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Studies on the sterilization of solutions of glucose and sucrose

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NOTE TO USERS

STUDIES ON THE STERILIZATION OF SOLUTIONS OF GLUCOSE AND SUCROSE

BY

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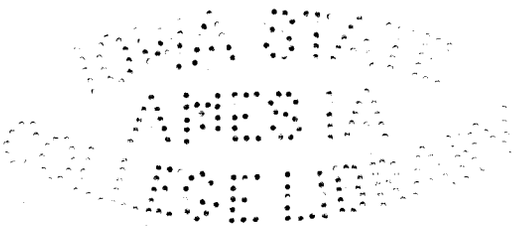

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STUDIES ON THE STERILIZATION OF SOLUTIONS OF GLUCOSE AND SUCROSE

INTRODUCTION

Glucose, the most widely known sugar of the monosaccharide group, is being economically produced at the present time by the hydrolysis of corn starch. This sugar, also known as dextrose, corn sugar, and cerelose, represents a product of 99.5% purity. The sale of corn sugar has been restricted in the past because the pure food law regulations required that food products sweetened with any other sugar than sucrose should have the fact stated on the label. However, this restriction was lifted in January, 1931, by the Secretary of Agriculture, thus opening a large field to the producers of glucose.

The principal industries that will be able to make use of this sugar are the canning and beverage industries. The beverage industry alone uses about 300,000 tons of sucrose annually. By using a cheaper sugar, such as dextrose, these industries may make a considerable saving.

Along with this economic feature there are two distinct advantages, especially to the manufacturer of carbonated beverages, in using glucose. The workers on the American Bottlers of Carbonated Beverages Fellowship have abundantly shown that the cause of spoilage of carbonated beverages is principally due to yeast (1,2,3,4). That cane sugar contains

yeast and may be the original source of these microörganisms in a majority of the cases of spoilage in carbonated beverages has been shown by several workers (3,4,5,6,7). Thus to eliminate the source of contamination by obtaining a sterile sugar will be a distinct advantage to the manufacturer of carbonated beverages. Glucose is relatively free from yeast. In an examination of samples of commercial dextrose sent to the Department of Food Chemistry, Hellwig (8) found no evidence of the presence of yeast.

The second advantage in the use of glucose is in the fact that the finished bottled product will not change in taste after it has been stored for several months. The presence of acid in carbonated beverages causes slow hydrolysis of the sucrose to invert sugar. Sale and Skinner (9) have shown that beverages made with invert sugar are not so sweet as when made with the same amount of sucrose. Since glucose is not changed in the presence of a moderate amount of acid, its use would prevent this change of flavor caused by the presence of increasing amounts of invert sugar.

There are two disadvantages in the use of glucose and the preliminary work was concerned with those problems: first, the low solubility of glucose in water (83 grams in 100 cc. of water at 17.5°C.), and secondly, the relatively low sweetening power of this sugar compared to sucrose.

Manufacturers of carbonated beverages prepare their syrups by two methods - hot process and cold process (10).

The heaviest glucose syrup that may be used at room temperature (23-25°C.) is of 27° Baumé strength. This amounts to a solution containing approximately 49% of sugar. The specific gravity of this solution at 20/20 is 1.2288. Those manufacturers using the hot process method will experience no difficulty in making 27° Baumé syrups if the necessary weight of glucose is added to the required volume of hot water. In attempting to make a similar syrup by the cold process method, the highest density syrup obtainable by simple mechanical stirring over a period of several hours was one of 20° Baumé. The disadvantage in using this concentration of glucose syrup is that such a large volume of syrup must be added to the bottle, that it is not possible to add sufficient carbonated water to give the desired volume of carbon dioxide. For example, three fluid ounces of flavored 20° Baumé syrup must be added to a six and one-half ounce bottle in order to give a sugar percentage corresponding in sweetness to the average bottle that contains 11-12% sucrose. This allows a volume of three ounces of carbonated water to give the desired carbonation to the finished product. The manufacturer usually uses from one to one and one-half ounces of flavored syrup to a six and one-half ounce bottle. By using a Lomax Syrup Maker of the mixer and filter type, it was possible fo produce a 27° Baumé syrup with several hours mixing.

The next problem was to determine what quality of beverage could be made by using a glucose syrup. A great many dif-

ferent values for the comparative sweetness of glucose and sucrose have been reported. One of the highest values found was that of 74.3 compared to a value of 100.0 for sucrose given by Biester, Wood and Wahlin (11). This value was found to be satisfactory in making up carbonated beverages of a good quality.

Having determined the conditions in which a glucose syrup could be prepared and utilized by the manufacturer of carbonated beverages, the problem arose as to the relative ease of sterilizing contaminated solutions of glucose and sucrose.

Briefly, the problem was as follows:

1. To make comparative studies of growth (or death) of yeasts in solutions of sucrose and glucose of the same densities at room temperature, using densities within the range that would be satisfactory for the manufacturer of carbonated beverages to use with glucose.

2. To study the effect of citric acid on the killing time of yeasts in solutions of glucose and sucrose of the same densities at room temperature.

3. To make comparative studies of the effect of temperatures on the killing times of yeasts in solutions of glucose and sucrose of the same densities.

4. To study the effect of the addition of citric acid to solutions of glucose and sucrose at high temperatures on the killing time of yeast.

REVIEW OF LITERATURE

A considerable amount of work has been reported on the fermentation of sugars, but very little has been published concerning the growth of yeast in high concentrations of glucose solutions. The only work reported on a high concentration of glucose was that of Brown (12). He found that there was a decrease in the amount of dextrose fermented when a 30% solution was used. In comparing fermentation of 10, 15, 20 and 30% solutions, a 20% solution appeared to be the optimum.

Owen and Denson (13) studied the effect of the accelerating action of vegetable carbons on the fermentation of solutions of 20% glucose and levulose. He found that while carbon stimulated the fermentation of levulose it had no accelerating action on the fermentation of glucose. The presence of carbon stimulated fermentation in sucrose solutions.

In working with high sucrose concentrations Dubourg (14) isolated a yeast that was active in an 80% sucrose solution. Owen (15) isolated Saccharomyces Zopfii from canned cane syrup having a density of 70-75 Brix (67-72% sugar) by plating out on a high sugar medium. He studied the effect of temperatures and densities of cane syrups on the fermentative action of this yeast by inoculating tubes of syrups of four different densities with cultures of S. Zopfii and exposing the tubes to varying temperatures for a definite length of time. After stopping the action of the heat by immersing in

cold water, the tubes were incubated until fermentation developed. He found that when syrups were heated to 90°C. for 10 minutes fermentation developed in the high density syrup (71.1 Brix) in 15 days, while with the lower densities (56.3 Brix, 41.5 Brix, and 31.5 Brix) no fermentation occurred. However, when tubes of syrups exposed to temperatures under 70°C. for 10 minutes were incubated, fermentation developed more rapidly in the lower density syrups than in the 71.1 Brix solution.

Owen (1,2) brought out the fact that the reason yeasts had not been isolated from heavy sugar syrups before was because of the medium used in plating them out. Yeasts are more sensitive to radical changes in the density of their environment than bacteria, and when a fermented heavy syrup was plated out on 10% sugar medium only bacteria were found to be present. When plated out on a 25% sugar medium both yeasts and bacteria were present - the bacteria in a greater number than the yeasts. By plating out on a 50% sugar medium, both yeasts and bacteria were found, but the former predominated.

Church, Paine and Hamilton (16) found abundant evidence that the bursting of chocolate-coated creams was due to yeast growth. These yeasts grew in a 76-77% sugar syrup.

McKelvey (4) carried out experiments to determine the length of time necessary to kill yeast spores in sugar syrups of 24, 27, 30 and 36° Baumé. Spores were killed in 24° Baumé syrups by heating to 70°C. for two minutes; 36° Baumé syrups

were heated for five minutes in order to kill all the yeast spores.

Peterson (17) worked on somewhat the same problem. The spores that he obtained required a considerably higher temperature to be killed than those spores of McKelvey, 30 minutes at 100°C. were necessary to sterilize a 36° Baumé syrup. Peterson's explanation (18) for the great difference in the killing of his spores and McKelvey's is that the latter added 1.5 cc. of saturated citric acid to 1000 cc. of his plating medium. Yeasts did not grow well in this medium after being exposed to heat.

Peterson used a mixture of spores from 28 different yeasts. The time necessary to kill 99.9% of yeast spores in sucrose syrups at a temperature of 100°C. was six minutes for 24° Baumé, 8-10 minutes for 30° Baumé, and 28 minutes for 36° Baumé. Upon the addition of 1 cc. of 7.074N citric acid to 100 cc. of 36° Baumé syrup, all the yeasts were killed in two minutes.

Toulouse (19) studied the effect of added acid to high density sucrose syrups on the killing of yeasts at room temperature. He obtained growth of yeasts on 24° syrups; but with the addition of citric acid at the rate of one ounce of 50% citric acid per gallon of syrup the yeasts were killed. He gives definite killing times for yeasts in syrups containing one, two and four ounces of citric acid per gallon. However, this applies only to the strain of vegetative cells of the yeast that he used.

EXPERIMENTAL

Source of Materials

1. The yeast.

In order to obtain yeasts that would be typical of those which would cause spoilage in the plants of the manufacturers of carbonated beverages, samples from spoiled beverages that had been sent to the American Bottlers of Carbonated Beverages Fellowship for analysis were plated out on Wort agar to determine the presence or absence of living yeasts. Twenty different samples were plated out, fourteen of which showed that living yeasts were present. To determine the presence of spore forming yeasts, typical colonies on each plate were inoculated into broth and incubated at 28°C. for 48 hours. Carrot juice calcium sulfate tubes were then inoculated and incubated for two weeks at 28°C. This carrot juice calcium sulfate agar was prepared as follows: two liters of distilled water were added to one kilogram of finely ground carrots, and the whole was boiled for ten minutes. The liquid was then extracted in a hand press, squeezing out as much of the juice as possible. To the pure solution was added 2% agar and 1% calcium sulfate, and the whole was boiled until the agar was dissolved. The media was placed in tubes and flasks and sterilized at 15 pounds pressure for 20 minutes.

After incubating the tubes for two weeks at 28°C., the

presence or absence of yeast spores was determined by Moller's method of spore staining. Seven of the fourteen tubes showed positive evidence of yeast spores.

To obtain a sufficient number of spores to carry out all of the experiments planned, Kolle flasks containing carrot juice calcium sulfate agar were inoculated from 48 hour broth cultures of the seven strains of yeast. After incubating for 20 days, when yeasts that were inoculated into tubes of the same agar at the same time showed the presence of abundant spores, the growth was scraped off by means of a small hoe-shaped spatula and placed in a sterile evaporating dish. The spores were then dried in a vacuum desiccator over sulfuric acid for 21 days.

In order to dilute the spores, they were ground in an agate mortar with three to four volumes of sterile powdered sugar. The resulting mixture was kept in a sample bottle in a desiccator containing sulfuric acid. A small amount of spores was plated out, and it was found that there were approximately 200 million yeasts per gram of material.

2. Inoculating material.

In the first few experiments on the growth of yeasts in syrups, the yeast suspension was prepared by adding the spores to sterile tap water; later, a 2% glucose solution was made up by the addition of the necessary amount of glucose to conductivity water. After sterilization, a weight of the spore

suspension was added which would give the desired number of yeasts in 5 cc. of the suspension. In experiments on the growing of yeasts in sugar solutions, it was desired to inoculate about one million yeasts in the flask. In the killing experiments, about four million yeasts per flask were employed.

In work with vegetative cells, a 48 hour malt extract broth culture, incubated at 28°C. was employed. Examination revealed the presence of some spores. One cubic centimeter of this broth contained approximately 30 million yeasts.

3. Plating medium.

All counts were made by plating out on Wort agar (Bacto). The formula, claimed by the manufacturers on the label, was as follows:

Maltose	12.75	parts
Malt Extract .	15.00	"
Dextrin	2.75	"
Glycerin . . .	2.35	"
K_2HPO_4	1.00	"
NH_4Cl	1.00	"
Peptone	0.78	"
Agar	15.00	"

Fifty and sixty-three hundredths grams were dissolved in 1000 cc. of distilled water. Since this mixture does not

solidify well, 10 grams of agar were added per 1000 cc. of medium. After solution, the medium was placed in bottles and sterilized at 15 pounds pressure for 15 minutes.

4. Sugars.

The glucose used was that obtained from a large producer of dextrose. A normal solution gave a reading of 99.6% in the saccharimeter.

The sucrose used was of a good grade of granulated cane sugar.

The syrups were prepared by adding the necessary weight of sugar to hot water. After cooling the syrups, the density was determined by means of the Westphal balance. A very small amount of water was usually added to make the solution the desired density.

5. Citric Acid.

The citric acid used was of C.P. grade and gave the following analysis on the label:

Heavy metals . .	0.0001	Per cent
Chlorine	0.002	" "
Non-volatile . .	0.04	" "
SO_3	0.0034	" "
Calcium	"Nil"	
H_2S metals . . .	"Nil"	

The 50% citric acid solution was prepared in the same manner as it is commercially - one pound of citric acid to one pint of water. This amounts to 453 grams of acid dissolved in 473 cc. of water. The solution was sterilized in the autoclave at 15 pounds for 15 minutes, and the amount of water which was lost during sterilization was made up by the addition of sterile distilled water.

Methods and Technique

1. Experiments conducted at room temperature.

A 100 cc. portion of the sugar syrup of the desired density was measured into a 300 cc. Pyrex Erlenmeyer flask and sterilized at 15 pounds pressure for 15 minutes. Upon cooling 5 cc. of a yeast spore suspension were added to the syrup.

In experiments with citric acid, the desired volume of acid was added to the sterile syrup shortly before inoculation with the test yeast.

At the desired time intervals, the flask, maintained at room temperature 26-28°C., was shaken and 5 cc. portions of the solution were transfered into 45 cc. of sterile tap water and the number of viable organisms determined by plating (Wort agar at 28°C. for 5 days). The initial count was obtained by taking a 5 cc. sample of the syrup within 10 minutes after inoculation.

2. Experiments conducted at 60°C.

The apparatus and techniques employed in these experiments were similar to those used by Hall (20), which were essentially the same as those employed by Levine, Buchanan and Lease (21).

A 100 cc. portion of syrup was measured into a flask. The stirring mechanism was placed in the flask and the whole sterilized for 15 minutes at 15 pounds pressure. The flask was then cooled to the desired temperature and placed in a DeKhotinsky water bath at 60°C. The stirring mechanism was connected and the flask left for thirty minutes before the citric acid or spores were added. When acid was added, a period of five minutes elapsed before the addition of the spores.

The initial count was obtained by placing 5 cc. of yeast suspension in 100 cc. of sterile tap water. Samples from this solution were plated out by the method used in the experiments conducted at room temperature.

Since the lowest dilution plated out was one-tenth, and since there was an inoculum of approximately four million yeasts, counts lower than 250 were unreliable.

Results

Experiments conducted at room temperature.

1. The effect of solutions of glucose and sucrose of the same densities on the growth of yeast spores.

To determine the effect of different sugars on the growth of the yeast three different concentrations of sugars were employed: 20° Baumé (sp.g. 1.1600 $\frac{20}{20}$), 24° Baume (sp.g. 1.1983 $\frac{20}{20}$), and 27° Baumé (sp.g. 1.2288 $\frac{20}{20}$)(22). These densities correspond to sugar percentages of 36, 44 and 49.5 respectively (23). The flasks were kept in the incubator (26-28°C.) except when samples were being removed. The results are given in tables I, II and III. Graphs, in which the per cent of initial count is plotted against the period of storage, are shown to illustrate the difference between the two sugars.

TABLE I

Growth of Yeasts in 20° Baumé Syrups at 28°C.

Date of Experiment	6/2/31	6/22/31	6/22/31	Average Number of Yeast per cc. of Syrup	Per Cent of Average Number of initial Count
Period of Storage	Yeast per cc. of Syrup				
			Glucose		
Initial	13,000	25,000	25,000	21,000	100
1 day	10,000	14,000	15,000	13,000	62
2 days	10,000	20,000	24,000	18,000	86
3 "	25,000	280,000	300,000	202,000	962
5 "	80,000	350,000	300,000	243,000	1,160
7 "	800,000	230,000	250,000	427,000	2,030
			Sucrose		
Initial	13,000	25,000	25,000	21,000	100
1 day	300,000	900,000	1,000,000	730,000	3,580
2 days	1,500,000	1,200,000	1,700,000	1,470,000	7,000
3 "	1,900,000	1,500,000	1,700,000	1,700,000	8,100
5 "	1,600,000	1,700,000	2,000,000	1,770,000	8,430
7 "	1,700,000	1,750,000	2,500,000	1,980,000	9,430

Form E-3

Plate I
Growth of Yeasts in 20° Baumé Syrups at 28° C.
Per Cent of Initial
Sucrose
Glucose
Period of Storage in Days

TABLE II

Growth of Yeasts in 24° Baumé Syrups at 28°C.

Date of Experiment	5/25/31	6/2/31	6/2/31	6/26/31	6/26/31	Average Number of Yeast per cc. of Syrup	Per Cent of Average Number of Initial Count
Period of Storage	Yeast per cc. of Syrup						
	Glucose						
Initial	30,000	13,000	13,000	25,000	25,000	21,000	100
1 day	20,000	9,000	9,000	20,000	21,000	16,000	76
2 days	15,000	7,000	6,500	20,000	18,000	13,000	62
3 "	60,000	10,000	8,000	18,000	18,000	23,000	110
5 "	1,400,000	150,000	150,000	210,000	40,000	390,000	1,860
7 "	1,400,000	550,000	550,000	450,000	280,000	650,000	3,100
	Sucrose						
Initial	30,000	13,000	13,000	25,000	25,000	21,000	100
1 day	800,000	100,000	100,000	60,000	115,000	235,000	1,120
2 days	1,100,000	500,000	500,000	1,300,000	1,500,000	980,000	4,700
3 "	1,200,000	1,900,000	1,900,000	1,300,000	1,450,000	1,550,000	7,400
5 "	1,550,000	1,800,000	1,700,000	1,700,000	1,700,000	1,700,000	8,100
7 "	1,400,000	1,500,000	1,700,000	1,800,000	1,500,000	1,590,000	7,600

Form E-3

Plate 2.
Growth of Yeasts in 24° Baumé Syrups at 28° C.
Per Cent of Initial
Sucrose
Glucose
Period of Storage in Days

TABLE III

Growth of Yeasts in 27° Baumé Syrups at 28°C.

Date of Experiment	5/19/31	5/19/31	5/28/31	5/28/31	Average Number of Yeast per cc. of Syrup	Per Cent of Average Number of Initial Count
Period of Storage	Yeast per cc. of Syrup					
	Glucose					
Initial	39,000	39,000	17,000	17,000	28,000	100
1 day	28,000	31,000	12,000	11,000	20,000	71
2 days	24,000	24,000	9,000	10,000	17,000	61
3 "	20,000	21,000	10,000	10,000	15,000	54
5 "	22,000	21,000	7,500	6,000	14,000	50
7 "	100,000	211,000	8,000	6,000	81,000	289
10 "	800,000	800,000	600,000	525,000	680,000	2,429
	Sucrose					
Initial	39,000	39,000	17,000	17,000	28,000	100
1 day	45,000	50,000	14,000	15,000	31,000	110
2 days	1,250,000	1,250,000	380,000	300,000	795,000	2,840
3 "	1,700,000	1,700,000	1,400,000	1,300,000	1,500,000	5,360
5 "	1,750,000	1,750,000	2,500,000	1,800,000	1,950,000	6,000
7 "	1,500,000	1,500,000	1,500,000	1,700,000	1,550,000	5,540
10 "	1,950,000	1,700,000	1,500,000	1,500,000	1,660,000	5,930

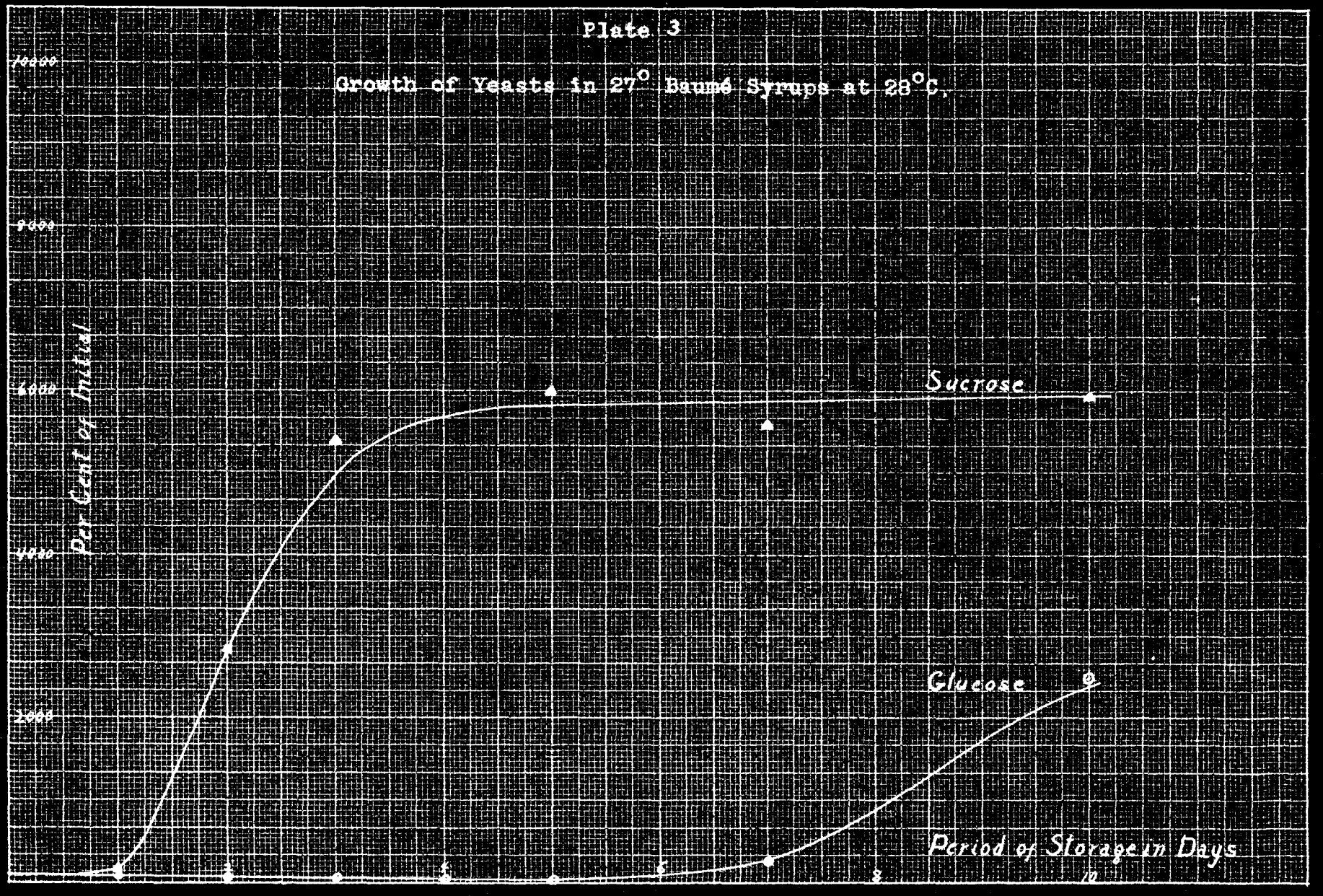


Plate 3
Growth of Yeasts in 27° Baumé Syrups at 28°C.
10000
8000
6000
4000
2000
Per Cent of Initial
Sucrose
Glucose
Period of Storage in Days
10

Form E-3

2. Effect of added citric acid in solutions of glucose and sucrose on the growth of yeast spores.

The next series of experiments were planned for the purpose of determining the effect of added citric acid on the growth or death of the yeasts. One concentration of citric acid was used - .04M. This corresponds to the bottlers two ounces of 50% citric acid per gallon of syrup. A .04M solution required the addition of 1.55 cc. of 50% citric acid to a total volume of 105 cc. of solution (100 cc. of syrup plus 5 cc. of spore suspension). Only one concentration of syrup was used - 27° Baumé.

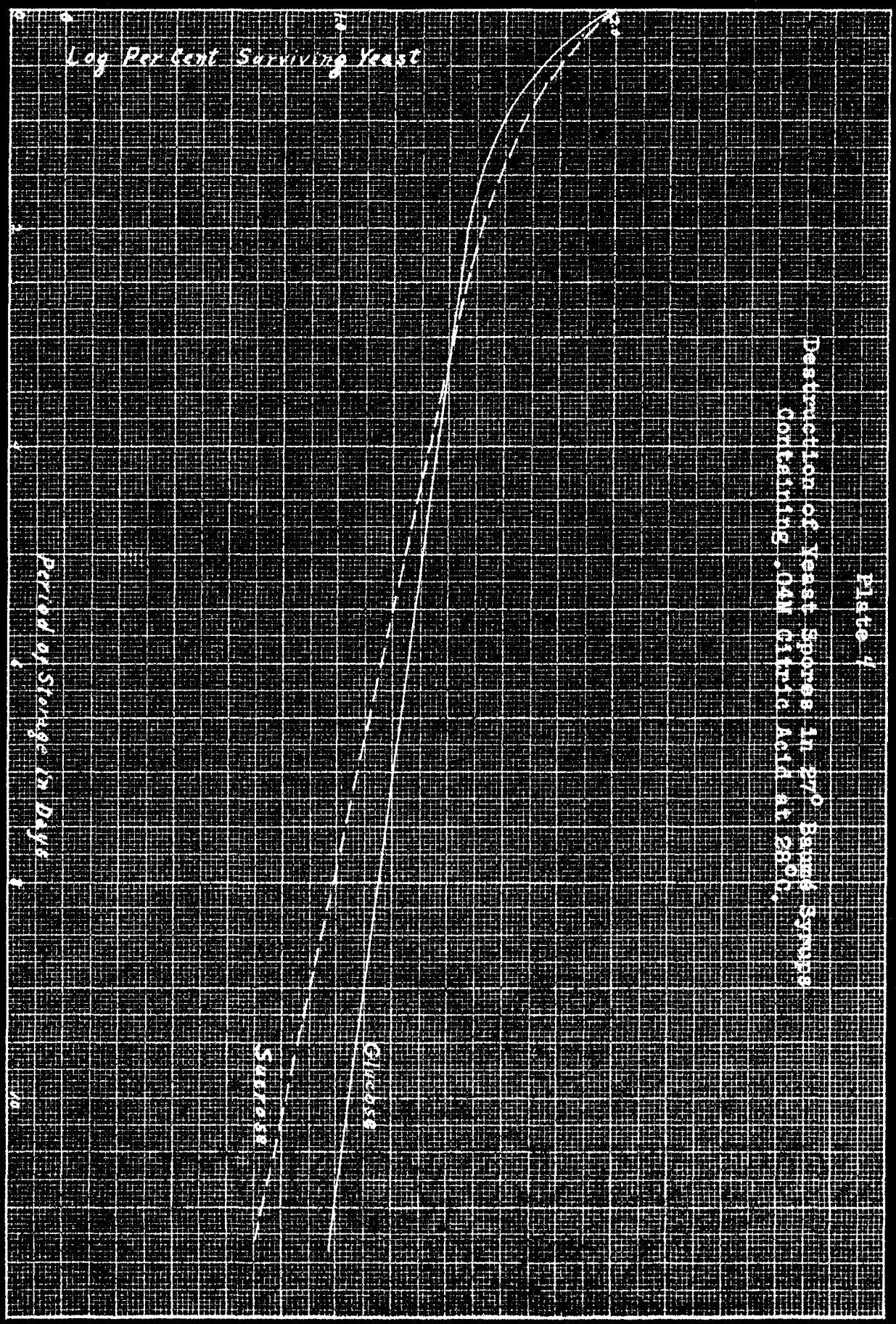
The results are given in table IV and shown graphically in plate 4.

TABLE IV

Destruction of Yeast Spores in Syrups Containing .04M Citric Acid at 28°C.

Sugar	Glucose		Sucrose	
Flask Number	37	80	39	83
Date of Experiment	7/10/31	7/25/31	7/10/31	7/25/31
Period of Storage	Yeasts per cc. of Syrup			
Initial	40,000	30,000	40,000	30,000
1 day	18,000	9,000	24,000	11,000
2 days	13,000	7,700	15,000	9,000
3 "	—	5,400	—	6,000
5 "	10,000	5,000	6,600	3,400
8 "	—	3,800	—	2,400
11 "	—	3,200	—	1,550
	Per Cent Surviving Yeast			
Initial	100.0	100.0	100.0	100.0
1 day	45.0	30.0	60.0	36.67
2 days	32.5	25.67	37.5	30.0
3 "	—	18.0	—	20.0
5 "	25.0	16.67	16.5	11.33
8 "	—	12.67	—	6.0
11 "	—	10.67	—	5.17

Form E-3


Log Per Cent Surviving Yeast
Periods of Standing in Days
Glucose
Sucrose
Fig. 1
Destruction of Yeast Cells at 77° F. by Sucrose and Glucose Solutions Containing Citric Acid at 25° C.

3. Action of vegetative cells in solutions of glucose and sucrose containing varying amounts of citric acid.

In an effort to correlate the work with that done by Toulouse (19,24) on vegetative cells, an experiment was run using a two day malt extract broth culture of the yeast suspension. An abundant growth of yeasts was obtained.

Toulouse obtained a killing time of forty-one hours for 99% of the yeasts in a 24° Baumé sucrose syrup containing .04M citric acid and fourteen and five-tenths hours for 30° Baumé sucrose syrup containing the same amount of acid. This experiment was run on 27° Baumé glucose and sucrose syrups. Two concentrations of acids were added - .02M and .04M. A one cubic centimeter suspension of a forty-eight hour broth culture was inoculated into the syrups. The temperature of the inocubator at times went as high as 32°C. Toulouse's experiments were run at 28°C.

These results are given in table V and illustrated graphically in plate 5.

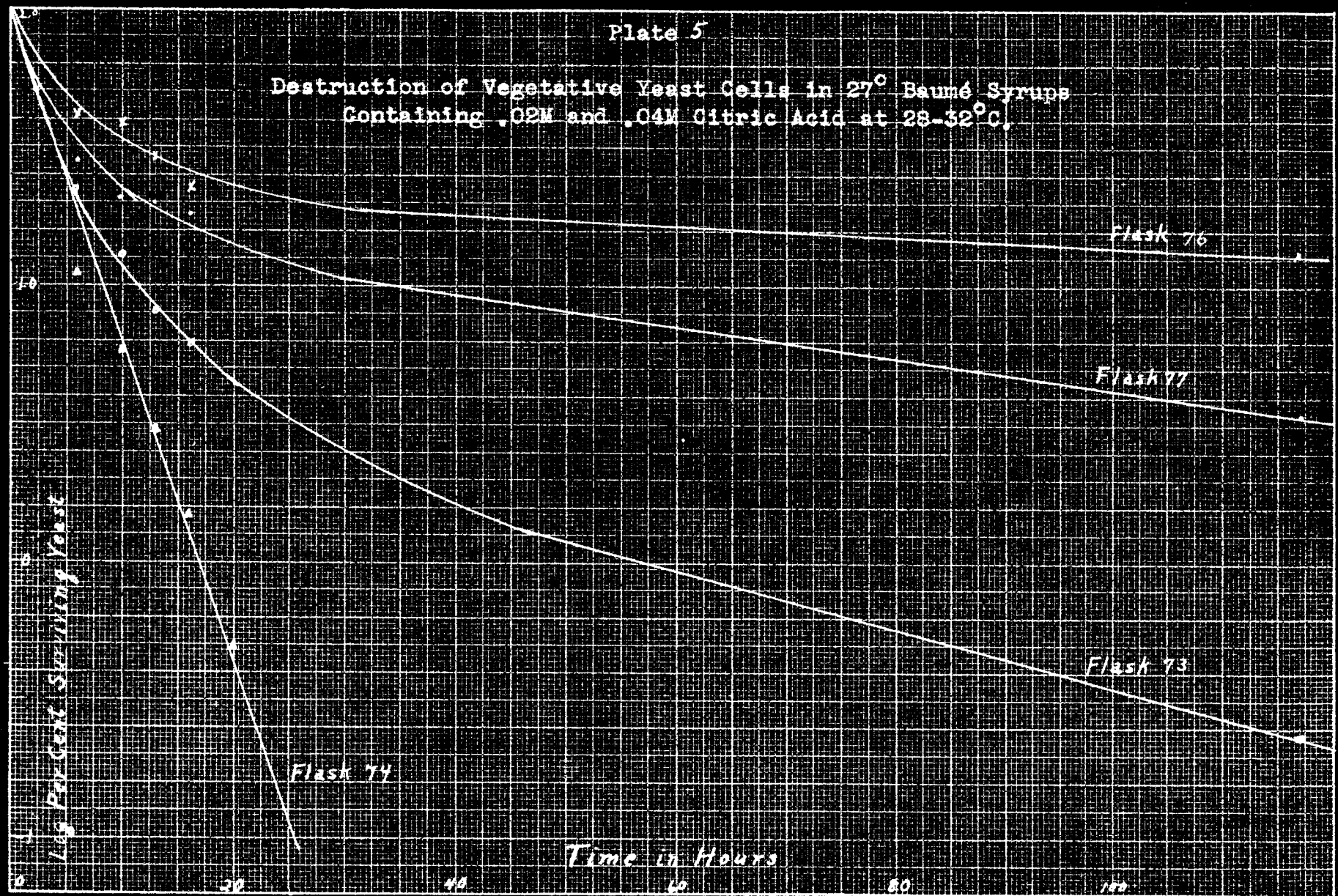
TABLE V

Effect of Varying Amounts of Citric Acid on Vegetative Cells in 27° Baumé Syrups at 28-34°C.

Sugar	Glucose		Sucrose	
Citric Acid Concentration	0.02M	0.04M	0.02M	0.04M
Flask Number	73	74	76	77
Time Elapsed	Surviving Yeast per cc. of Syrup			
Initial	320,000	320,000	320,000	320,000
6 hours	68,000	35,000	134,000	91,000
10 "	40,500	18,500	125,000	68,000
13 "	26,000	9,800	95,000	65,000
16 "	20,000	5,000	75,000	58,000
20 "	—	1,650	—	—
117 "	780	250	53,000	10,000
	Per Cent Surviving Yeast			
Initial	100.0	100.0	100.0	100.0
6 hours	21.25	10.9	41.7	28.4
10 "	12.7	5.8	39.1	21.2
13 "	8.1	3.0	29.7	20.3
16 "	6.25	1.5	23.4	18.1
20 "	—	0.55	—	—
117 "	0.24	0.01	16.6	3.4

Plate 5

Destruction of Vegetative Yeast Cells in 27° Baumé Syrups Containing .02M and .04M Citric Acid at 28-32°C.



Form E-3

Experiments conducted at 60°C.

4. Effect of temperature on the killing of yeast spores in solutions of glucose and sucrose.

In preliminary experiments conducted at 50°C., 27° Baumé syrups containing .04M citric acid showed a killing of only 80% over a period of four hours. In similar preliminary experiments conducted at 60°C. the flasks were sterile after two hours exposure.

Two concentrations of syrup were used - 24° and 27° Baumé.

Since the water bath held but two flasks, each experiment consisted of one flask of glucose and one flask of sucrose of the same densities.

Due to the small number of yeasts used per unit volume, and to the fact that the 1 - 10 dilution was the lowest dilution plated out, plates that contained less than 25 yeasts (250 per cc. of syrup) were regarded as unreliable.

The results of these experiments are given in tables VI and VII and shown graphically in plates 6 and 7.

TABLE VI

Destruction of Yeast Spores in 24° Baumé Syrups at 60°C.

Sugar	Glucose		Sucrose	
Flask Number	54	60	55	61
Date of Experiment	7/17/31	7/21/31	7/17/31	7/21/31
Time Elapsed	Surviving Yeast per cc. of Syrup			
Initial	40,000	48,000	40,000	48,000
5 min.	13,000	20,300	12,000	10,200
10 "	9,000	14,000	5,700	5,500
15 "	5,500	8,200	3,800	4,100
25 "	2,800	4,350	2,000	1,650
45 "	1,050	1,700	750	560
	Per Cent Surviving Yeast			
Initial	100.0	100.0	100.0	100.0
5 min.	32.5	42.3	30.0	21.25
10 "	22.5	29.2	14.2	11.46
15 "	13.75	17.1	9.5	8.54
25 "	7.00	9.06	5.0	3.44
45 "	2.62	3.54	1.9	1.17

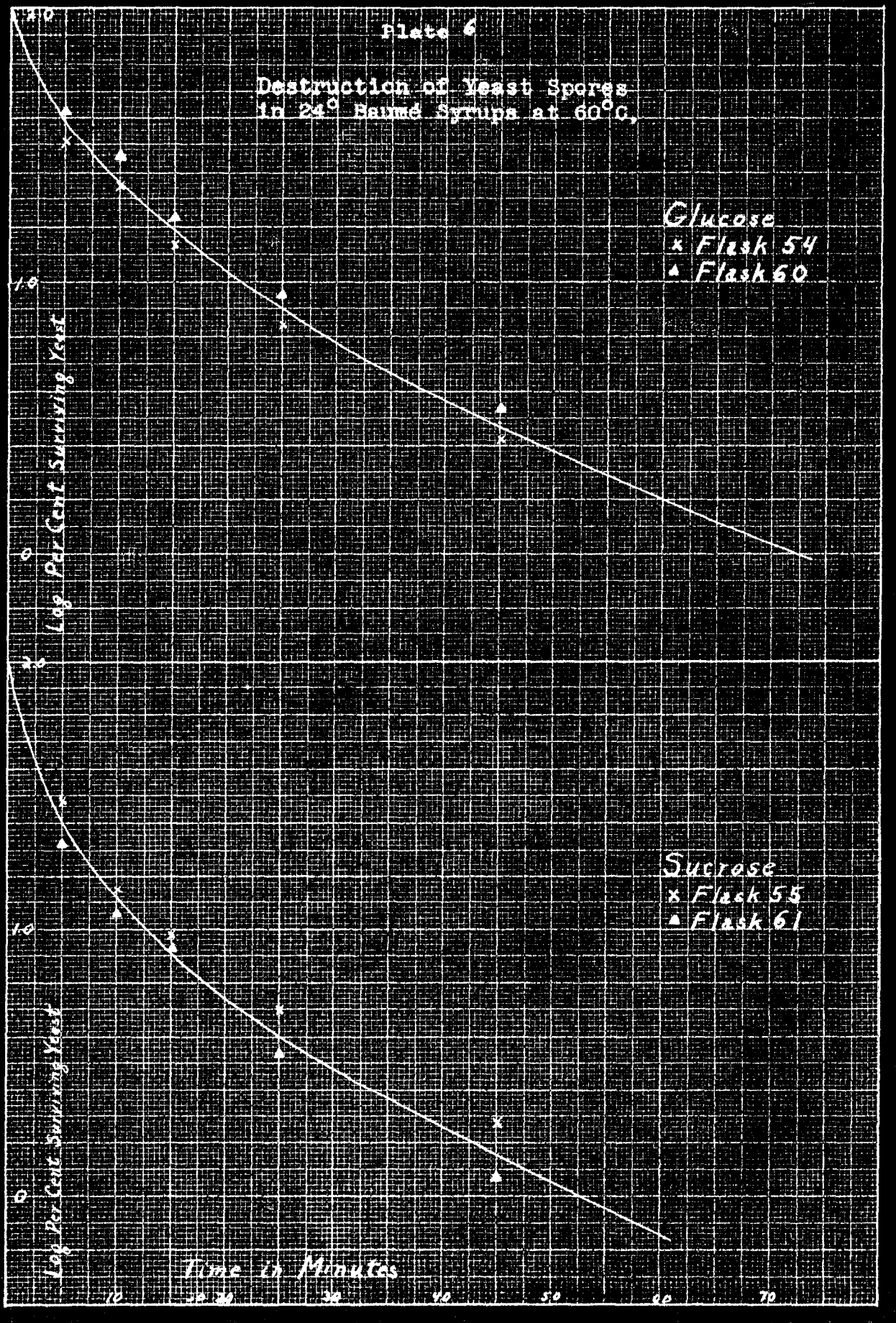


Plate 6
Destruction of Yeast Spores
in 24° Baumé Syrups at 60° C.
Glucose
× Flask 54
▲ Flask 60
Sucrose
× Flask 55
▲ Flask 61
Log Per Cent Surviving Yeast
Log Per Cent Surviving Yeast
Time in Minutes
10
30
40
50
60
70

TABLE VII

Destruction of Yeast Spores
in 27° Baumé Syrups at 60°C.

Sugar	Glucose		Sucrose	
Flask Number	56	70	57	71
Date of Experiment	7/20/31	7/23/31	7/20/31	7/23/31
Time Elapsed	Surviving Yeast per cc. of Syrup			
Initial	38,000	57,000	38,000	57,000
5 min.	15,500	29,000	12,000	28,000
10 "	14,000	25,000	8,000	19,000
15 "	11,000	20,500	5,000	10,500
25 "	7,000	9,000	3,000	5,500
40 "	—	4,700	—	2,650
45 "	3,600	—	1,750	—
60 "	—	2,400	—	1,750
70 "	1,500	—	655	—
	Per Cent Surviving Yeast			
Initial	100.0	100.0	100.0	100.0
5 min.	40.79	50.87	31.58	49.12
10 "	36.84	43.86	21.21	33.33
15 "	28.95	35.97	13.15	18.42
25 "	18.42	15.79	7.89	9.65
40 "	—	8.24	—	4.65
45 "	9.47	—	4.60	—
60 "	—	4.21	—	3.07
70 "	3.95	—	1.72	—

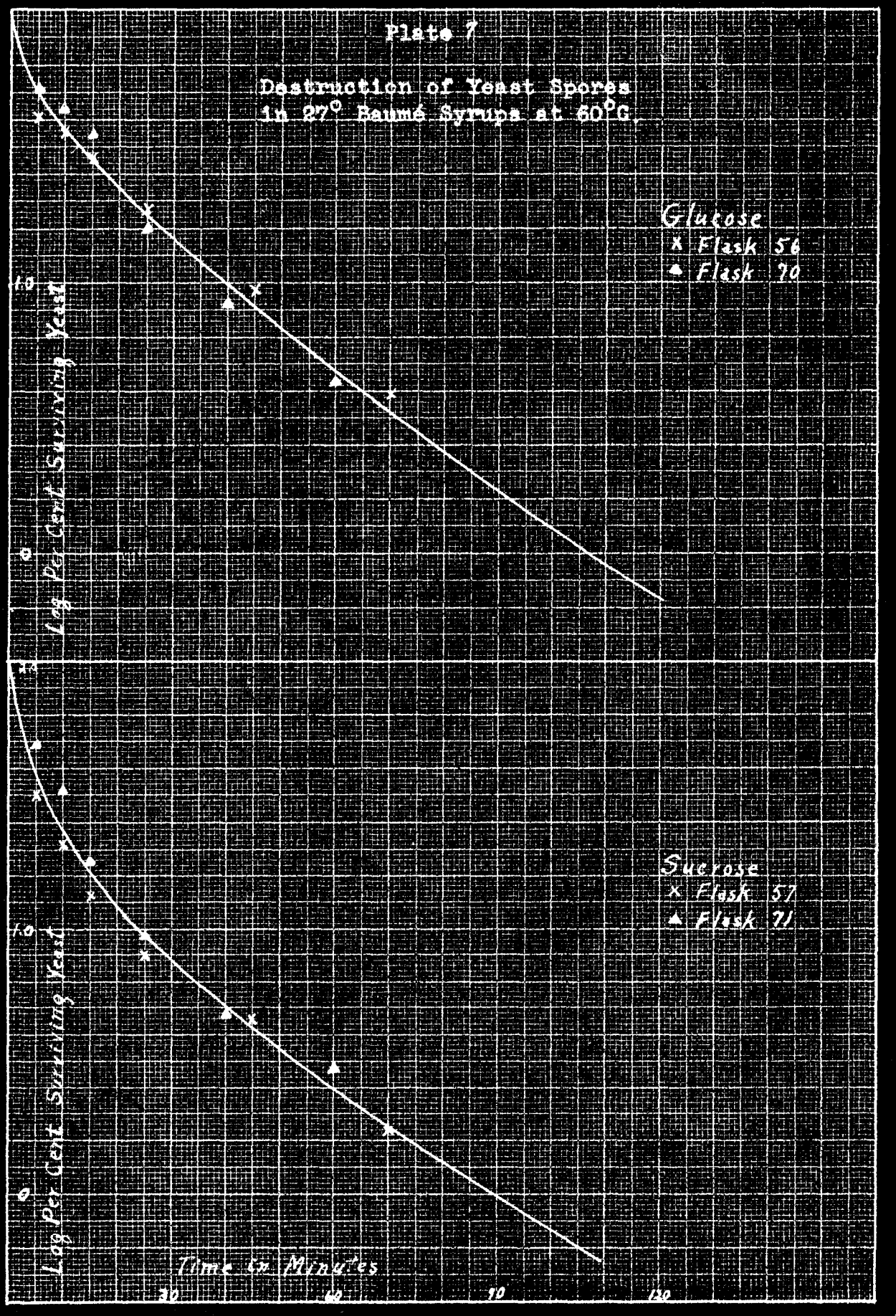


Plate 7
Destruction of Yeast Spores
in 27° Baumé Syrups at 60°C
Glucose
× Flask 56
▲ Flask 70
Sucrose
× Flask 57
▲ Flask 71
Per Cent Living Yeast Spores
100
0
Time in Minutes
30
60
90
120

5. Effect of temperature and varying amounts of citric acid on the killing of yeast spores in solutions of glucose and sucrose.

In studying the effect of temperature and citric acid on the yeast spores two densities of glucose and sucrose syrups were used - 24° Baumé and 27° Baumé. Two different amounts of citric acid were added - 1.55 cc. of 50% citric acid to make a .04M solution and .77 cc. to make a .02M solution.

These results are given in tables VIII, IX, X, and XI and shown graphically in plates 8, 9, 10 and 11.

TABLE VIII

Destruction of Yeast Spores in 24° Baumé Syrups Containing .02M Citric Acid at 60°C.

Sugar	Glucose		Sucrose	
Flask Number	52	64	53	65
Date of Experiment	7/17/31	7/21/31	7/17/31	7/21/31
Time Elapsed	Surviving Yeast per cc. of Syrup			
Initial	39,000	60,000	39,000	60,000
5 min.	6,300	8,000	4,600	5,000
10 "	3,800	4,000	2,400	2,400
15 "	2,200	2,500	1,200	1,400
25 "	650	1,680	400	300
	Per Cent Surviving Yeast			
Initial	100.0	100.0	100.0	100.0
5 min.	16.15	13.33	11.79	8.33
10 "	9.74	6.67	6.15	4.00
15 "	5.64	4.17	3.08	2.33
25 "	1.67	2.80	1.03	0.50

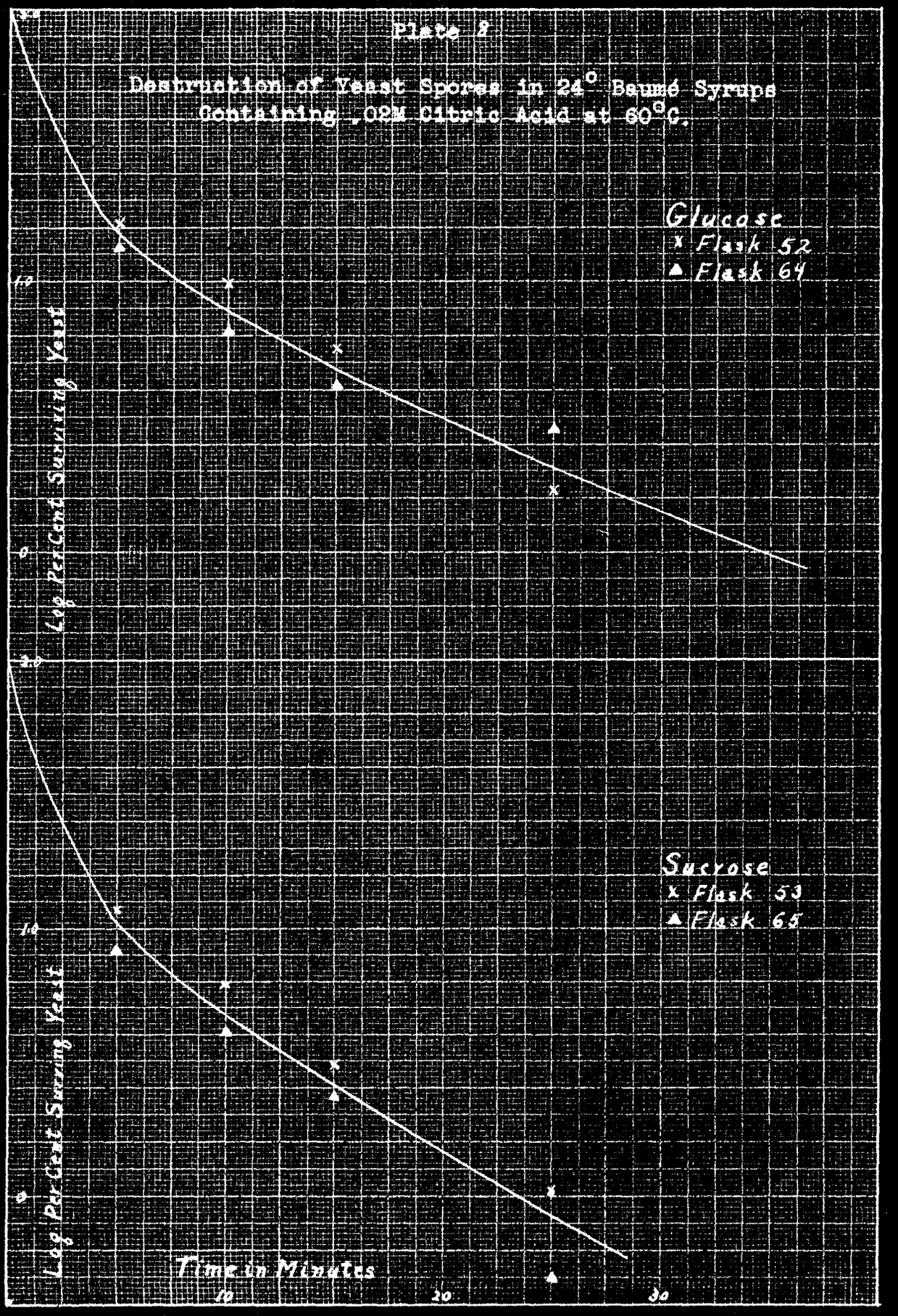


Plate 8
Destruction of Yeast Spores in 24° Baumé Syrups
Containing .02M Citric Acid at 60°C.
Glucose
x Flask 52
▲ Flask 64
Sucrose
x Flask 53
▲ Flask 65
Log Per Cent Surviving Yeast
Time in Minutes
10
20
30

TABLE IX

Destruction of Yeast Spores in 24° Baumé Syrups Containing .04M Citric Acid at 60°C.

Sugar	Glucose		Sucrose	
Flask Number	50	62	51	63
Date of Experiment	7/17/31	7/21/31	7/17/31	7/21/31
Time Elapsed	Surviving Yeast per cc. of Syrup			
Initial	48,000	50,000	48,000	50,000
5 min.	5,200	5,350	3,850	4,000
10 "	2,100	3,100	1,300	1,750
15 "	1,200	2,100	500	910
25 "	300	1,000	—	390
	Per Cent Surviving Yeast			
Initial	100.0	100.0	100.0	100.0
5 min.	10.8	10.7	8.02	8.0
10 "	4.4	6.2	2.7	3.5
15 "	2.5	4.2	1.04	1.82
25 "	0.6	2.0	—	0.78

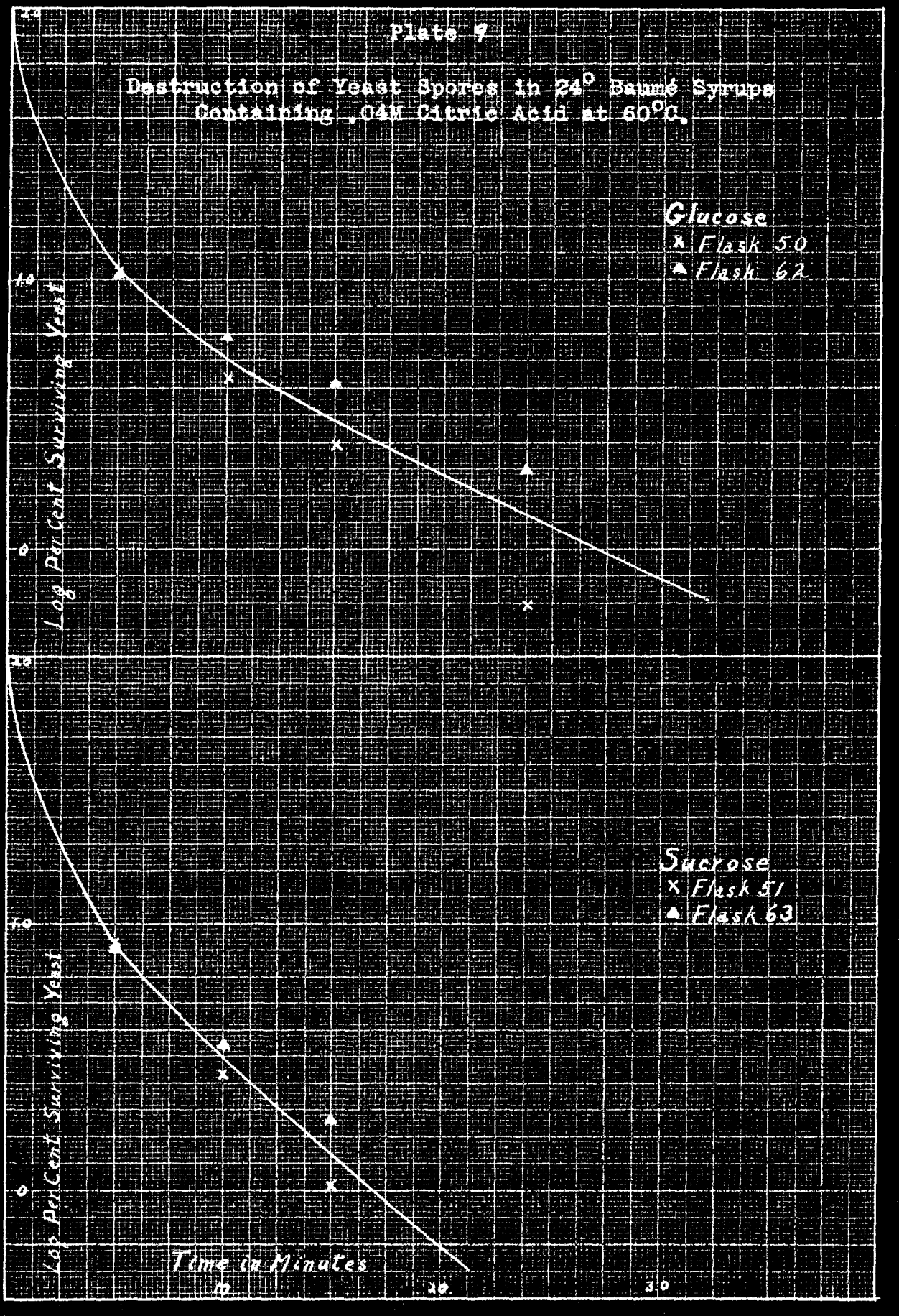


Plate 9
Destruction of Yeast Spores in 24° Baumé Syrups
Containing .04M Citric Acid at 60°C.
Glucose
X Flask 50
▲ Flask 62
Log Per Cent Surviving Yeast
10
0
Sucrose
X Flask 51
▲ Flask 63
Log Per Cent Surviving Yeast
10
0
Time in Minutes
10
20
30

TABLE X

Destruction of Yeast Spores in 27° Baumé Syrups Containing .02M Citric Acid at 60°C.

Sugar	Glucose		Sucrose	
Flask Number	46	68	47	69
Date of Experiment	7/14/31	7/23/31	7/14/31	7/23/31
Time Elapsed	Surviving Yeast per cc. of Syrup			
Initial	57,000	53,000	57,000	53,000
5 min.	14,000	15,000	8,800	8,000
10 "	6,500	5,200	4,000	5,200
15 "	5,350	4,000	1,900	2,200
25 "	2,000	1,900	900	1,200
40 "	—	700	—	500
	Per Cent Surviving Yeast			
Initial	100.0	100.0	100.0	100.0
5 min.	24.56	28.30	15.44	15.09
10 "	11.40	9.81	7.02	9.81
15 "	9.38	7.55	3.33	4.15
25 "	3.51	3.58	1.58	2.26
40 "	—	1.32	—	0.94

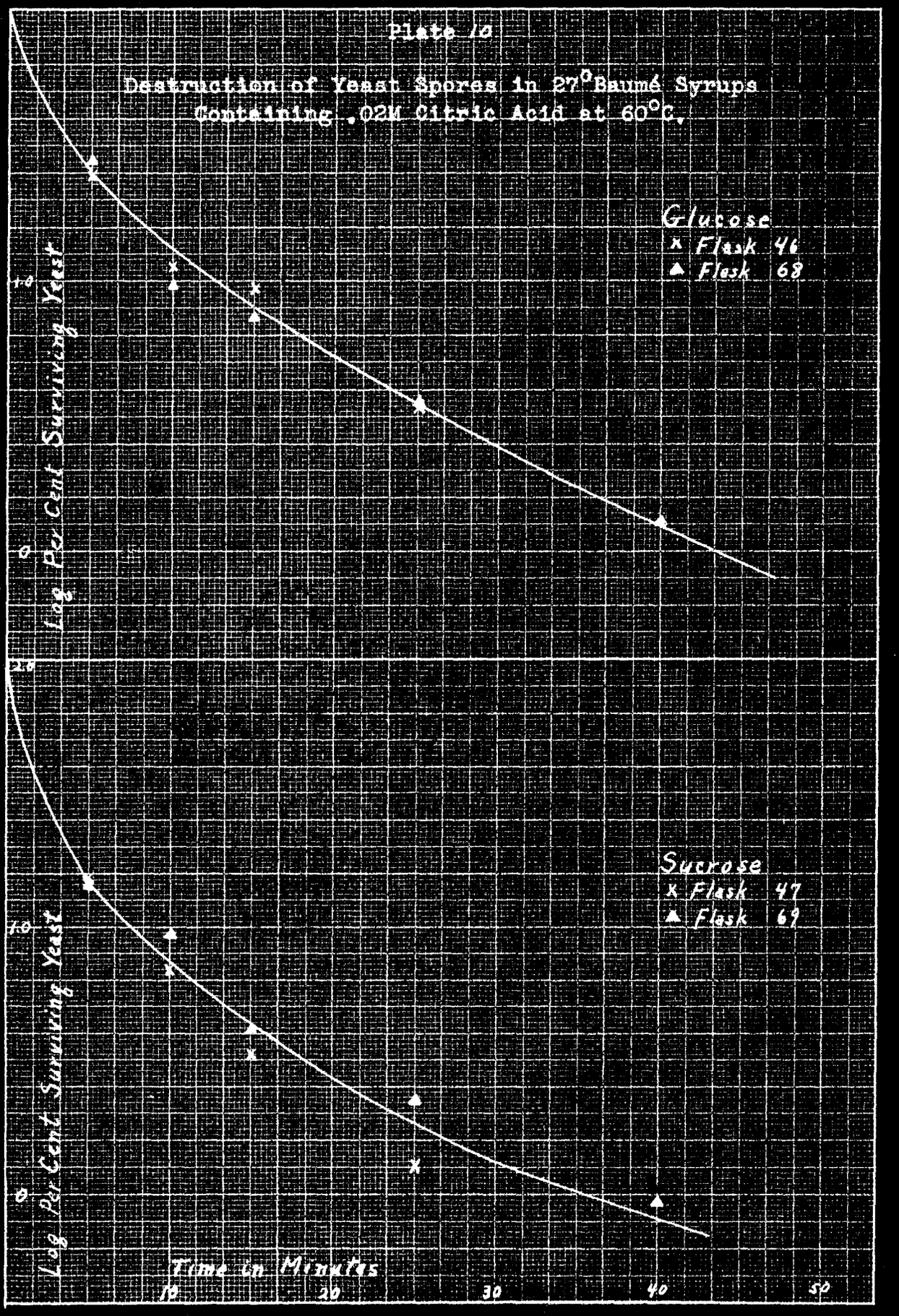


Plate 10
Destruction of Yeast Spores in 27° Baumé Syrups
Containing .02M Citric Acid at 60° C.
Glucose
x Flask 46
▲ Flask 68
Log Per Cent Surviving Yeast
1.0
0.5
Sucrose
x Flask 47
▲ Flask 69
Log Per Cent Surviving Yeast
1.0
0
Time in Minutes
10
20
30
40
50

TABLE XI

Destruction of Yeast Spores in 27° Baumé Syrups Containing .04 M Citric Acid at 60°C.

Sugar	Glucose		Sucrose	
Flask Number	58	66	59	67
Date of Experiment	7/20/31	7/23/31	7/20/31	7/23/31
Time Elapsed	Surviving Yeast per cc. of Syrup			
Initial	40,000	53,000	40,000	53,000
5 min.	6,500	8,000	4,600	7,000
10 "	4,200	3,400	2,300	2,700
15 "	2,900	2,600	1,600	1,700
25 "	1,200	1,200	780	800
	Per Cent Surviving Yeast			
Initial	100.0	100.0	100.0	100.0
5 min.	16.25	15.1	11.5	13.21
10 "	10.52	6.41	5.75	5.09
15 "	7.25	4.9	4.0	3.21
25 "	3.0	2.26	1.95	1.51

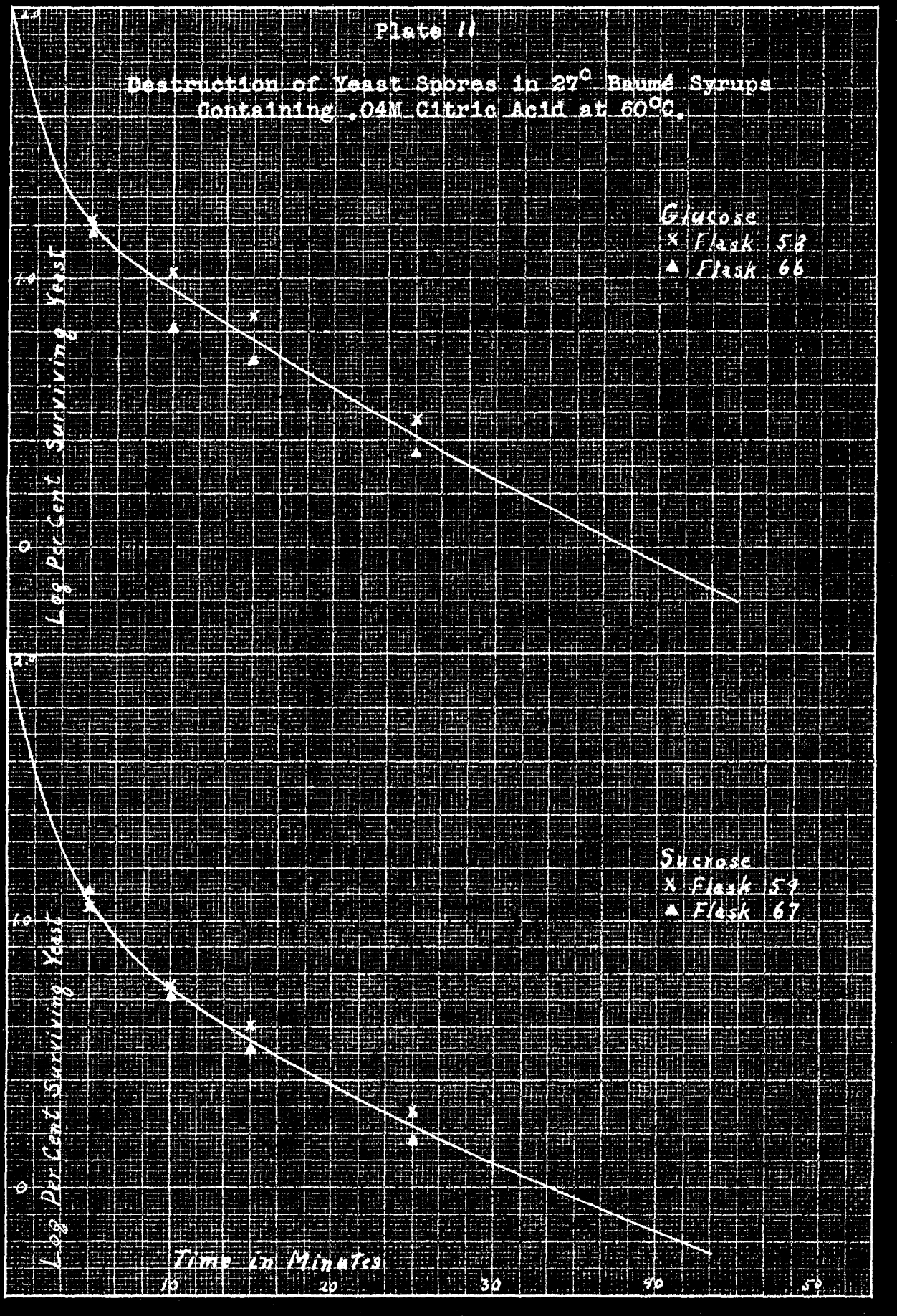


Plate II
Destruction of Yeast Spores in 27° Baumé Syrups
Containing .04M Citric Acid at 60°C.
Glucose
x Flask 58
▲ Flask 66
Sucrose
x Flask 59
▲ Flask 67
Log Percent Surviving Yeast
Time in Minutes
10
20
30
40
50

DISCUSSION

Plates I, II and III bring out the fact that glucose and sucrose, compared at densities of 20°, 24° and 27° Baumé, show a decided difference in their actions toward yeast. In all concentrations, sucrose supported growth much better than glucose. The lag period for glucose varied from a period of two to three days in 20° Baumé glucose to five to seven days in 27° Baumé glucose. In 20° Baumé sucrose solutions there was no noticeable lag period; 24° Baumé sucrose showed a lag period of less than 24 hours; and 27° Baumé sucrose showed a distinct period of no growth during the first 24 hours.

An interesting fact shown in tables I, II and III, but which is not brought out in the corresponding plates, is the amount of decrease in the number of yeasts in the glucose syrups during the first few days of the experiment. In 20° Baumé syrups, the lowest number to which the yeast count dropped was 62% of the initial; 24° Baumé showed the same minimum; and in 27° Baumé the count dropped to 50% of the initial.

This initial killing is probably due to the effect of high osmotic pressure on the vegetative cells. The length of time elapsing before growth takes place in heavy glucose syrups may be attributed to the time that it takes the cells to become acclimated to the high osmotic pressure. There appears to be a relationship between the osmotic pressure of

the solutions and the length of the lag period. This is brought out in the following table.

TABLE XII

Relation of Calculated Osmotic Pressure to Lag Period in Sugar Syrups

Density of Sugar (Baumé)	Sugar	Osmotic Pressure (Atmospheres)	Lag Period
27°	Glucose	113	5 - 7 days
24°	"	89	3 "
20°	"	66	2 "
27°	Sucrose	65	1 "
24°	"	51	Distinguishable
20°	"	38	Negligible

However, the difference between the osmotic pressure of 20° Baumé glucose and 27° Baumé sucrose and the length of the lag period seems to indicate that there is some other factor involved that is peculiar to the sugar used.

Table IV shows that the addition of .04M citric acid to 27° Baumé syrups prevents the growth of yeast spores. The curves in plate 4 show a slight difference between the two sugars, but since the period of storage extends for such a long time (11 days) this difference is not significant.

The effect of varying concentrations of citric acid in

syrups is brought out very sharply by using vegetative cells instead of spores. Table V and plate 5 show that it is easier to sterilize glucose syrups containing acid than sucrose syrups containing the same amount of acid.

The slow killing of the cells in sucrose containing .04M citric acid does not check with the rather rapid killing that Toulouse (24) obtained. However, it must be remembered that he used a single culture of yeast when inoculating his broth.

The killing of the vegetative cells in this experiment appears to be due to a combination of three factors - osmotic pressure, temperature and citric acid concentration. The effect of osmotic pressure has been discussed above. The temperature factor can not be great since it is but five degrees above the optimum (28°C.). However, the effect of pH alone can not account for the curves, because a glucose solution of this density containing .02M citric acid has a pH of 2.12, while sucrose containing .04M citric acid had a pH of 2.05; yet it killed more slowly than the former solution. However, in comparing the two acid concentrations in each sugar, the effect of citric acid is brought out clearly - the higher acid concentration killing more rapidly than the lower.

Tables VI and VII show the effect of temperature on the yeast spores in the sugar solutions. An entirely opposite effect for the two sugars from that at room temperature is brought out in these tables and in plates 6 and 7. While sucrose was more favorable toward the growth of yeast spores

than glucose at 28°C., at higher temperatures (60°C.) the former sugar is a more favorable medium for killing the spores.

TABLE XIII

Killing Times for 99% of Yeast in Syrups at 60°C.

Density (Baumé)	Sugar	Time to Kill 99% of Spores
24°	Glucose	72 minutes
	Sucrose	52 "
27°	Glucose	106 "
	Sucrose	90

This table shows that in both concentrations of sugar employed, yeast spores may be killed more rapidly in sucrose than in glucose. This is in accord with other observed facts. It is much easier to kill spores by moist heat than by dry heat. Hence, the amount of water and the osmotic pressure of the solution will be the deciding factors in the killing of the yeast spores. The higher the osmotic pressure, the greater will be the tendency on the part of the solutions to hold the water, thus preventing the passage of water into the cell. There is a more or less protecting action of the sugars on the killing of yeasts because of the fact that a solution of tap water showed a killing time of 5.2 minutes

for 99% of the yeast at 60°C.

This relationship between osmotic pressure and killing times does not hold exactly when comparing different sugars. For example, a 27° Baumé sucrose solution with an osmotic pressure of 65 atmospheres shows a killing time of 90 minutes, while a 24° Baumé glucose solution with a higher osmotic pressure (89 atmospheres) shows a killing time of 72 minutes. This seems to indicate, as in the experiments conducted at room temperature on growth of yeasts in syrups, that there is some specific property of the sugar influencing the growth and death of yeasts.

Plates 8 - 11 show that with the addition of citric acid to these solutions a much shorter killing time is obtained.

TABLE XIV

Killing Time of 99% of Yeast Spores in Syrups Containing Citric Acid at 60°C.

Density (Baumé)	Sugar	Citric Acid Concentration	Time to Kill 99% of Yeasts
24°	Sucrose	.02M	23 minutes
		.04M	17 "
	Glucose	.02M	34 "
		.04M	27 "
27°	Sucrose	.02M	35 "
		.04M	33 "
	Glucose	.02M	43 "
		.04M	38 "

Here again, shorter killing times are always obtained in sucrose solutions than in the corresponding solutions of glucose. Evidently the lower pH of the glucose solution (less than .1) is not great enough to overcome the greater protecting action of osmotic pressure.

The difference in killing time of the varying amounts of acid concentration in each sugar is emphasized more in the 24° Baumé syrups than in the 27° Baumé syrups. The addition of twice as much acid lowers the killing times about 20-25% in 24° Baumé syrups, while at 27° Baumé, the killing times decrease about 10%.

SUMMARY AND CONCLUSIONS

1. At lower temperatures (28°C.) glucose syrups have a greater inhibiting action toward the growth of yeast spores than sucrose syrups of the same density.

2. There is no difference between the actions of 27° Baumé glucose and sucrose solutions toward yeast spores upon the addition of .04M citric acid at 28°C.

3. Concentrations of .02M and .04M citric acid in 27° Baumé solutions are toxic toward vegetative yeast cells to a greater extent in glucose than in sucrose at low temperatures.

4. At high temperatures (60°C.), yeast spores are killed more easily in sucrose solutions than in glucose solutions of the same densities.

5. Upon the addition of small amounts of citric acid to sugar solutions, yeast spores are killed in a shorter length of time, but the time is shorter in solutions of sucrose than in glucose.

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