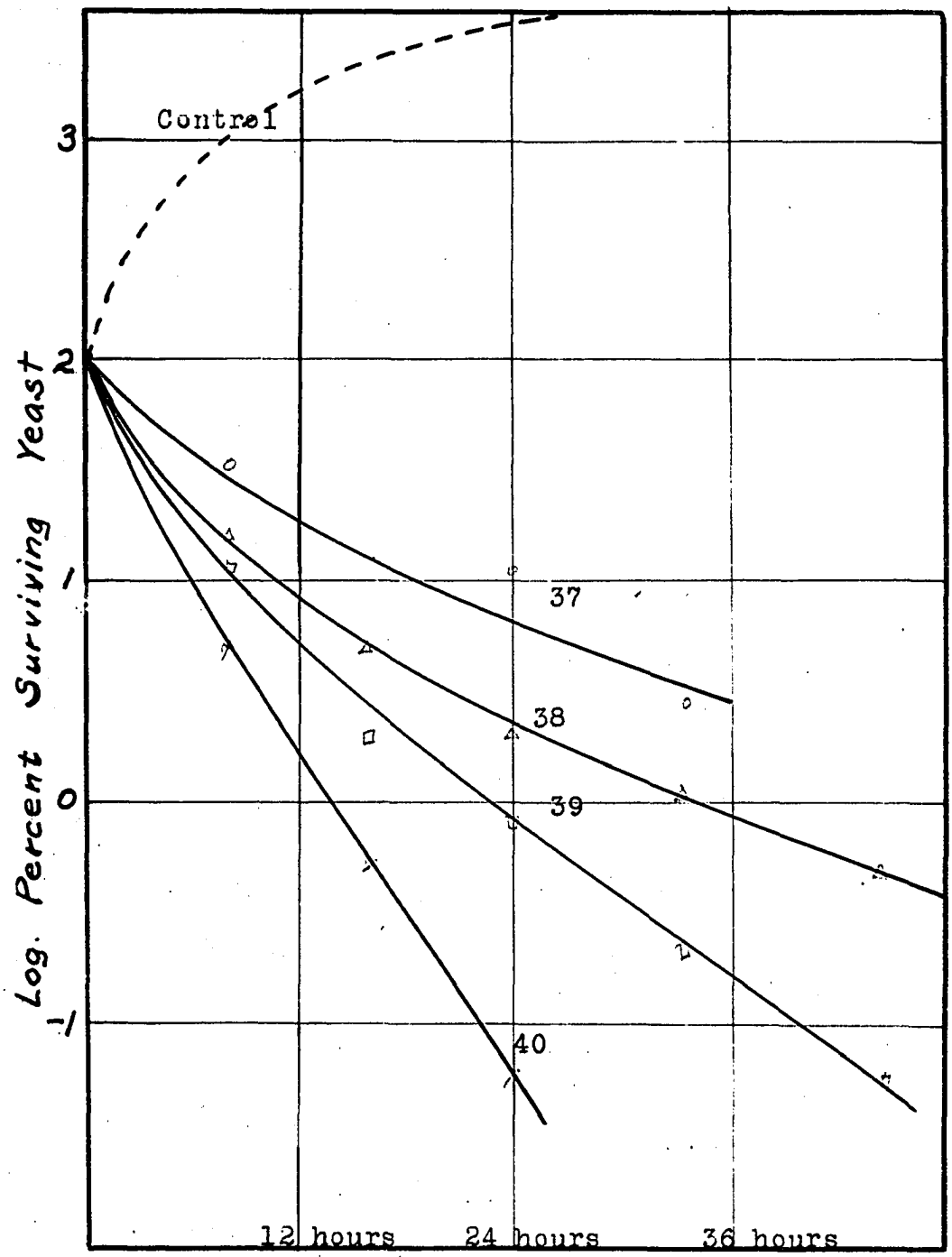
TABLE XXIII

30° Be. Syrup with no added acid or salt.

Solution number	67	68
Original count	210,000	232,000
Count after 2 days	1625,000	2,162,000
4 "	3,560,000	3,235,000
6 "	3,545,000	3,640,000
10 "	5,930,000	-
16 "	-	4,876,000
21 "	4,080,000	4,924,000
Percent survivors		
2 days	772	923
4 "	1,695	1,382
6 "	1,688	1,554
10 "	2,816	-
16 "	-	2,080
21 "	1,944	2,104

The series following was with 30° Be. syrup but different salt combinations. It had been noted that the acid-salt combination giving a theoretical content of NaH_2 Citrate was not antagonistic to yeast life, while citric acid was. It was therefore decided to make up a

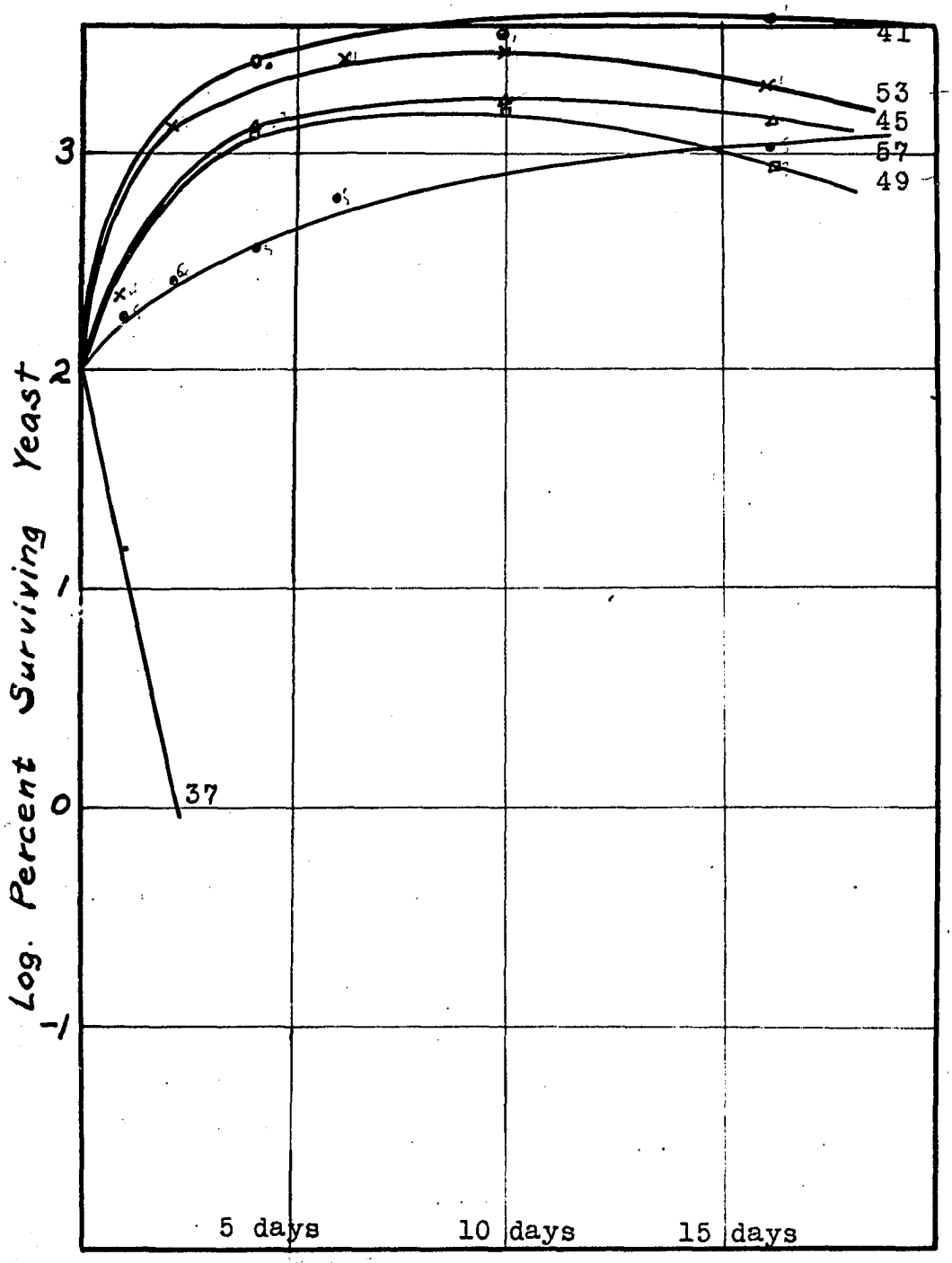
Plate 16.



The influence of increasing acid strength on killing of yeast in 30° Baume syrup.

Solutions number 37, 38, 39, and 40.

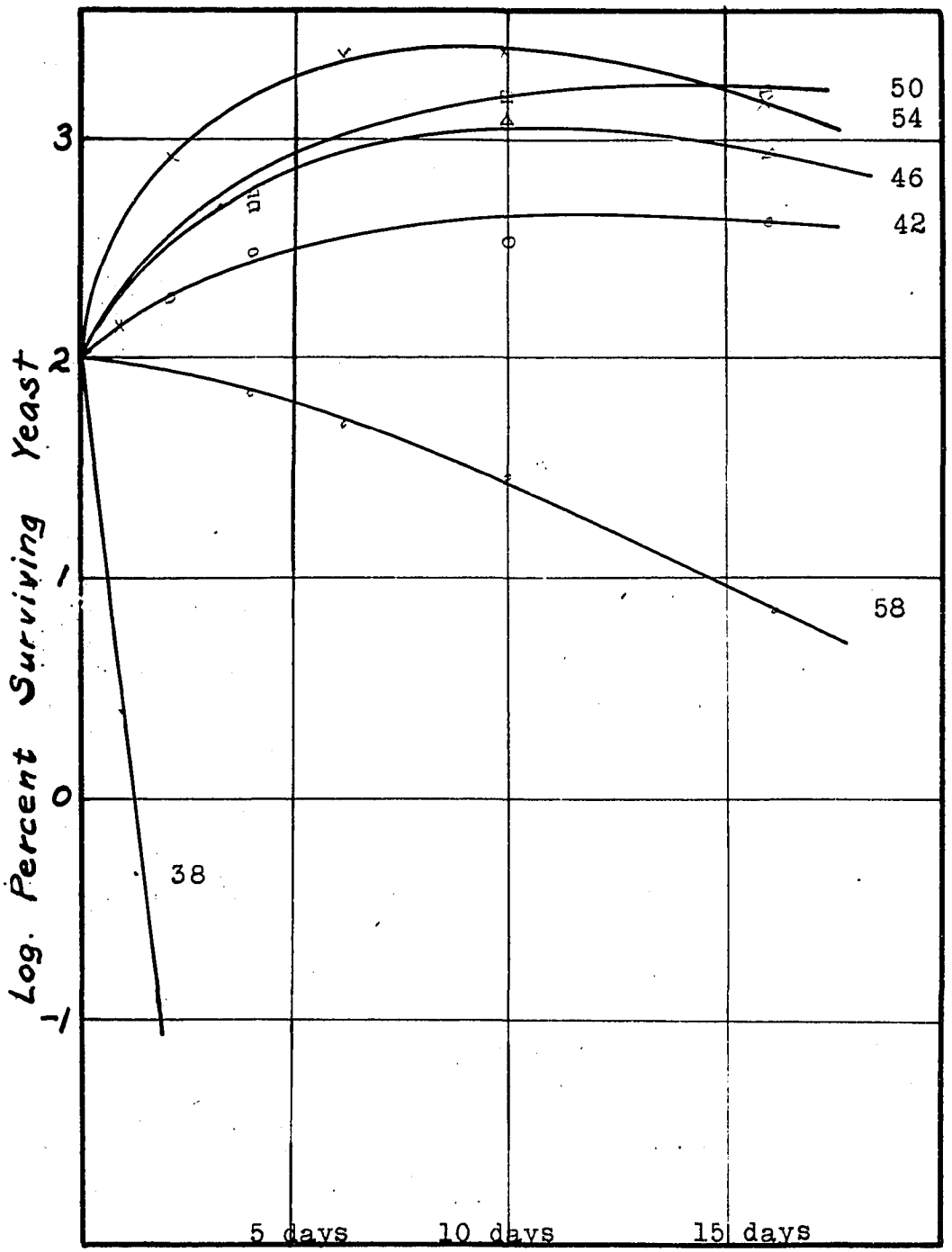
Plate 17.



The effect of added sodium citrate on 0.01 M citric acid in the killing of yeast in 30°Be. syrup.

Solutions number 37, 41,45, 49, 53, and 57.

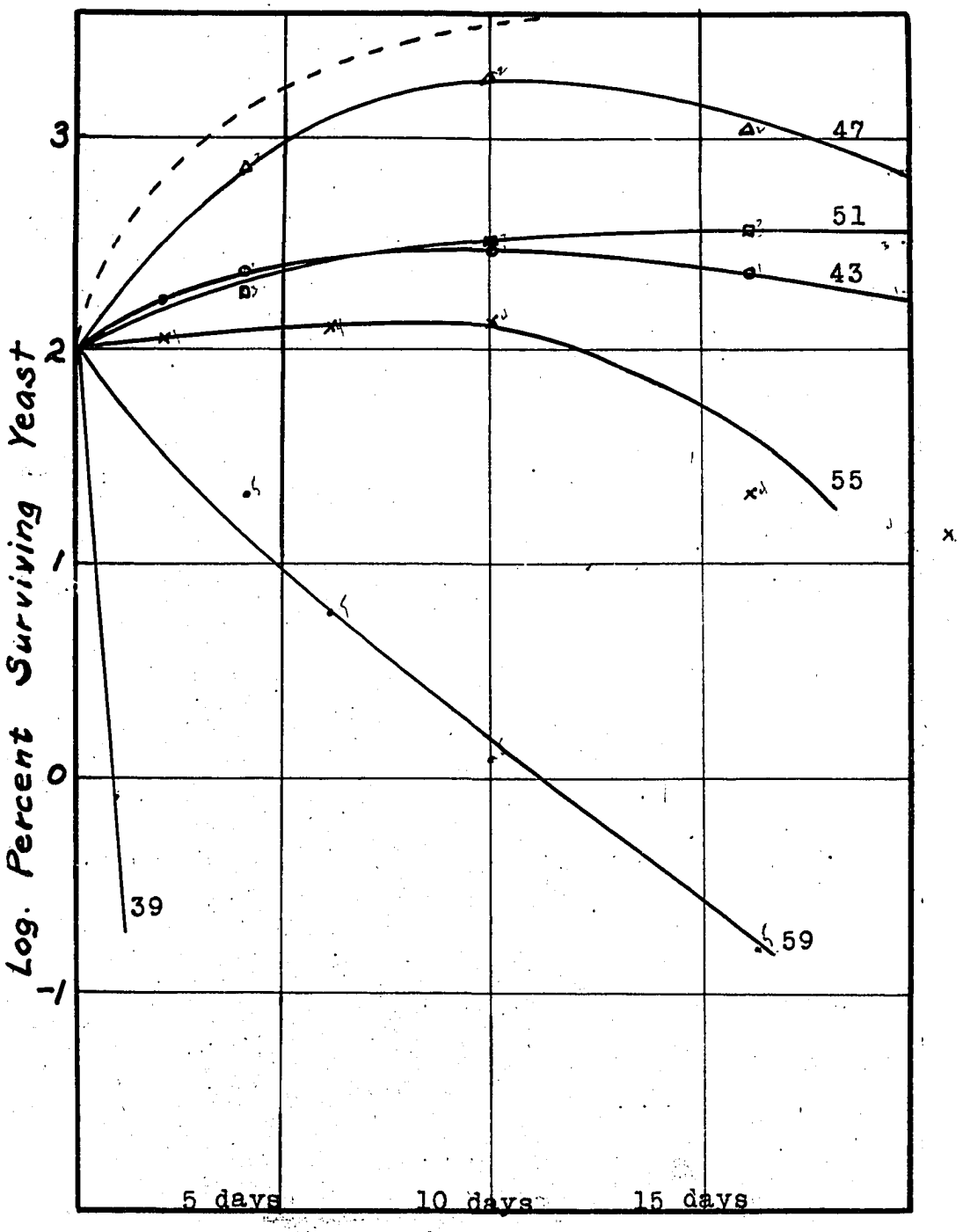
Plate 18.



The effect of added sodium citrate on 0.02 M citric acid in the killing of yeast in 30° Be. syrup.

Solutions number 38, 42, 46, 50, 54, and 58.

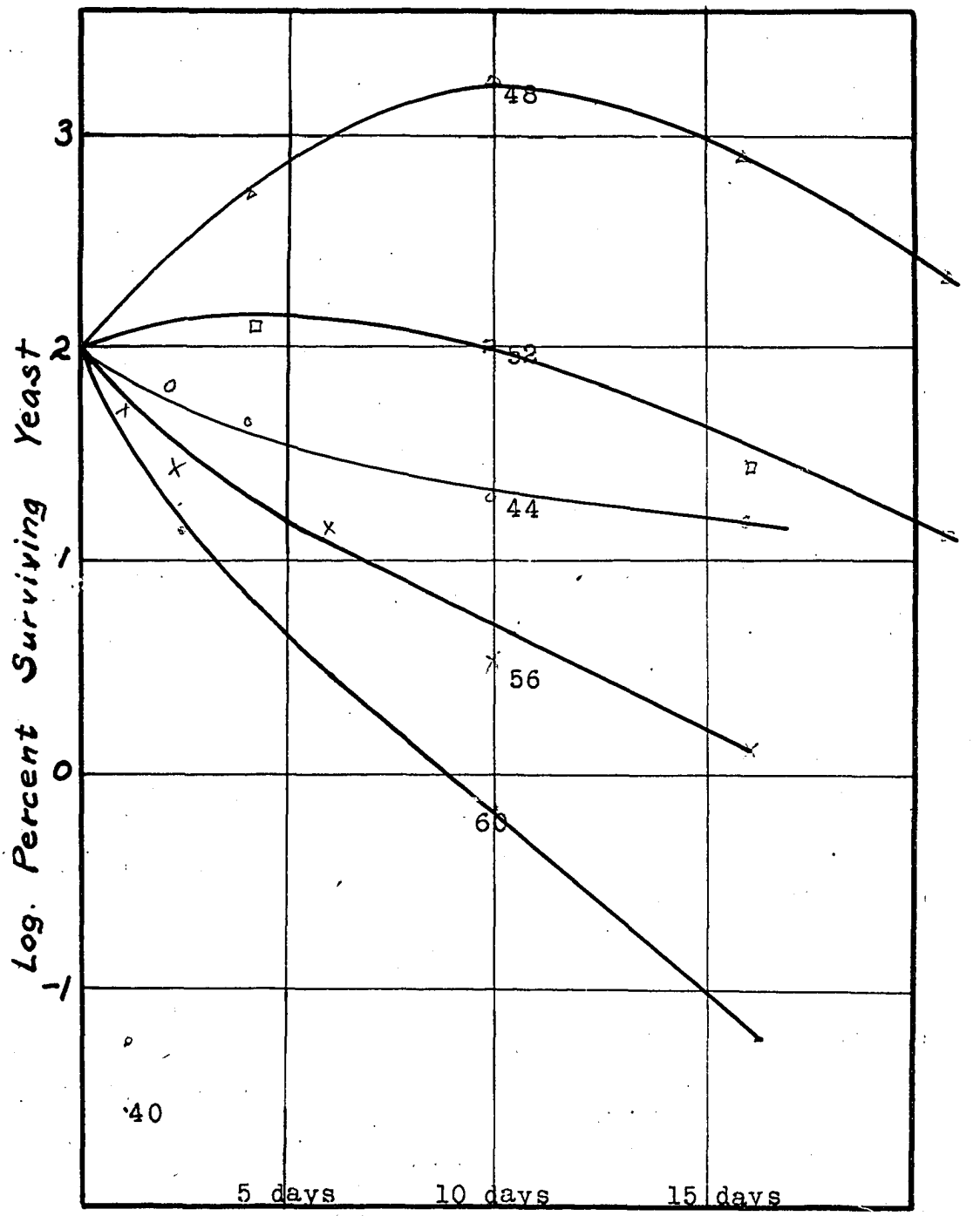
Plate 19.



The effect of added sodium citrate on 0.04 M citric acid in the killing of yeast in 30°Be. syrup.

Solutions number 39, 43, 47, 51, 55, and 59.

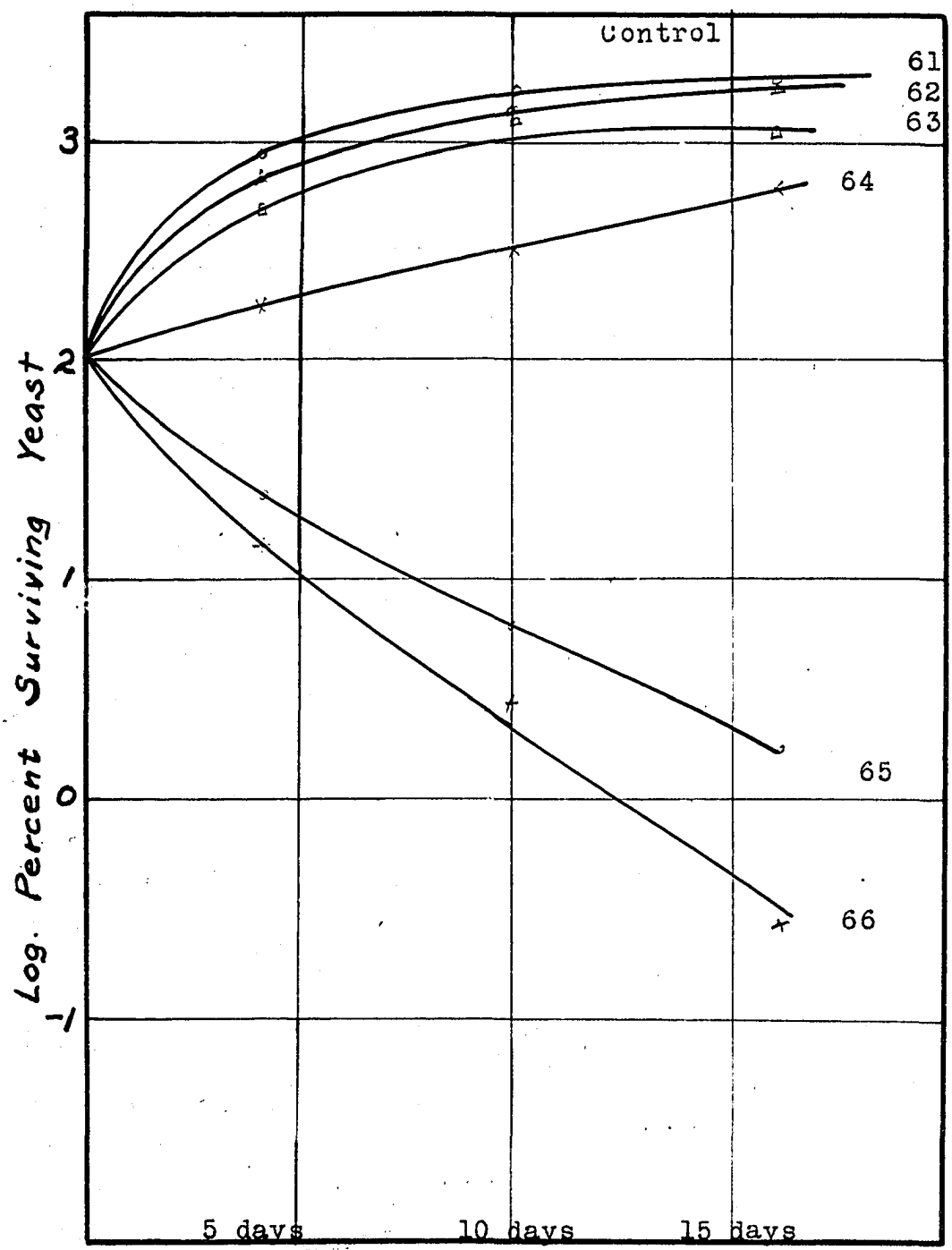
Plate 20.



The effect of added sodium citrate on 0.08 M citric acid in the killing of yeast in 30°Be. syrup.

Solutions number 40, 44, 48, 52, 56, and 60.

Plate 21.



The influence of increasing concentration of sodium citrate in the killing of yeast in 30°Be. syrup.

Solutions number 61, 62, 63, 64, 65, and 66.

series in which NaH_2 Citrate was added to citric acid. This series differed from the proceding in that no acid was used up in making NaH_2 Citrate since the acid salt was added as such. Hence these solutions were stronger in acid than those whose death rates were recorded in Table XVI.

TABLE XXIV

30° Be Syrup, with citric acid.

Solution number	69	70	71
Molarity of acid	0.02	0.04	0.08
Original count	130,000	130,000	130,000
Count after			
4 hours	15,450	8,300	5,500
8 "	4,210	1,200	750
12 "	860	185	120
16 "	235	62	17
20 "	117	3	0
24 "	40	0	
Percent survivors			
4 hours	11.9	6.38	4.23
8 "	3.24	.922	.577
12 "	.662	.142	.092
16 "	.181	.0477	.0131
20 "	.090		
24 "	.0308		

All work up to, and including, Table XXIV was with solutions inoculated with 1c.c. of a 2 day broth culture of yeast. Only a few spores could be found in a culture of this age. A 7 day culture showed a large percentage of spores even in malt extract broth. The procedure is varied from this point on, making comparison between results of seeding with 2 day and 7 day broth cultures of yeast.

TABLE XXV

30°Be. Syrup, seeded from a 7 day broth culture of yeast.

Solution number	72	73	74
Molarity of acid	0.02	0.04	0.08
Original count	322,000	322,000	322,000
Count after 3 hours	318,000	309,000	295,000
" " 6 hours	221,000	153,000	78,800
" " 9 hours	86,700	17,640	4,300
" " 12 hours	14,280	1,950	832
" " 15 hours	2,420	241	160
" " 18 hours	630	60	0
Percent survivors			
3 hours	98.8	96.0	91.6
6 hours	68.6	47.5	24.5
9 hours	26.9	5.48	1.33
12 hours	4.43	.605	.258
15 hours	.752	.0748	.0497
18 hours	.196	.0186	

TABLE XXVI

30° Be. Syrup with citric acid and sodium di-hydrogen citrate in molar ratio 5:1. Inoculated with a two day broth culture of yeast.

Solution number	75	76	77
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.004	0.008	0.016
Original count	130,000	130,000	130,000
Count after			
4 hours	-	-	5,964
8 "	6,430	2,900	1,246
12 "	-	250	120
16 "	622	35	
24 "	140		
Percent survivors			
4 hours	-	-	4.58
8 "	4.95	2.23	.954
12 "	-	.192	.0922
16 "	.478	.0269	
24 "	.0108		

TABLE XXVII

30° Be. Syrup with citric acid and sodium dihydrogen citrate in ratio 5:1. Cultures inoculated with a seven day broth culture of yeast.

Solution number	78	79	80
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.004	0.008	0.016
Original count	306,000	306,000	306,000
Count after			
3 hours	299,700	271,800	253,900
6 "	196,800	116,000	72,400
9 "	68,800	20,800	3,890
12 "	21,200	2,508	853
15 "	4,880	295	173
18 "	1,424	82	
21 "	142		
Percent survivors			
3 hours	97.9	88.5	82.6
6 "	64.2	36.4	23.6
9 "	22.5	6.8	1.27
12 "	6.92	.842	.262
15 "	1.60	.0963	.0565
18 "	.464	.0268	
21 "	.046		

TABLE XXVIII

30° Be. Syrup with citric acid and sodium dihydrogen citrate in ratio 5:2. Inoculated with a seven day broth culture of yeast.

Solution number	81	82	83
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.008	0.016	0.032
Original count	224,000	171,000	224,000
Count after			
3 hours	219,100	160,000	209,400
6 "	163,300	91,800	67,600
9 "	66,600	20,900	9,840
12 "	28,600	5,640	693
15 "	14,840	561	160
18 "	1,680	22	
21 "	196		
24 "	14		
Percent survivors			
3 hours	97.7	93.5	93.2
6 "	72.2	53.7	30.1
9 "	29.7	12.2	4.38
12 "	12.76	3.3	.309
15 "	6.62	.328	.0713
18 "	.75	.013	
21 "	.0874		
24 "	.0062		

TABLE XXIX

30° Be. Syrup with citric acid and sodium dihydrogen citrate in ratio 5:3. Inoculated with seven day broth culture of yeast.

Solution number	84	85	86
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.012	0.024	0.032
Original count	206,000	206,000	206,000
Count after			
3 hours	190,300	179,000	151,400
6 "	106,700	68,500	56,100
9 "	54,400	20,800	18,500
12 "	14,440	7,660	2,808
15 "	5,630	600	260
18 "	1,820	70	
21 "	214	14	
24 "	66		
Percent survivors			
3 hours	92.3	86.8	73.4
6 "	51.7	33.2	27.2
9 "	26.4	10.1	8.97
12 "	6.98	3.71	1.36
15 "	2.76	.291	.126
18 "	.882	.034	
21 "	.104	.0068	
24 "	.032		

TABLE XXX

30° Be. Syrup with citric acid and sodium dihydrogen citrate in ratio 5:4. Inoculated with seven day broth culture of yeast.

Solution number	87	88	89
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.016	0.032	0.0640
Original count	206,000	206,000	206,000
Count after			
3 hours	192,700	171,300	164,000
6 "	84,800	55,200	53,900
9 "	56,800	21,800	13,600
12 "	40,000	9,100	2,360
15 "	12,720	768	360
18 "	3,280	166	
21 "	530	38	
24 "	142		
Percent survivors			
3 hours	93.5	83.0	79.5
6 "	41.2	26.8	26.1
9 "	27.6	10.6	6.6
12 "	19.4	4.42	1.144
15 "	6.17	.373	.175
18 "	1.59	.0805	
21 "	.257	.0184	
24 "	.069		

TABLE XXXI

30° Be. Syrup with citric acid and sodium dihydrogen citrate in equal molar quantities. Inoculated with a seven day broth culture of yeast.

Solution number	90	91	92
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.02	0.04	0.08
Original count	192,000	192,000	192,000
Count after			
3 hours	181,500	163,500	156,500
6 "	73,200	46,200	42,100
9 "	50,400	24,800	26,300
12 "	15,840	9,600	7,120
15 "	2,704	1,364	488
18 "	624	196	
21 "	113	68	
24 "	34		
Percent survivors			
3 hours	94.2	85.2	81.5
6 "	38.1	24.1	21.9
9 "	26.2	12.9	13.7
12 "	8.25	5.00	3.71
15 "	1.41	.71	.254
18 "	.325	.101	
21 "	.0593	.0354	
24 "	.0177		

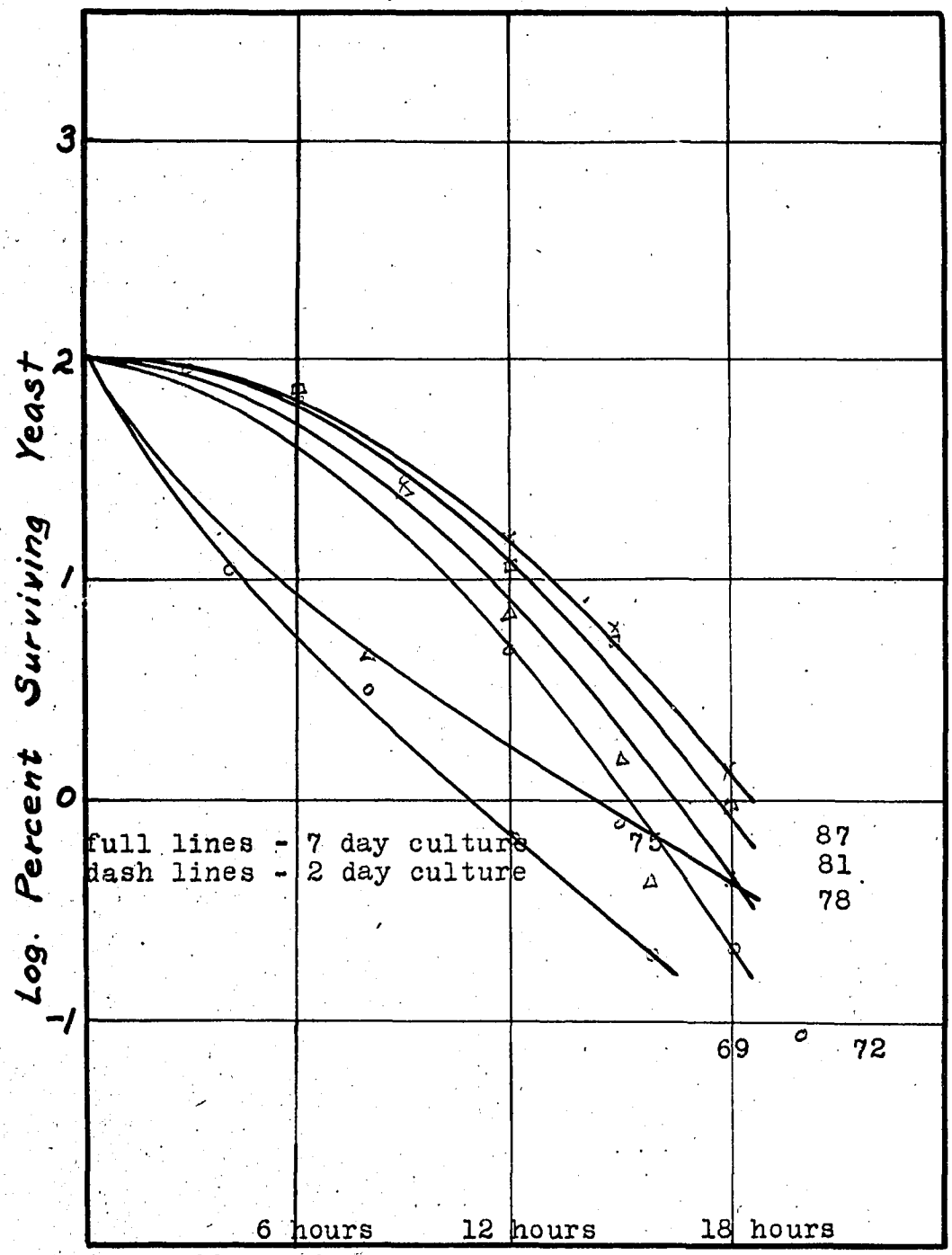
TABLE XXXII

30° Be. Syrup with no acid or salt.

Inoculated with seven day broth culture of yeast.

Solution number	93	94
Original count	393,000	232,000
Count after		
1 hour	385,000	254,000
6 "	694,000	485,000
12 "	630,000	550,000
18 "	830,000	640,000
24 "	1,340,000	685,000
percent survivors		
1 hour	98	109.4
6 "	177	209
12 "	160	237
18 "	211	276
24 "	341	295

Plate 22.

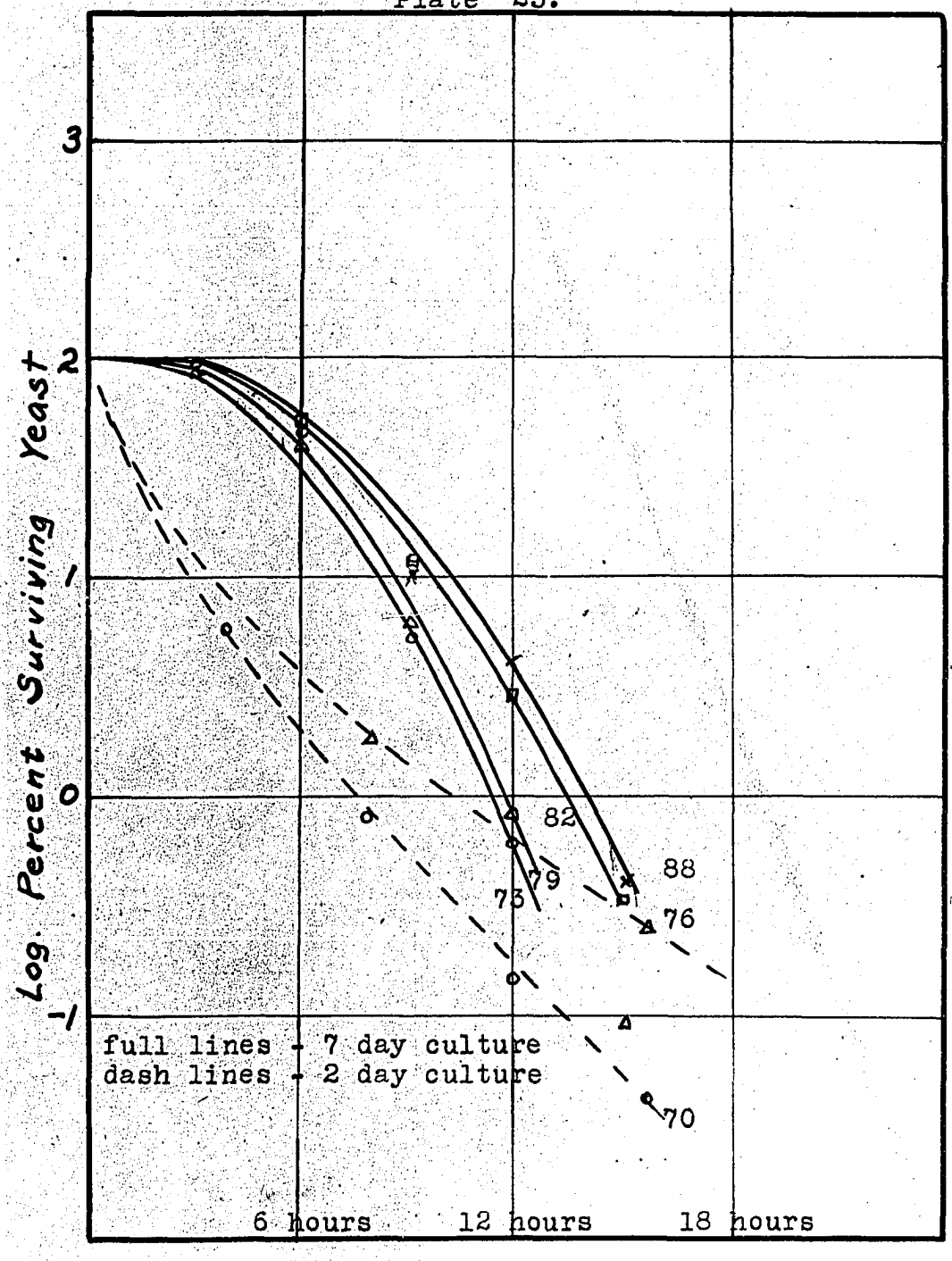


The effect of age of seeding culture on death rate.

The effect of added sodium di-hydrogen citrate on 0.02 M citric acid in the killing of yeast in 30° Be. syrup.

Solutions number 69, 72, 75, 81, and 87.

Plate 23.

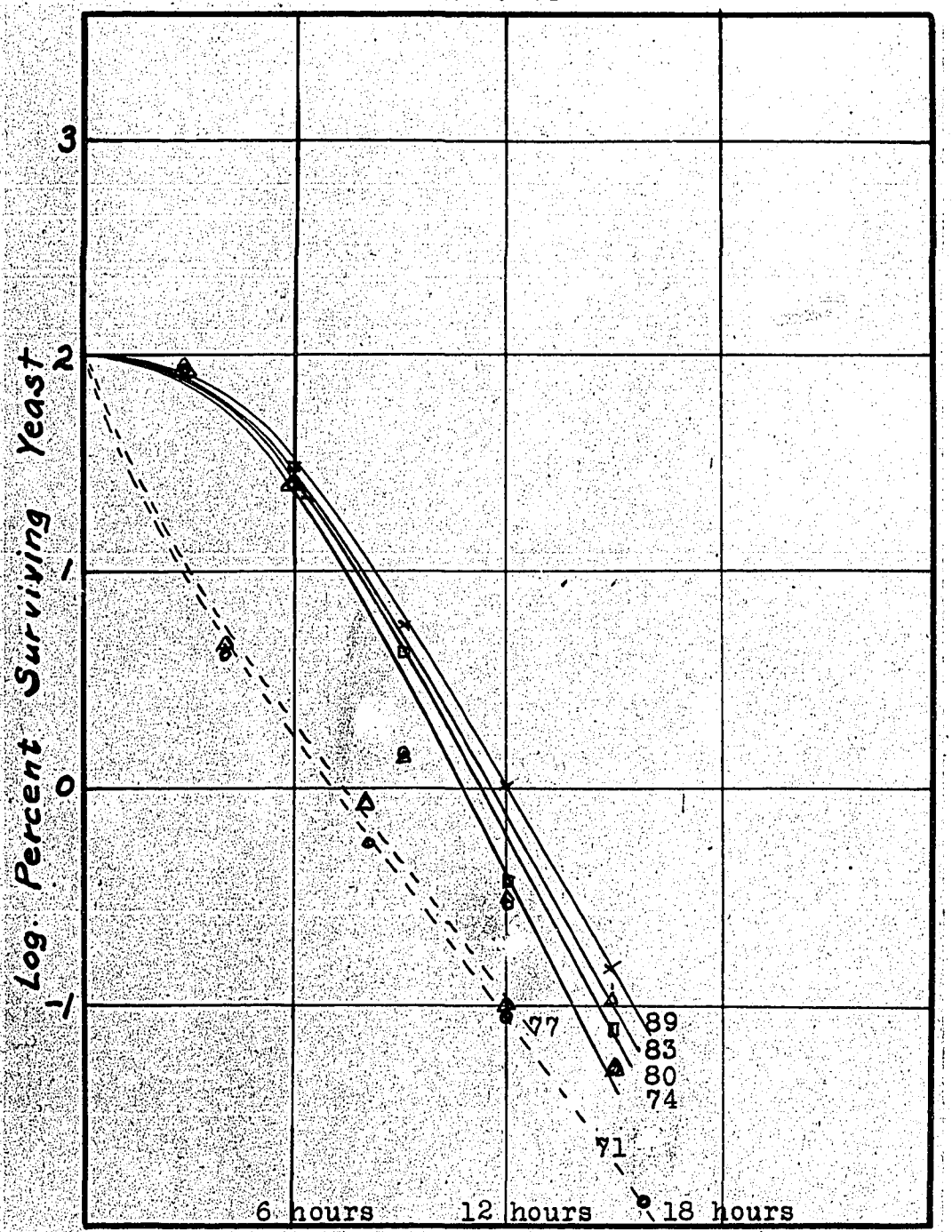


The effect of age of seeding culture on death rate.

The effect of added sodium di-hydrogen citrate on 0.04 M citric acid in the killing of yeast in 30°Be. syrup.

Solutions number 70, 73, 76, 79, 82, and 88.

Plate 24



The effect of age of seeding culture on death rate.

The effect of added sodium di-hydrogen citrate on 0.08 M citric acid in the killing of yeast in 30°Be. syrup.

Solutions number 71, 74, 77, 80, 83, and 89.

This same procedure was followed with 27° Be. syrup, with results which are given in tables following those for the 30° Be. syrup.

TABLE XXXIII

27° Be. Syrup with citric acid, inoculated with a seven day broth culture of yeast.

Solution number	95	96	97
Molarity of acid	0.02	0.04	0.08
Original count	292,000	292,000	292,000
Count after 6 hours	219,000	176,000	128,000
10 "	-	25,800	
12 "	26,300	-	
13 "	-	9,150	6,240
16 "	-	1,159	608
18 "	3,750	578	97
25 "	820		
30 "	137		
Percent survivors			
6 hours	64.	60.2	43.8
10 "	-	8.82	
12 "	9.0	-	-
13 "	-	3.13	2.14
16 "	-	.397	.208
18 "	1.28	.198	.033
25 "	.281		
30 "	.0469		

TABLE XXXIV

27° Be. Syrup with citric acid and sodium dihydrogen citrate in molar ratio 5:1. Inoculated with seven day broth culture of yeast.

Solution number	98	99	100
Molarity of acid	0.02	0.04	0.08
molarity of salt	0.004	0.008	0.016
Original count	292,000	292,000	292,000
Count after 6 hours	222,000	181,000	175,000
10 "	-	40,400	47,600
12 "	27,200	-	-
13 "	-	13,925	10,831
16 "	-	1,665	993
18 "	4,170	616	152
25 "	708		
30 "	158		
Percent survivors			
6 hours	76.0	62.0	60.0
10 "	-	13.8	16.3
12 "	9.3	-	-
13 "	-	4.76	3.71
16 "	-	.57	.339
18 "	1.43	.211	.052
25 "	.242		
30 "	.054		

TABLE XXXV

27° Be. syrup with citric acid and sodium di-hydrogen citrate in molar ratio 5:2. Inoculated with seven day broth culture.

Solution number	101	102	103
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.008	0.016	0.032
Original count	292,000	292,000	292,000
Count after 6 hours	230,000	187,400	148,000
10 "	-	69,500	55,600
12 "	36,000	-	-
13 "	-	12,266	13,292
16 "	-	1,656	1,697
18 "	4,560	730	165
25 "	920		
30 "	220		
Percent survivors			
6 hours	78.7	63.6	50.6
10 "	-	23.8	19.0
12 "	12.3	-	-
13 "	-	4.19	4.55
16 "	-	.567	.58
18 "	1.56	.25	.0564
25 "	.314		
30 "	.075		

TABLE XXXVI

27° Be. Syrup with citric acid and sodium dihydrogen citrate in molar ratio 5:4. Inoculated with seven day broth culture of yeast.

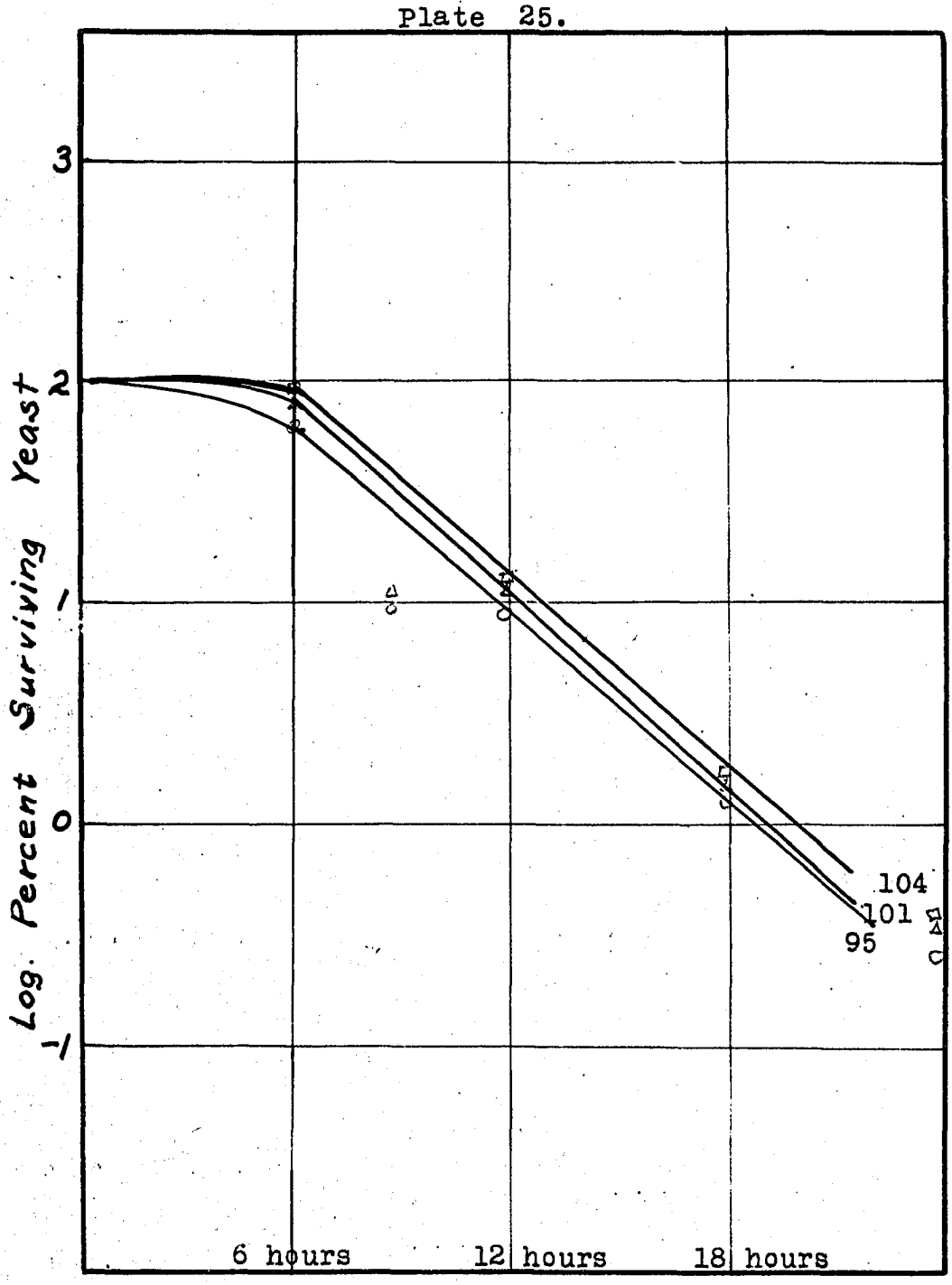
Solution number	104	105	106
Molarity of acid	0.02	0.04	0.08
Molarity of salt	0.016	0.032	0.064
Original count	292,000	292,000	292,000
Count after 6 hours	268,000	255,000	210,000
10 "	-	110,000	54,200
12 "	37,000	-	-
13 "	-	18,500	13,962
16 "	-	2,247	1,652
18 "	4,850	868	161
25 "	1,208		
30 "	197		
Percent survivors			
6 hours	91.7	87.3	72.0
10 "	-	37.7	18.6
12 "	12.7	-	-
13 "	-	6.33	4.78
16 "	-	.77	.567
18 "	1.61	.298	.055
25 "	.412		
30 "	.0675		

TABLE XXXVII

27° Be. Syrup without the addition of acid or salt. Inoculated with seven day broth culture of yeast.

Solution number	107	108
Original count	88,000	75,000
Count after 6 hours	105,000	147,000
12 "	138,000	235,000
25 "	180,000	280,000
48 "	672,000	598,000
Percent survivors		
6 hours	119.4	196
12 "	157.0	313.2
25 "	204.8	373
48 "	764.	798

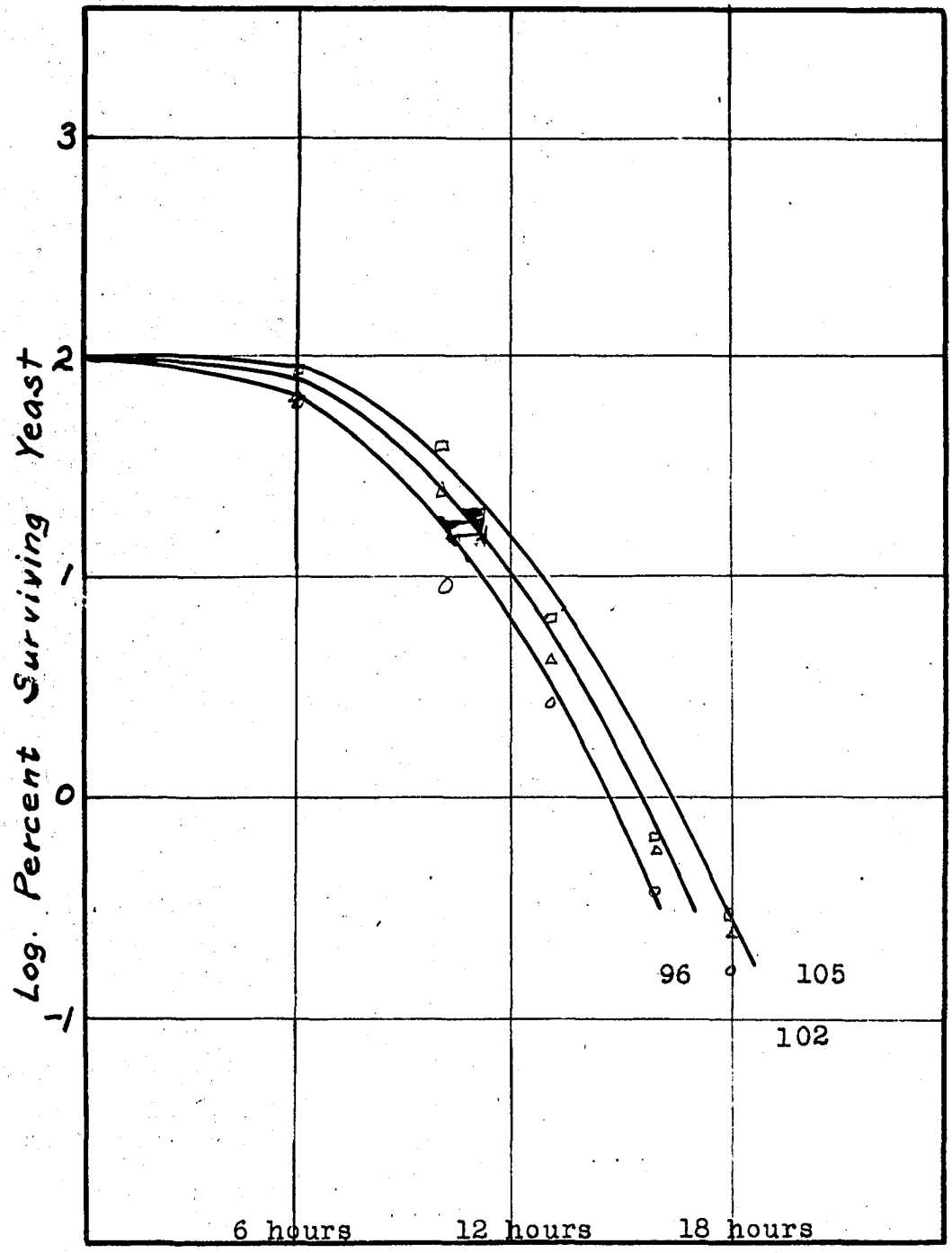
Plate 25.



The effect of added sodium di-hydrogen citrate on 0.02 M citric acid in the killing of yeast in 27° Be. syrup.

Solutions number 95, 101, and 104.

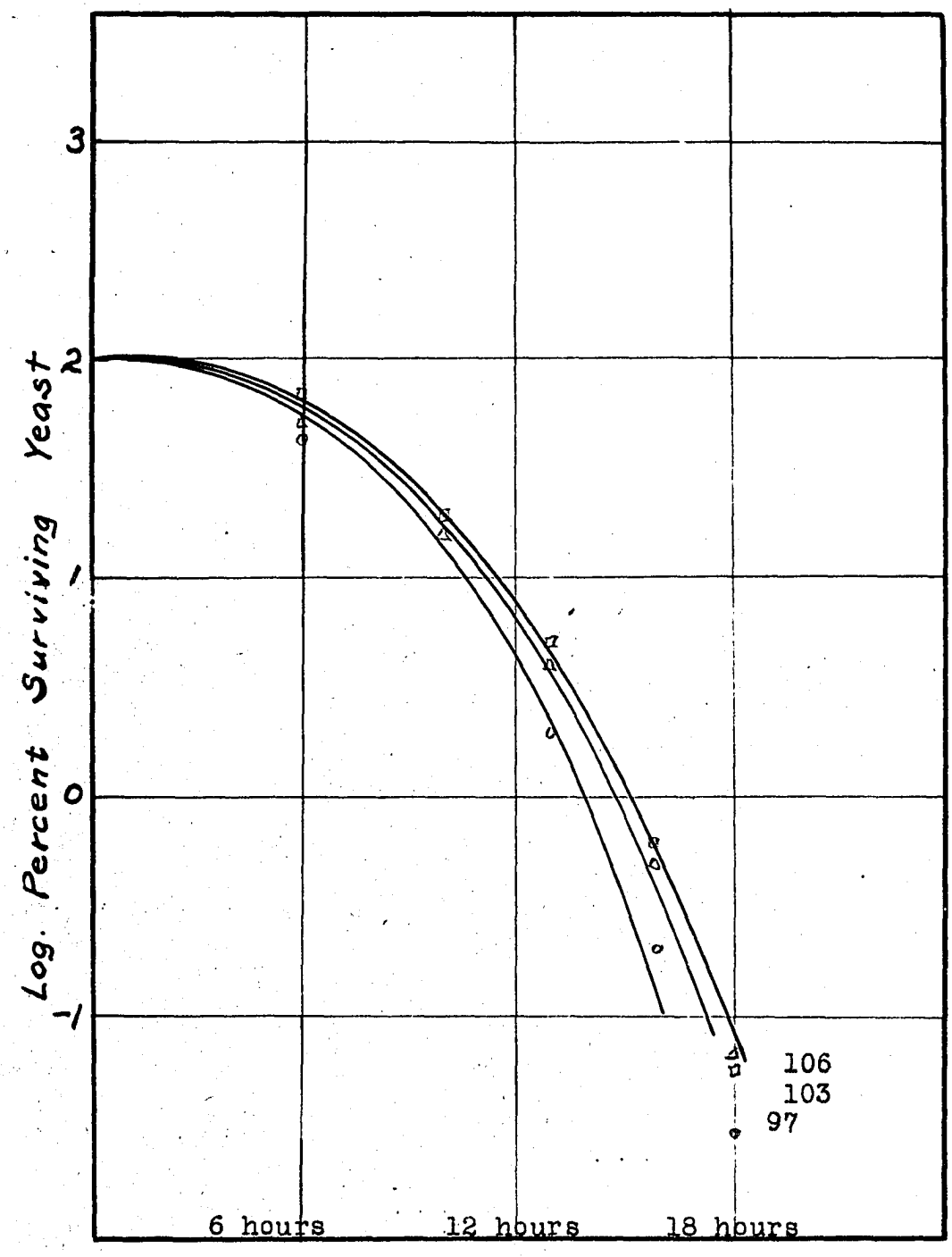
Plate 26.



The effect of added sodium di-hydrogen citrate on 0.04 M citric acid in the killing of yeast in 27° Be. syrup.

Solutions number 96, 102, and 105.

Plate 27.



The effect of added sodium di-hydrogen citrate on 0.08 M citric acid in the killing of yeast in 27°Be. syrup.

Solutions number 97, 103, and 106.

A few miscellaneous runs were made, with the object of comparing the action in syrups containing larger amounts of sodium di-drogen citrate than those solutions already dealt with. The sugar used in these runs was heavily inoculated with the sediment of bottom yeast, quickly dried, ground to pass thru a 20 mesh sieve, and well mixed before using.

The syrups from the two tables which follow were diluted to a uniform sugar content of 12 percent, and determinations made as to taste, pH, and amount of inversion of the sucrose. The pH of the original solution also was determined.

Taste was found to vary. In cases where high acid content was used, and where high salt content as well as high acid was used, there was little difference between the two corresponding solutions. Where the acid and salt contents were low, a great difference in taste was shown. In almost all cases the added salt gave an astringency not found in the case of acid alone. The effect could be best described as more nearly approaching the sourness of natural fruits. With acid alone there was a bland, but penetrating, acidity that was hardly palatable in the absence of flavor.

The values for pH, etc. for these solutions appear in table XL.

TABLE XXXVIII

24° Baume syrup, with added citric acid and sodium di-hydrogen citrate. Made with inoculated sugar, and used without sterilizing.

Solution number	109	110	111	112
Molarity of acid	0.02	0.02	0.04	0.04
Molarity of salt	0.0	0.04	0.0	0.08
Original count	8862	9464	7648	7720
Count after 9 hours	2400	6200	1120	4000
18 "	400	1260	-	-
28 "	130	810	70	300
36 "	70	482	58	140
46 "	44	-	15	-
60 "	8	142		37
Percent survivors				
9 hours	28.2	65.6	14.7	51.8
18 "	4.52	13.3	-	-
28 "	1.47	8.56	.915	2.88
36 "	.79	5.1	.76	1.81
46 "	.496	-	.196	-
60 "	.09	1.5		.48

TABLE XXXIX

30° Be. syrup, with added citric acid and sodium di-hydrogen citrate. Source of yeast same as in table XXXVIII.

Solution number					
113	114	115	116	117	118
Molarity of acid					
0.02	0.02	0.04	0.04	0.08	0.08
Molarity of salt					
0.0	0.04	0.0	0.08	0.0	0.16
Original count					
7300	7820	8820	7600	7500	7200
Count after 20 hours					
200	1400	200	960	136	622
Count after 32 hours					
18	111	7	61	4	32
Count after 44 hours					
	50		8		
Count after 56 hours					
	19				
Percent survivors					
After 20 hours					
3.56	17.9	2.27	12.64	1.81	8.63
After 32 hours					
.246	1.42	.08	.80	.053	.445
After 44 hours					
-	.64		.105		
After 56 hours					
	.283				

TABLE XL

Inversion and electrometric data on syrups made with citric acid and sodium di-hydrogen citrate. Inversion after 10 minutes at 100°. Diluted values at 12% sugar.

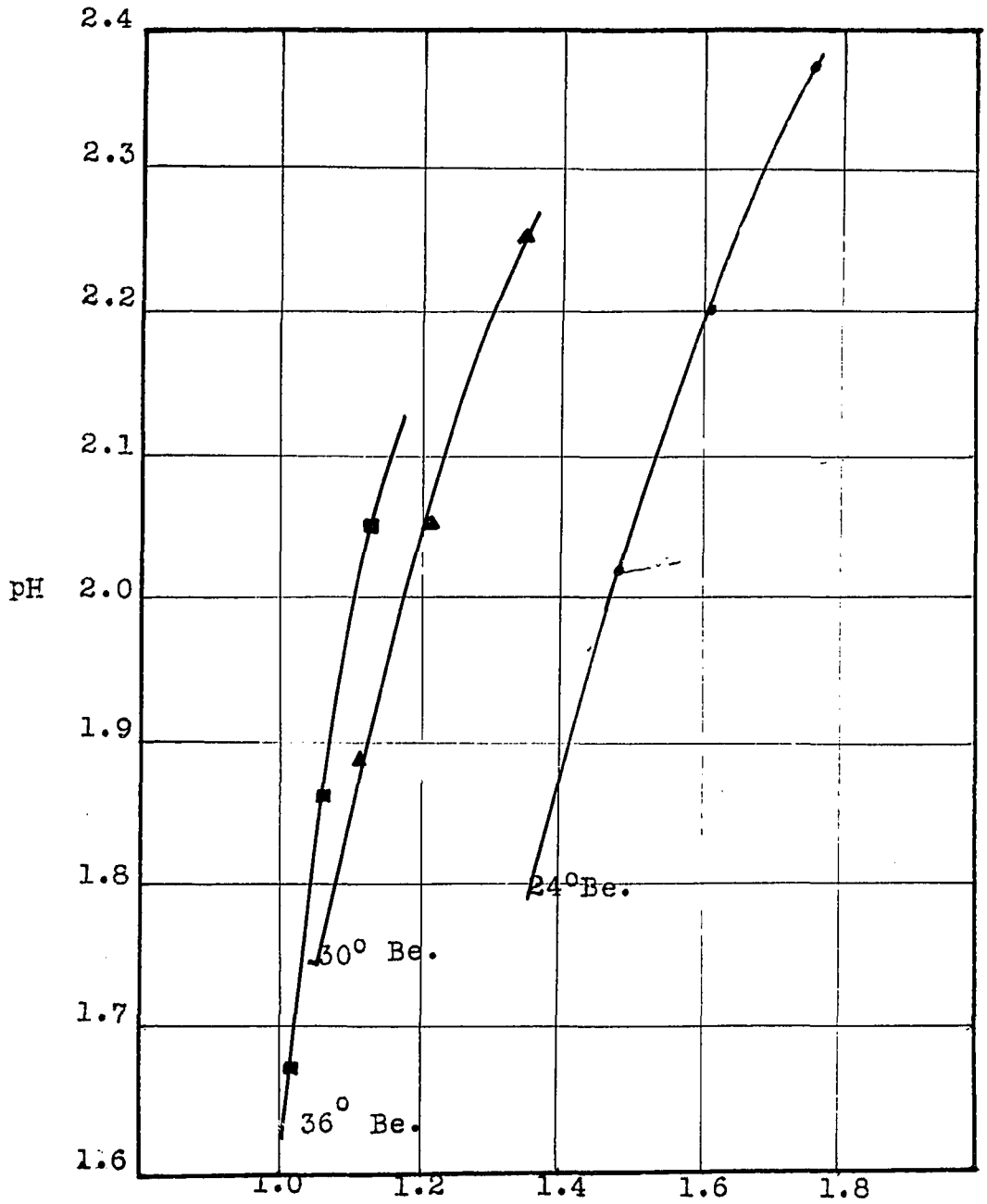
Syrup Baume	Molarity of acid	Molarity of salt	Percent inversion	Syrup pH after inversion	pH after dilution
24	0.02	0.0	94.43	2.32	2.66
	0.02	0.04	73.5	3.22	3.25
	0.04	0.0	97.42	2.28	2.58
	0.04	0.08	88.48	3.10	3.19
27	0.02	0.0	96.09	2.42	2.73
	0.02	0.02	74.12	3.27	3.29
	0.04	0.0	97.42	2.34	2.60
	0.04	0.04	82.84	3.14	3.20
	0.08	0.0	98.41	2.20	2.32
	0.08	0.08	92.45	3.04	3.07
30	0.02	0.0	97.08	2.53	2.76
	0.02	0.04	61.0	3.28	3.34
	0.04	0.0	97.75	2.46	2.62
	0.04	0.08	73.5	3.17	3.22
	0.08	0.0	98.74	2.28	2.37
	0.08	0.16	83.5	3.08	3.14
33	0.04	0.0	97.42	2.56	2.64
	0.04	0.08	68.9	3.23	3.25
	0.08	0.0	97.75	2.35	2.42
	0.08	0.16	79.5	3.10	3.17

TABLE XLI

Relation of killing time for 99% of initial yeast to density of syrup, citric acid content, pH, and conductivity.

Syrup degrees Baume	Acid concentration molar	Killing time hours	pH	Equivalent conductance
24°	0.02	58	2.37	13.34
	0.04	41	2.20	10.25
	0.08	31	2.02	8.22
30°	0.02	22.4	2.25	4.93
	0.04	16.4	2.05	3.90
	0.08	12.8	1.88	3.32
36°	0.02	13.6	2.05	1.158
	0.04	11.6	1.86	.947
	0.08	10.2	1.67	.912

Plate 28



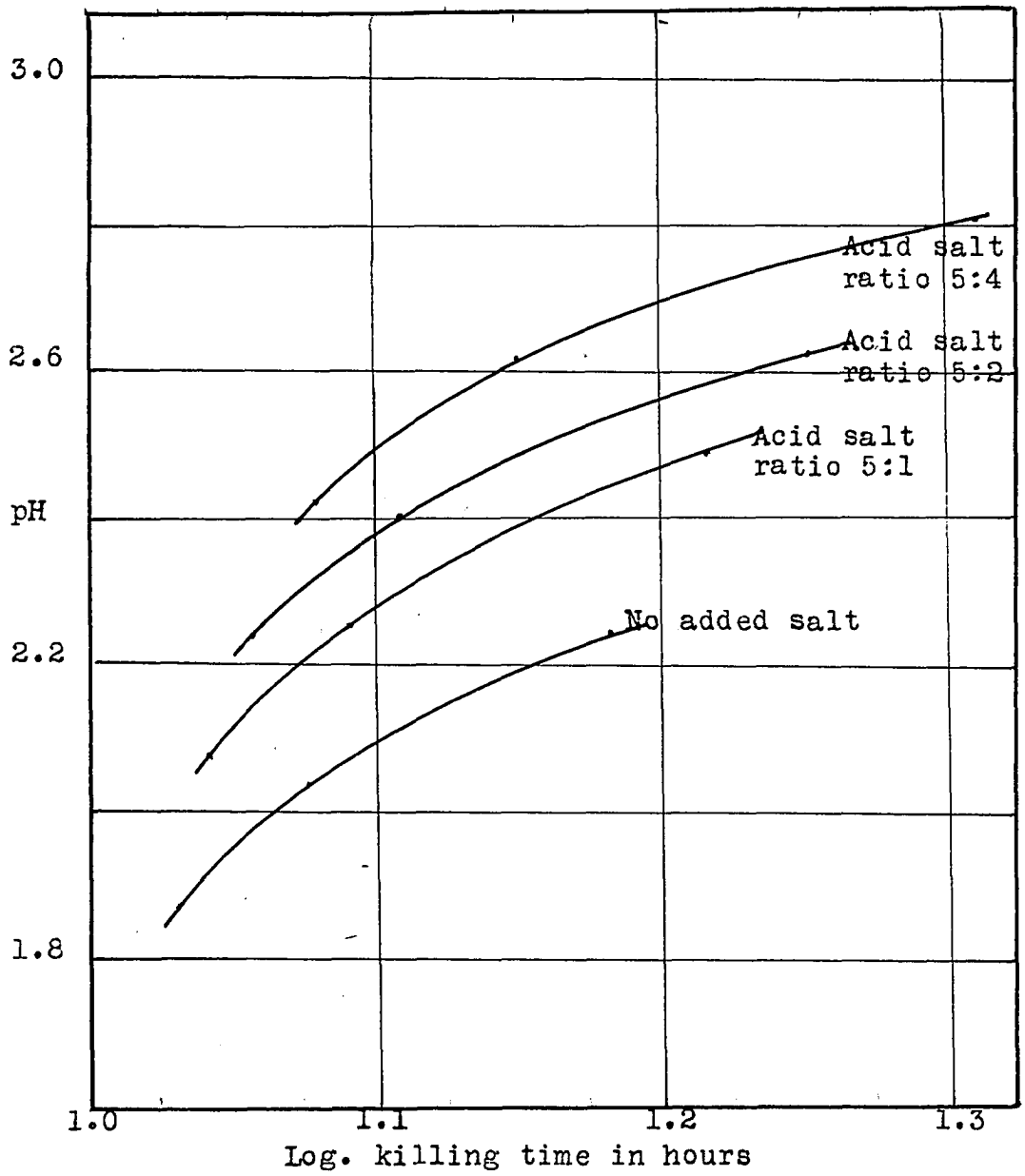
The relation between pH and killing time for yeast in syrups of different densities.

TABLE XLII

Relation of killing time for 99% of initial yeast in 30° Be. syrup, to citric acid and sodium dihydrogen citrate content, pH, and conductivity.

Acid Concentration molar	Salt Concentration molar	Killing time hours	p H	Specific conductance
0.02	0.0	15.2	2.25	.0001064
	0.004	16.4	2.49	.0001128
	0.008	18.0	2.61	.0001214
	0.012	-	2.76	.0001343
	0.016	21.2	2.81	.0001483
	0.020	-	2.85	.0001705
0.04	0.0	11.8	2.05	.0001277
	0.008	12.2	2.27	.0001305
	0.016	12.8	2.41	.0001581
	0.024	-	2.57	.0001810
	0.032	14.1	2.62	.0002148
	0.040	-	2.65	.0002580
0.08	0.0	10.7	1.88	.0001643
	0.016	11.0	2.09	.0001662
	0.032	11.4	2.24	.0002160
	0.048	-	2.38	.0002708
	0.064	12.0	2.43	.0003108
	0.080	-	2.46	.0003467

Plate 29.



Effect of salts on killing time of yeast in citric acid solutions in 30° Be. syrup.

DISCUSSION

It will be seen from the foregoing plates and tables that there is a distinct change in the death rate of yeast in sucrose syrups when certain factors are varied. These factors may be sucrose concentration, acid concentrations, or the amount and kind of added salt. The cultural state of the organism, whether it is in a spore or vegetative phase, has a marked effect on the shape and slope of the curve for the death rate of the organism.

The plates from 1 to 15 inclusive cover a series in which three quantities of added citric acid were used with 24^{o}, 30^{o}, and 36^{o} Baume syrup. These acid concentrations were 0,02, 0,04, and 0.08 molar citric acid and corresponded to the manufacturers designations of one, two, and four "ounces of citric acid per gallon" of syrup. To these acid concentrations, various amounts of (tri) sodium citrate were added. The result was that in addition to solutions containing acid alone, there was for each acid strength of each syrup density, one solution of NaH_2 Citrate, one of Na_2H Citrate, and one containing equal quantities of both these acid salts.

For each syrup concentration involving acid and acid-salt concentrations the same general facts were noted, which can be observed from an inspection of the curves:

1. Acid alone is found to be toxic to yeast in all of the concentrations employed.

2. NaH_2 Citrate was not toxic to yeast in any of the concentrations employed. Curves, for 36° Baume syrups show deaths which can be explained as due to the high concentration of the syrup, rather than to the salt alone.

3. Na_2H Citrate was found to inhibit yeast growth in the lower concentrations, and to cause a slow death rate at higher concentrations. That its effect is not due to pH can be shown by the fact that its range of acidity was that of the usual optimum for the yeast studied (pH 4.5 to 5.9).

Plates 13, 14, and 15 show the same results arranged in the order of increasing syrup concentration at the same acid concentrations. It will be noted that the increase of death rate is regular with increased syrup concentrations. In passing from 24° to 30° Baume the time is more than halved without change of acid strength. In passing from 30° to 36° Baume, the decreased death time is very slight. This is another example of the protective action of heavy syrups.

Reference to plates 1, 5, and 9, as well as to table XLI will show the relation between killing powers of different acid concentrations at the same sugar concentration. In the weaker syrup concentrations (plates 1, and 5; 24° and 30° Be.). The killing time is halved by increasing the citric acid concentration from 0.02 M to 0.08 M. It is decreased one third by doubling the acid content. With the heavier syrup strength (36°Be. plate 9) the relationship between acid

and death time does not hold. The time by which sterilization is shortened in this syrup through the addition of acid, is only a small fraction of the total time. In the case of 36° Baume syrup various factors appear to modify the process of sterilization by acid as observed in the weaker syrup. These factors are the greater osmotic pressure, greater viscosity, changes in the hydration of the sucrose present, or other changes in the system, sucrose-water, which become important in syrup densities above 30° Baume. There seems to be a protective action for yeast as a result of the increased syrup density. It is observed here in the sense of protection against the killing power of acid. Earlier work was reported in which the heavy syrups seemed to protect yeast from the affects of heat.

This protective action results in the approach of a minimum death time beyond which increase in acid or sucrose concentration becomes increasingly less effective. This will be seen in the curves in plate 28. It is there evident that a killing time of 10 hours (log.1) will not be far from this minimum.

Hydrogen ion concentration showed a regular and almost proportionate increase in value when the same amount of acid was added to sugar solutions of increasing density. Although this is in line with the change in death rates, there is not a simple proportionate relation. Reference to plate 28

will show three different killing times for pH values between pH 1.7 and 2.4. At pH 2.05 for example, the killing times are 13.6 hours for 36° Baume syrup, 16.4 hours for 30° Baume syrup, and 33 hours (interpolated) for 24° Baume syrup. Thus it is evident that pH alone is not an index of the killing power, for at the same pH the killing times were markedly different with different amounts of sugar.

At pH values less than 1.7 a change takes place since the line for 36° Baume syrup crosses through the curve for 30° Baume syrup. That this result is perhaps justified and not due to experimental error may be gathered from certain physical data obtainable for this system:-

1. Sucrose is believed to become hydrated with 6 to 8 mols of water, and in 36° Baume syrup this is enough to account for practically all the water in the system.

2. The further addition of acids or salts, with their demand of hydration or solution, will remove more water from the system.

3. When the demands of both the sucrose-water and acid-salt-water sub-systems cannot be fully supplied by the water present, they enter into competition for the water.

4. Unless some active solvent is present, no means will be available for the acid or salt to enter through the membrane of the micro-organism. Since it is capable of maintaining the spore state under adverse conditions, the result

will be that the organism will not be killed, but will remain dormant.

5. This results in a "protective action" on the part of the heavier syrups. Evidence of this is found in the very small change in killing time for all combinations of acid and salt in 36° Baume syrup.

The rate of inversion of sucrose had little bearing on the rate of death of yeast at the same acid or salt concentration. Inspection of appendix D will show positive inversion taking place but little less rapidly at salt concentrations which served as actual stimuli to yeast growth, than at concentrations of acid which killed yeast rapidly.

Attempts to apply such physical constants as rate of inversion of sucrose, change in pH as measured by the quinhydrone electrode, or conductivity directly to the explanation of the change in death time failed. It was not possible to obtain definite data on the unionized citric acid in heavy sugar systems. Such data as could be obtained will be found in the appendix.

The conductivity data obtained was directly opposed to that found in the absence of sugar. On the basis of replacement of water by sucrose, a concentrating effect, tending to increase the conductance, would be expected. Owing probably to the high viscosity, this factor was reversed in so far as measurable conductance was concerned. In the light

of uncertain corrections for viscosity, or for space factors because of replacement of water by sucrose, or for change of ionization, the conductivity data taken was not found entirely suitable for application to the citric acid-sucrose-water systems as an explanation for the killing of yeast.

Plates 16 to 21 are a repetition of the work of the first series, but attention was brought to bear upon sodium citrate in sugar syrups without acid, and upon solutions of acid containing NaH_2 Citrate.

Sodium citrate alone was found to be toxic to yeast. Others have reported it as antagonistic to other cell structures and harmful in the blood stream. In addition to the growth data presented, it was found that concentrated sodium citrate and Na_2H Citrate solutions used as stock solutions for this work were not easily contaminated with mold, where as citric acid solutions as high as 1.5 molar readily supported mold growth.

The addition of NaH_2 Citrate to citric acid, as will be seen from the above tables, resulted in a marked reduction in death rates. That NaH_2 Citrate, either ionized or undissociated, has no toxic effect in itself is readily evident from those curves for solutions containing this salt alone.

Plates 22 to 27 gives some results of adding NaH_2 Citrate to citric acid. There was found to be a distinct

increase in killing time over that for the acid alone. Whatever effect that undissociated citric acid might have had may be balanced by other factors. There was no apparent relation to the sodium hydroxide, sodium carbonate system mentioned in the historical part. Several points of difference between these two systems may be mentioned. Chief of these might be the fact that the pH of the acid solutions was quite close (pH2) to the optimum reaction for the yeast (pH 4.5 - 5.1). In the case of the alkaline solution the reaction was about pH 13. Thus a small change in pH might have but little effect in the latter case and more in the former.

SUMMARY

1. The rate of death of yeast in sugar solutions of the same density increases with increasing acid concentration.

2. The rate of death of yeast in acid solutions of the same concentration increases with increasing sucrose concentration. Sucrose solutions above 30^0 Baume (Sp.gr. 1.2309) exhibit a protective action against acid in that the death time approaches a minimum.

3. The rates of death of yeast in sucrose solutions of the same hydrogen ion concentration vary with the density of the syrups In general, the more concentrated syrup will bring about a quicker death.

4. The addition of sodium di-hydrogen citrate to citric acid-syrup solutions increases the death time for yeast in sugar solutions. The salt itself was practically non-toxic to yeast in the concentrations employed.

5. Sodium citrate has a definite toxic action, but this is not as great as that of citric acid.

6. The effect of the addition of citrates to citric acid-syrup solutions is less marked at higher syrup concentrations. The effect of increasing either acid or salt concentrations is also less marked as syrup concentration increases.

CONCLUSION

By means of the addition of citric acid it is possible to sterilize sugar syrup in the cold. The time varies for different syrup concentrations, being shorter with heavier syrups. This makes possible a process of preparing syrups for bottling, canning, and other industries by simply mixing and storing a solution of sugar-citric acid-and water. The acid can be maintained at a concentration sufficient to meet the needs of the product to which the syrup is added.

Storage time will vary, but on the basis of the work which is here reported, and with allowance for a factor of safety, the following can be recommended:--

1. 24° Baume syrup with one ounce of 50% citric acid solution per gallon, seven days; with two ounces per gallon, five days; and with four ounces per gallon, four days. Conditions governing the taste desired in the finished product, would limit the amount of acid to less than two ounces per gallon.

2. 30° Baume syrup with the same acid quantities three days, two days, and thirty hours respectively.

3. 36° Baume syrup with all three acid quantities, thirty hours.

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Appendix A

Conductivity data for the system:- sucrose-citric acid-water.

Sugar degrees Baume	Citric acid mols per Liter	Cell resistance (cell constant .156)	Molar equivalent conductance	Visosity coefficient
0	0.01	54.10	36.0	1
	0.02	73.49	52.5	
	0.04	114.16	68.25	
	0.08	166.70	93.7	
12	0.01	92.6	21.1	1.38
	0.02	141	27.7	
	0.04	207	37.7	
	0.08	302	51.7	
24	0.01	229	8.22	2.23
	0.02	380	10.25	
	0.04	589	13.34	
	0.08	830	18.8	
30	0.01	613	3.324	3.62
	0.02	1000	3.90	
	0.04	1581	4.93	
	0.08	2066	7.55	
3 36	0.01	2136	.912	22.4
	0.02	4114	.947	
	0.04	6732	1.158	
	0.08	8405	1.855	

Appendix B

Ionization of citric acid at 25° C.

Concentration of acid Gr. mols	Resistance of cell	Specific conductivity	Equivalent conductance	Percent ionized
.16	22.65	.00408	8.5	6.89
.08	32.52	.00284	11.8	9.57
.04	44.46	.00208	17.3	14.03
.02	66.96	.00138	23.0	18.64
.01	100.1	.000923	30.8	25.0
.005	150.1	.000615	41.0	33.2
.002	265.7	.000357	59.5	47.3
.001	414	.000223	74.3	60.3
.0005	692	.0001345	89.7	72.7
.0002	1382	.0000669	111.5	90.4
.0001	1888	.0000490	163.3	132.6
.00002	8260	.0000112	186.7	151.3
.00001	13812	.00000669	223.0	181

Appendix C

pH of citric acid solutions at 25° C.

Sugar degrees Baume	pH at citric acid concentrations 0.08M	0.04 M	0.02 M	0.01 M
0.0	2.10	2.27	2.44	2.62
12	2.07	2.25	2.41	2.59
24	2.02	2.20	2.37	2.54
30	1.88	2.05	2.25	2.42
36	1.67	1.86	2.05	2.23

pH of NaH_2 Citrate solutions at 25°C.

Sugar degrees Baume	pH at NaH_2 concentrations 0.08 M	0.04 M	0.02 M	0.01M
H_2O	3.66	3.73	3.77	3.81
12	3.64	3.71	3.76	3.79
24	3.62	3.69	3.75	3.78
30	3.56	3.64	3.70	3.73
36	3.48	3.56	3.62	3.66

Appendix D

Rate of inversion of 6 Be. sugar at 25° C. by citric acid, NaH_2 citrate, and Na_2H citrate.

Molar concentration	9 days	17 days	34 days
	Citric acid		
0.02	47	74.0	99.3
0.04	52.4	78.0	99.3
0.08	63.7	84.9	99.9
	NaH_2 Citrate		
0.03	22.1	63.7	95.4
0.06	38.3	75.6	97.5
0.12	34.6	82.7	99.7
	Na_2H Citrate		
0.06	0	0	2.67
0.12	0	.49	3.21
0.24	0	0	3.76

Appendix E

pH of solutions of citric acid and its sodium salts.

Concentration M/Liter	Citric acid	Citric acid NaH_2Citrate ratio 2:1	NaH_2 Citrate	Na_2H Citrate	Na_3 Citrate
1.5				4.05	
1.0			3.44		7.64
.6				4.13	
.5	1.75				
.4			3.52		7.635
.37		2.54			
.3				4.22	
.24				4.24	
.2	1.91		3.60		7.63
.16			3.61		7.625
.15		2.64			
.12				4.32	
.1	2.06				
.08	2.12		3.66		
.075		2.69			
.06		2.71		4.39	
.04	2.26		3.73		7.62
.03		2.76		4.47	
.02			3.77		7.55
.015		2.83		4.51	
.01	2.61		3.81		7.30
.0075		2.93			
.005	2.79				
.004		3.04			