THE PRESERVING AND GERMICIDAL ACTION OF VARIOUS SUGARS AND ORGANIC ACIDS ON YEASTS AND BACTERIA

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Introduction*

Sugars and organic acids, either natural or added, play an important role in food preservation. The amount and kind of sugar or organic acid to be used for best results frequently is in question. Many food manufacturers consider all acids and sugars equally valuable for preserving purposes. The influence of sugars, acids, and a combination of the two on microorganisms has not been studied extensively. The great expansion of the food industry in recent years and the emphasis being placed upon the microbiological aspects of food indicated the need for such a study.

Literature Review

Influence of Sugars

One of the earlier workers in this country on the preserving action of sugars was Bitting (2), who in 1909 studied the effect of sugar on both molds and yeasts in tomato juice. He found no effect until the concentration of sugar had reached 25 grams per 100 ml., at which point growth of both yeasts and molds occurred as readily but less abundantly than at lower concentrations. There was less development as the sugar was increased up to a concentration of 40 grams. The yeast was completely inhibited in concentrations above 80 grams per 100 ml. Mold growth became slower up to 170 grams per 100 ml. and above that concentration required two months to develop.

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In 1919, Sackett (16), while studying the longevity of members of the colon typhoid group in pure honey, found that Eberthella typhosa remained alive for 48 hours in pure honey, but was dead in 24 hours in dilutions above 50 per cent but in a 10 per cent dilution was sterile after 4 days. Sackett believed the failure of the organism to die out as readily in concentrated honey as in the diluted solutions to be due to the fact that the former is a saturated colloidal solution, therefore, having a low osmotic pressure and in such a solution the plasmolysis would take place relatively slowly. When water was added, some of the sugar would form a molecular solution increasing the osmotic pressure and thereby increase the rate of plasmolysis.

Fabian and Quintet (5) found that 21 per cent moisture was the critical point for fermentation in honey. Honey containing less moisture than this rarely fermented while a greater amount of moisture than this usually caused trouble.

In 1926, Pederson and Breed (15) showed a sugar concentration of 35 per cent was ineffective as a preservative in ketchup as it inhibited only certain types of microorganisms found in spoiled tomato products. It was found, however, that combinations of sugar and salt were effective. A combination of 15 per cent sugar and 3.5 per cent of salt was sufficient to stop the growth of all organisms except one, yeast. Combinations of sugar or salt with acid, on the other hand, did not lower appreciably the amount of acid required.

Nunheimer and Fabian (12) found that dextrose exerted an inhibiting effect in a concentration of 30 to 40 per cent and a germicidal effect at 40 to 60 per cent when using typical strains of food poisoning staphylococci, whereas, sucrose was less active and a concentration of 50 to 60 per cent was required for inhibition and 60 to 70 per cent for germicidal action. In this work the sugar concentrations were made up by volume.

Influence of Acids

As early as 1898 Kahlenberg and True (8) found that many of the weaker organic acids were antiseptic and bactericidal at pH values far higher than the highly dissociated acids. They suggested that the undissociated molecules and the anions may exert a toxic effect in the case of the weaker acids.

Clark (3) observed that acetic acid at a dilution which was only two per cent ionized showed a higher retarding effect than highly dissociated acids on the germination of the spores of a group of filamentous fungi. He attributed the activity of the weakly dissociated acids to the undissociated molecule.

Kronig and Paul in 1897 (9) carried out experiments upon the disinfectant action of various salts, bases and acids upon _Staphylococcus aureus_ and the spores of _Bacillus anthracis_. They found that the number of organisms, or spores, which developed after treatment for a given time varied inversly with the amount of dissociation. Solutions of mercuric chloride, silver nitrate, etc., in alcohol, where no dissociation occurs, showed almost no disinfectant action. The investigators concluded that there is a general relation between the action of the acids and the amount of dissociated hydrogen ions present; but there appeared many exceptions to a strict parallelism. The authors, however, attributed these exceptional effects to the anion or the undissociated molecule.

In 1902 Bial (1) made a study of the antiseptic action of the hydrogen ion of dilute acids upon yeasts. The yeasts were cultivated in fermentation tubes filled with grape-sugar solution to which various amounts of acid had been added and the antiseptic action was inversely registered by the amount of gas produced. Bial did not make exact calculations of the amount of dissociated hydrogen necessary to inhibit the yeast, but found that a general relation existed between the ionization and the antiseptic action. The highly dissociated acids, -hydrochloric, sulfuric, nitric and trichloracetic-, entirely stopped the action of yeast in concentrations between 0.005 and 0.008 normal. Acids of intermediate dissociation, -phosphoric, formic, oxalic-, accomplished the same effect at 0.01 normal; while acids still less dissociated, -acetic, benzoic, and butyric-, stopped all fermentation only when 0.04 to 0.07 normal. The most striking feature of Bial's work was a series of experiments showing the diminution of the antiseptic action of acids by the addition of neutral salts whose action is to decrease the dissociation of the acidic hydrogen. A solution of 0.01 normal formic acid and 0.3 normal sodium formate showed active fermentation. The same action was noticed with salts of the other acids used.

In 1906 Winslow and Lochridge (18) found the mineral acids, hydrochloric and sulfuric, were fatal to <u>Escherichia coli</u> and <u>Eberthella typhosa</u> in concentrations at which they are highly dissociated. Their action runs parallel, not to their normal strength, but to the number of free hydrogen ions per unit volume. The organic acids, acetic and benzoic, are fatal to the typhoid and colon bacilli at a strength at which they are only slightly dissociated. The effect here appears to be due to the whole molecule and is specific for each acid, acetic having only 10-20 per cent the toxicity of benzoic.

Paus (14) in his work with media for Escherichia coli and Eberthella typhosa concluded that there was little relation between the hydrogen ion concentration and growth, but that the kind of acid as well as the acidity was responsible for the germicidal value.

In 1912 Johannessohn (7) found that yeast fermentation is increased in the presence of acetic acid and in its higher homologs when in a sufficiently dilute solution. He concluded that the action of the acid depends chiefly on the undissociated molecule and not on the ions.

Wolf and Harris (19) were of the opinion that the degree of acidity rather than the nature of the acid was the controlling factor in the germicidal and antiseptic actions of the acids.

Norton and Hsu in 1916 (13) found that acids act as disinfectants through the agency of the hydrogen ions produced by electrolytic dissociation. They also found that the addition to an acid of a salt containing an anion common to this acid, dimishes its disinfecting power, as a result of a decrease in the hydrogen ion concentration and an increase in the concentration of the undissociated acid molecules. They came to the conclusion that the disinfecting power of an acid is approximately proportional to the hydrogen ion concentration.

Pederson and Breed (15) found that one per cent of acetic acid was required to stop growth of all the bacteria and yeasts isolated from spoiled tomato products.

Reid in 1932 (16) concluded that the resistance of Bacillus pyocyaneous to acids is not constant, but varies with the kind of acid. The mono-basic acids, the least dissociated of the acids used, inhibited growth at a much lower H-ion concentration than the highly dissociated acids such as oxalic. A wide difference was found to exist between the ability of an acid to exert a bactericidal effect and to inhibit growth as acids which were strongly bactericidal frequently exhibited weak inhibiting powers in liquid media.

A study of the preserving value of acetic and lactic acids by Fabian and Wadsworth (6) showed the preserving value of acetic was superior to that of lactic acid. This confirmed the work of Fabian and Johnson (4) who found that 0.2 acetic was equivalent to 0.3 per cent lactic acid in their action on certain pectin decomposing bacteria.

Levine and Fellers (10) showed that acetic acid was more toxic than either lactic or hydrochloric acid to Salmonella aertrycke, Saccharomyces cereviseae, and Aspergillus niger. These organisms were inhibited or destroyed at a higher pH value with acetic acid than with lactic or hydrochloric acids. The mold utilized relatively high amounts of lactic acid to develop a growth heavier than that obtained from the acetic acid or the hydrochloric acid series.

In studies on food poisoning staphylococci Nunheimer and Fabian (11) found the decreasing order of germicidal action of the acids studied to be acetic > citric > lactic > malic > tartaric > hydrochloric. The decreasing order of antiseptic action was found to be acetic > lactic > citric >

malic > tartaric > hydrochloric. They concluded that although the action of the highly dissociated mineral acid is due mainly to the hydrogen ion concentration, the organic acids exerted a germicidal and antiseptic effect disproportionate to the hydrogen ion concentration produced. Therefore, it is apparent that the observed effects are due to factors in addition to the hydrogen ion, presumably either the un-ionized molecule or the anion or both.

Influence of Sugar plus Acids

Not much information is available on the effect of concentrated sugar solutions plus acids upon bacteria.

Levine and Fellers (11) found that appart from the indirect effect in altering the hydrogen-ion, the salt and sugar aided the acetic acid but little in its toxic effect on bacteria and yeast. Similarly, the added salt and sugar exerted little, if any, effect on the minimum percentage required for total destruction of these organisms.

The results secured by Nunheimer and Fabian (12) did not agree with the above work. They found that the amount of dextrose which was required to exert a germicidal action could be reduced 50 per cent when used in the presence of one-half the inhibiting concentration of acid. On the other hand, sodium chloride and sucrose could be reduced 30 and 20 per cent respectively and still bring about a germicidal effect.

Experimental Work

In studying the effects of the sugars and acids, the following bacteria and yeasts were used.

Organism	Source of culture	Opt. temp. of growth
1. *Bacillus calidolactis*	Hammer collection Iowa State College	55° C.
2. *Bacillus coagulans*	Hammer collection Iowa State College	55° C.
3. Thermophile--milk powder	Isolated from milk powder	55° C.
4. Thermophile--raw milk	Isolated from raw milk	55° C.
5. *Streptococcus lactis*	Hammer collection Iowa State College	35° C.
6. *Streptococcus liquefaciens*	Hammer collection Iowa State College	25° C.
7. *Saccharomyces ellipsoideus*	Tanner collection Univ. of Illinois	25° C.
8. *Saccharomyces cerevisiae*	Tanner collection Univ. of Illinois	25° C.
9. *Zygosaccharomyces mellis*	Tanner collection Univ. of Illinois	25° C.
10. *Torula lactis-condensi*	Tanner collection Univ. of Illinois	25° C.
11. Yeast--pickles	Isolated from sweet pickles	25° C.

The basic medium used for the bacteria and the yeasts had the following composition:

For bacteria

15 grams--Bacto peptonized milk
1.5 grams--Bacto yeast extract
1.5 grams--Bacto meat extract
1000 ml.--water
pH 6.4

For yeasts

30 grams--Trommer's plan malt extract
1 gram--NH_4Cl
1 gram--K_2HPO_4
30 ml.--N/10 citric acid
1000 ml.--water
pH 5.4

The acids were made up in normalities ranging from 0.1 to 5 and were sterilized by filtration. They were then added in various amounts to sterile test tubes and made up to 10 ml. with sterile broth. The sugars were made up by weight in 100 gram quantities in the respective broths and sterilized by autoclaving at 12 pounds for fifteen minutes. They were then pipetted in 10 ml. portions to sterile test tubes. The test tubes were then implanted with either bacteria or yeasts and incubated at the optimum temperature for the organism. At intervals the number of viable organisms was determined by the plate method using Standard milk agar for the bacteria and wort agar for the yeast.

The H-ion concentration was determined with a Beckman pH meter.

Influence of Sugars on the Various Bacteria and Yeasts

Fructose, dextrose, lactose, and sucrose, were used in determining the effect of the sugars upon the organisms. They were added to sterile broth and autoclaved at 12 pounds in order to keep the hydrolysis at a minimum. All of the sugar concentrations were made up by weight. The highest concentration used for lactose was 30 per cent and for sucrose 60 per cent as above these percentages crystallization of the sugars took place in two or three days.

Using a sterile 10 ml. pipette, ten ml. portions of each concentration were placed in sterile test tubes. The tubes were inoculated with one or two drops of an actively growing 24 hour culture from a 5 ml. pipette, depending on the growth in the broth. For the bacteria, the range of the inoculum was such that when a drop or two was added to 10 ml. of sugar solution there was from 50,000 to 150,000 organisms per ml., and for the yeast from 100,000 to 200,000 per ml.

These tubes were incubated at the optimum temperature for each organism; and at the end of seven days a sub-culture of 0.5 ml. was transferred into broth to determine the presence or absence of growth. The preserving percentage was taken as that percentage where there was no growth in the original but there was growth in the sub-culture broth. The germicidal percentage was that in which there was no growth in the original tube or in the sub-cultured broth. All experiments were repeated until four checks had been obtained. The results are found in Tables 1-4.

After determining the germicidal concentration of the various sugars, the work was repeated and at intervals the number of viable organisms was determined by plate count using Standard milk agar for the bacteria and wort agar for the yeasts.

Table 1. Per cent of fructose, dextrose, lactose, and sucrose exerting a preserving effect on the various bacteria.

Bacteria	Per cent			
	Fructose	Dextrose	Lactose	Sucrose
Strept. lactis	25.0	30.0-32.5	None*	50-60
Strept. liquefaciens	25.0	30.0-32.5	None	50-60
Bacillus coagulans	>15.0 <17.5	25.0	None	45.0
Bacillus calidolactis	>15.0 <17.5	25.0	None	40.0-45.0
Thermophile--milk powder	15.0	25.0	None	>42.5<45.0
Thermophile--raw milk	>15.0 <17.5	>25.0<27.5	None	>42.5-45.0

*None=could not get high enough concentration of lactose in solution to be preserving.

Table 2. Per cent of fructose, dextrose, lactose, and sucrose exerting a germicidal effect on the various bacteria.

Bacteria	Per cent			
	Fructose	Dextrose	Lactose	Sucrose
Strept. lactis	27.5	35.0	None*	None*
Strept. liquefaciens	27.5	35.0	None	None
Bacillus coagulans	17.5	27.5	None	47.5
Bacillus calidolactis	17.5	27.5	None	47.5
Thermophile--milk powder	17.5	27.5	None	45.0
Thermophile--raw milk	17.5	27.5	None	47.5

*None=could not get a high enough concentration of sugar in solution to be germicidal. Saturated solution of lactose (30%) and sucrose (60%) did not kill in 7 days.

Table 3. Per cent of fructose, dextrose, lactose, and sucrose exerting a preserving effect on the various yeasts.

Yeast	Per cent			
	Fructose	Dextrose	Lactose	Sucrose
Sacch. cerevisiae	45.0	42.5-47.5	None*	57.5
Sacch. ellipsoideus	42.5-45.0	45.0	None	60.0
Zygo. mellis	45.0-55.0	47.5-55.0	None	55.0-60.0
Torula lactis-condensi	55.0	55.0	None	60.0
Yeast--pickles	52.5-55.0	52.5-55.0	None	60.0

* None=could not get high enough concentration of lactose in solution to be preserving.

Table 4. Per cent of fructose, dextrose, lactose, and sucrose exerting a germicidal effect on the various yeasts.

Yeast	Per cent			
	Fructose	Dextrose	Lactose	Sucrose
Sacch. cerevisiae	47.5	50.0	None*	60.0
Sacch. ellipsoideus	47.5	47.5	None	None*
Zygo. mellis	60.0	60.0	None	None
Torula lactis-condensi	60.0	60.0	None	None
Yeast--pickles	60.0	60.0	None	None

*None=could not get a high enough concentration of sugar in solution to be germicidal. Saturated solution of lactose (30%) and sucrose (60%) did not kill in 7 days.

Discussion

Table 1 shows the order of the preserving action of the sugars on the bacteria is fructose > dextrose > sucrose > lactose. From 15 to 17.5 per cent of fructose was required to exert a preserving action compared with 25 to 32.5 per cent dextrose, and 40 to 60 per cent of sucrose. Lactose, with a solubility of only 30 per cent, did not have any preserving action. This table also shows that the preserving percentage varies with the different bacteria. For example, *Streptococcus lactis* and *Streptococcus liquefaciens* required 27.5 per cent fructose while the thermophiles required only 17.5 per cent fructose. This same relationship held true for dextrose where the preserving percentage for the milk streptococci was 35 and for the thermophiles 27.5 per cent, and likewise for sucrose where the former group had a preserving percentage of 60 while the latter group was inhibited at 47.5 per cent.

Table 2 shows that the order of the germicidal action of the sugars was the same as the preserving action with the exception of *Streptococcus lactis* and *Streptococcus liquefaciens*, where sucrose did not exert any germicidal action. Except for these two organism, there was only a difference of 2.5 per cent between the germicidal and preserving concentrations. That *Streptococcus lactis* and *Streptococcus liquefaciens* are more resistant to sugars than the thermophiles is again demonstrated since the former required a higher concentration of all sugars for a germicidal action than the later.

Table 3 shows that the yeasts are more tolerant than bacteria to the sugars since it required 45 to 60 per cent to bring about a preserving action with yeasts, whereas, bacteria required only 15 to 45 per cent. The order of activity of the sugars for the yeasts differed slightly from that of the bacteria in that fructose and dextrose exerted a preserving action at the same concentration of 45 to 55 per cent, while 60 per cent of sucrose was required to bring about the action.

Table 4 shows that only fructose and dextrose exerted a germicidal action on the yeasts. These sugars required a concentration of 47.5 to 60 per cent to kill all of the yeasts while a concentration of 60 per cent sucrose was germicidal to only one yeast, _Saccharomyces cerevisiae_.

The graphs in Figures 1-11 and the data in Tables 16-26 indicate there is a difference in the action of the sugars on the viability of bacteria and yeasts. They show that the viability of _Streptococcus lactis_, _Streptococcus liquefaciens_ and all of the yeasts continually decreased during the seven day period in the germicidal percentage of dextrose and fructose, while the thermophiles were killed within the first 24 hours.

The fact that both dextrose and fructose exerted a germicidal effect at a lower concentration than sucrose and lactose may be explained by the fact that the action of each, is at least in part, due to plasmolysis of the microbial cells. Since this effect depends upon the number of particles present in solution, it is natural that fructose and dextrose, each with a molecular weight of 180, would contain more molecules per unit weight than would sucrose and lactose with molecular weights of 342. Therefore, the activity should tend to decrease as the molecular weight increases.

Since fructose and dextrose have the same molecular weight, the difference in reactivity of these sugars in respect to bacteria must be explained on a chemical rather than a physical basis. The difference in their action on bacteria may be explained by the fact that dextrose is an aldehyde sugar while fructose is a keto sugar. It is a well known fact in biochemistry that fructose is more reactive than dextrose since many of the chemical tests may be carried out in the cold with fructose while heat has to be applied in the case of dextrose. Another factor influencing the reactivity of the two sugars is that fructose has the reactive radical nearer the center of the molecule than dextrose which increases its reactivity as well as its solubility over that of dextrose. These differences in chemical structure apparently account for the chemical as well as the biological differences noted in the two sugars.

Influence of Acids on the Various Bacteria and Yeasts

In order to study the preserving and germicidal action of the acids on the bacteria and yeasts, the acids were made up in normalities ranging from 0.1 to 5 and were sterilized by filtration. They were then added in various amounts to sterile test tubes and made up to 10 ml. with sterile broth. The tubes were inoculated with one or two drops, depending on the amount of growth in the broth, of an actively growing broth culture. For the bacteria, the range of the inoculum was such that when a drop or two was added to 10 ml. there was from 50,000 to 150,000 organisms per ml. and for the yeast from 100,000 to 200,000 per m.

The tubes were incubated at the optimum temperature for each organism and at the end of seven days, a sub-culture of 0.5 ml. was transferred into suitable broth to determine the presence or absence of growth. The preserving quantity of acid was taken as that quantity where there was no visible growth in the original broth contraining the acid but where there was growth in the sub-culture broth containing no acid. The germicidal quantity was that in which there was no growth in the original tube containing the acid or in the sub-cultured broth containing no acid. The results are found in Tables 5-9.

After determining the germicidal quantity of the various acids, the work was repeated and at intervals the number of viable organisms was determined by plate count using Standard milk agar for the bacteria and wort agar for the yeasts.

Table 5. The number of milliliters of acid necessary to exert a preserving effect on the various bacteria.

Bacteria	Mls. of acid made up to 10 mls. with broth		
	Acetic	Citric	Lactic
Strept. lactic	1.5-2.25 (0.3N)	1.5-2.25 (0.3N)	1.0-1.75 (0.2N)
Strept. liquefaciens	1.5-2.25 (0.2N)	1.5-2.75 (0.2N)	1.5(0.2N)
Bacillus coagulans	2.1(0.1N)	1.7(0.1N)	1.1(0.1N)
Bacillus calidolactis	1.7(0.1N)	1.4(0.1N)	1.1(0.1N)
Thermophile-milk powder	2.1(0.1N)	1.4(0.1N)	1.25(0.1N)
Thermophile-raw milk	2.9(0.1N)	1.8(0.1N)	1.25(0.1N)

Table 6. The number of milliliters of acid necessary to exert a germicidal effect on the various bacteria.

Bacteria	Mls. of acid made up to 10 mls. with broth		
	Acetic	Citric	Lactic
Strept. lactis	2.5(0.3N)	2.5(0.3N)	2.0(0.2N)
Strept. liquefaciens	2.5(0.2N)	3.0(0.2N)	1.75(0.2N)
Bacillus coagulans	2.25(0.1N)	2.0(0.1N)	1.25(0.1N)
Bacillus calidolactis	2.0(0.1N)	1.5(0.1N)	1.25(0.1N)
Thermophile-milk powder	2.25(0.1N)	1.5(0.1N)	1.4(0.1N)
Thermophile-raw milk	3.0(0.1N)	1.9(0.1N)	1.4(0.1N)

Table 7. The number of milliliters of acid necessary to exert a preserving effect on the various yeasts.

Yeast	Mls. of acid made up to 10 mls. with broth		
	Acetic 0.5N	Citric	Lactic 1N
Sacch. cerevisiae	1.0-1.5	2.0(1N)-3.0(5N)	2.0-3.75
Sacch. ellipsoideus	1.25-1.75	2.0(1N)-1.5(5N)	1.75-3.75
Zygo. mellis	1.5	2.75(1N)-2.5(5N)	1.75-3.25
Torula lactis-condensi	1.5-1.75	3.0(1N)-2.5(5N)	2.0-4.0
Yeast--pickles	1.25-2.0	2.5(1N)-4.0(5N)	2.25-4.5

Table 8. The number of milliliters of acid necessary to exert a preserving effect on the various yeasts.

Yeast	Mls. of acid made up to 10 mls. with broth		
	Acetic 0.5N	Citric 5N	Lactic 1N
Sacch. cerevisiae	1.75	3.5	4.0
Sacch. ellipsoideus	2.0	2.0	4.0
Zygo. mellis	1.75	3.0	3.5
Torula lactis-condensi	2.0	3.0	4.5
Yeast--pickles	2.25	4.5	5.0

Table 9. The pH values of the various acids for the various bacteria and yeasts at the points where there is growth, preserving and germicidal action.

Organism	Acetic Acid			Citric Acid			Lactic Acid		
	Growth	Preserving	Germicidal	Growth	Preserving	Germicidal	Growth	Preserving	Germicidal
Strept. lactis	4.27	4.12-3.94	3.89	3.96	3.81-3.54	3.4	4.46	3.96-	3.46
Strept. liquefaciens	4.31	4.23-4.13	4.09	4.41	4.13-3.63	3.38	3.96	3.63	3.55
Bacillus coagulans	4.52	4.5	4.48	4.5	4.48	4.32	4.4	4.34	4.3
Bacillus calidolactis	4.62	4.6	4.52	4.68	4.52	4.5	4.4	4.34	4.3
Thermophile-milk	4.52	4.5	4.48	4.68	4.52	4.5	4.34	4.3	4.2
Thermophile-raw milk	4.37	4.32	4.29	4.49	4.46	4.42	4.34	4.3	4.2
Sacch. cerevisiae	4.01	3.79-3.6	3.52	2.28	2.23-1.73	1.7	2.4	2.39-2.21	2.2
Sacch. ellipsoideus	3.79	3.68-3.52	3.48	2.28	2.23-1.92	1.82	2.42	2.4-2.21	2.2
Zygo. mellis	3.79	3.6	3.52	2.17	2.13-1.79	1.73	2.42	2.4-2.27	2.24
Torula lactis-condensi	3.68	3.6-3.52	3.48	2.13	2.1-1.79	1.73	2.4	2.39-2.2	2.17
Yeast-pickles	3.79	3.68-3.48	3.42	2.2	2.17-1.68	1.65	2.39	2.38-2.17	2.12

Discussion

Two methods may be used to determine the preserving and germicidal value of the acids. If the same method is used for evaluating the preserving action of acids as was used for sugars, viz., the number of grams of each acid in 10 mls. of broth, then the preserving value of the acids for the bacteria is lactic > acetic > citric (Table 5). If, however, the preserving values of the acids are based on pH (Table 9), the order is as follows: acetic > citric > lactic.

The germicidal value of the acids (Table 6) is as follows: lactic > acetic > citric if the number of grams of acid in 10 mls. of broth is used as the basis of comparison, whereas, if pH is used as the basis the order is acetic > citric > lactic. The results secured with the acids showed that the same relationship existed between the streptococci and the thermophiles as was found with the sugars. The streptococci were considerably more resistant to acids than were the thermophiles.

On the basis of pH, the order of activity of the acids agrees with the findings of Levine and Fellers (10) and Nunheimer and Fabian (11) since they compared the acids in relation to their pH and not on the basis of the amount of acid added.

The order of the activity of the acids on the basis of pH is in keeping with the dissociation constants of the acids as acetic acid with a dissociation constant of 1.86×10^{-5} takes more acid to lower the pH than does citric acid with a dissociation constant of 1.38×10^{-4} or lactic acid with a dissociation constant of 8.0×10^{-4}. These pH values show that each acid does not depend on the hydrogen-ion activity alone for its action on the bacteria. For example, acetic acid with a lower hydrogen-ion must depend on the un-ionized molecule and the acetate radical as well as the hydrogen-ion of the acid.

The yeasts were more tolerant to the acids than the bacteria since it required an acid strength of 0.5 to 5 normal (Table 7) to bring about a preserving action while the bacteria (Table 5) required a normality of only 0.1 to 0.3. The germicidal quantity (Table 8) of the acids was likewise greater for yeasts than for bacteria. (Table 6). However, the order of effectiveness of the acids in the preserving and germicidal range was acetic > lactic > citric for the yeasts. Furthermore, the order remained the same irrespective of whether it was based on pH or the per cent acid added. There was a considerable difference in the pH needed to kill the yeasts as they were killed at pH 3.5 in the case of acetic acid and at pH 1.75 for citric acid.

Influence of Mixtures of the Preserving Quantity of the Acids with the Sugars

A study was next made of a combination of the acids with the sugars to determine the influence of the various combinations of acids and sugars upon the different microorganisms. For this purpose, the amount of acid which preserved but did not kill within seven days was arbitrarily chosen as the starting point at which the sugars would be added to the acids. The experiments were carried out by pipetting the preserving amount of the various acids (given in Tables 5 & 7) into sterile test tubes and making up to 10 ml. with sterile sugars solutions of varying concentration. These sugar solutions were made to a percentage so that when they were diluted with the acid, the desired concentrations were obtained. These tubes were inoculated and incubated the same as for the acid and sugar experiments.

Table 10. The final percentage of fructose, dextrose, lactose, and sucrose exerting a germicidal effect on the bacteria in the presence of the preserving concentration of acetic acid.

Organism	Ml. of acid*	Per cent acidity**	Per cent			
			Sucrose	Lactose	Dextrose	Fructose
Strept. lactis	2.0(0.3N)	0.36	20.0	None***	12.5	25.0
Strept. liquefaciens	2.0(0.2N)	0.24	40.0	None	22.5	35.0
Bacillus coagulans	2.1(0.1N)	0.126	15.0	None	<2.5	<2.5
Bacillus calidolactis	1.7(0.1N)	0.102	<2.5	<2.5	<2.5	<2.5
Thermophile--milk powder	2.1(0.1N)	0.126	20.0	None	<2.5	<2.5
Thermophile--raw milk	2.9(0.1N)	0.175	<2.5	<2.5	<2.5	<2.5

* Made up to 10 ml.

** Calculated

*** Saturated solution of lactose in combination with acid was not germicidal.

Table 11. The final percentage of fructose, dextrose, lactose, and sucrose exerting a germicidal effect on the bacteria in the presence of the preserving concentration of citric acid.

Organism	Ml. of acid*	Per cent acidity**	Per cent			
			Sucrose	Lactose	Dextrose	Fructose
Strept. lactis	1.8(0.3N)	0.378	30.0	None***	17.5	17.5
Strept. liquefaciens	2.0(0.2N)	0.28	45.0	None	22.5	30.0
Bacillus coagulans	1.7(0.1N)	0.119	10.0	<2.5	5.0	<2.5
Bacillus calidolactis	1.4(0.1N)	0.098	<2.5	<2.5	<2.5	<2.5
Thermophile--milk powder	1.4(0.1N)	0.098	10.0	10.0	<2.5	<2.5
Thermophile--raw milk	1.8(0.1N)	0.126	<2.5	5.0	<2.5	<2.5

* Made up to 10 ml.

** Calculated

*** Saturated solution of lactose in combination with acid was not germicidal.

Table 12. The final percentage of fructose, dextrose, lactose, and sucrose exerting a germicidal effect on the bacteria in the presence of the preserving concentration of lactic acid.

Organism	Ml. of acid*	Per cent acidity**	Per cent			
			Sucrose	Lactose	Dextrose	Fructose
<u>Strept.</u> <u>lactis</u>	1.75(0.2N)	0.315	10.0	15.0	<2.5	<2.5
<u>Strept.</u> <u>liquefaciens</u>	1.5(0.2N)	0.27	30.0	5.0	<2.5	<2.5
<u>Bacillus</u> <u>coagulans</u>	1.1(0.1N)	0.099	<2.5	5.0	<2.5	<2.5
<u>Bacillus</u> <u>calidolactis</u>	1.1(0.1N)	0.099	<2.5	<2.5	<2.5	<2.5
Thermophile--milk powder	1.25(0.1N)	0.113	<2.5	<2.5	<2.5	<2.5
Thermophile--raw milk	1.25(0.1N)	0.113	<2.5	<2.5	<2.5	<2.5

* Made up to 10 ml.

** Calculated

Table 13. The final percentage of fructose, dextrose, lactose, and sucrose exerting a germicidal effect on the yeasts in the presence of the preserving concentration of acetic acid.

Yeast	Ml. of acid*	Per cent acidity**	Per cent			
			Sucrose	Lactose	Dextrose	Fructose
Sacch. cerevisiae	1.25(0.5N)	0.375	55.0	None***	40.0	40.0
Sacch. ellipsoideus	1.5(0.5N)	0.45	55.0	None	35.0	25.0
Torula lactis-condensi	1.75(0.5N)	0.525	25.0	None	10.0	15.0
Zygo. mellis	1.5(0.5N)	0.45	45.0	None	35.0	20.0
Yeast-pickles	1.75(0.5N)	0.525	30.0	None	15.0	15.0

* Made up to 10 ml.

** Calculated

*** Saturated solution of lactose in combination with acids was not germicidal.

Table 14. The final percentage of fructose, dextrose, lactose, and sucrose exerting a germicidal effect on the yeasts in the presence of the preserving concentration of citric acid.

Yeast	Ml. of acid*	Per cent acidity**	Per cent			
			Sucrose	Lactose	Dextrose	Fructose
<u>Sacch</u>. <u>cerevisiae</u>	3.5(1N)	2.45	50.0	None[2]	45.0	30.0
<u>Sacch</u>. <u>ellipsoideus</u>	3.5(1N)	2.45	40.0	None	25.0	30.0
<u>Torula</u> <u>lactis-condensi</u>	3.5(1N)	2.45	None[1]	None	50.0	50.0
<u>Zygo</u>. <u>mellis</u>	3.5(1N)	2.45	None	None	50.0	45.0
Yeast--pickles	3.5(1N)	2.45	None	None	50.0	45.0

* Made up to 10 ml.

** Calculated

1 Highest possible concentration of sucrose with acid (55%) was not germicidal

2 Saturated solution of lactose in combination with acid was not germicidal.

Table 15. The final percentage of fructose, dextrose, lactose, and sucrose exerting a germicidal effect on the yeasts in the presence of the preserving concentration of lactic acid.

Yeast	Ml. of acid*	Per cent acidity**	Per cent			
			Sucrose	Lactose	Dextrose	Fructose
Sacch. cerevisiae	3.0(1N)	2.7	35.0	None***	20.0	20.0
Sacch. ellipsoideus	3.0(1N)	2.7	25.0	None	15.0	20.0
Torula lactis-condensi	3.0(1N)	2.7	40.0	None	30.0	40.0
Zygo. mellis	2.5(1N)	2.25	35.0	None	25.0	25.0
Yeast--pickles	3.5(1N)	5.15	40.0	None	30.0	30.0

* Made up to 10 ml.

** Calculated

*** Saturated solution of lactose in combination with acid was not germicidal.

Discussion

Tables 10-12 show that the percentage of sugar required to bring about a germicidal action on the bacteria was reduced when combined with the preserving quantity of acid. For bacteria, the order of effectiveness of the acids in combination with sugars was lactic > acetic > citric. Fructose and dextrose were more effective than either sucrose or lactose in combination with the acids. Streptococcus lactis and Streptococcus liquefaciens still retained their resistance since a higher percentage of the sugars in combination with the acids was needed to kill them than for the thermophiles. The thermophiles were not able to withstand a combination of fructose or dextrose with any of the acids as it took less than 2.5 per cent of the sugars to exert a germicidal action. Bacillus coagulans and the thermophile isolated from milk powder required from 10 to 20 per cent sucrose in combination with the preserving quantity of citric or acetic acid for a germicidal action while less than 2.5 per cent sucrose was needed to bring about the same germicidal action in combination with the preserving quantity of lactic acid.

It was found for the yeasts that dextrose and fructose in combination with the preserving quantity of the respective acids was germicidal at a lower percentage than either sucrose or lactose in combination with the same amount of acid (Tables 13-15). In general, it took less dextrose and fructose to bring about a germicidal action with acetic acid than with lactic acid and less with lactic than with citric acid.

With the preserving quantity of acid, sucrose was more effective with lactic than with acetic acid. Sucrose in combination with the preserving quantity of citric acid exerted a germicidal action on only two of the yeasts, namely, Saccharomyces cerevisiae and Saccharomyces ellipsoideus. No germicidal action was exerted by lactose in combination with any of the preserving quantities of the acids.

The results agree with those of Nunheimer and Fabian (12) who found that the germicidal amount of dextrose could be reduced by 50 per cent and sodium chloride and sucrose by 30 and 20 per cent respectively when used with one-half the inhibiting concentration of acid.

Summary

1. The order of preserving and germicidal action of the sugars for the bacteria studied is fructose > dextrose > sucrose > lactose. The thermophiles were more susceptible to sugar than _Streptococcus lactis_ and _Streptococcus liquefaciens_.

2. The yeasts were more resistant than bacteria to all the sugars studied. The preserving concentrations of fructose and dextrose were the same for yeasts while for sucrose it required from a 5 to 15 per cent greater concentration. Lactose had no preserving action on the yeasts.

3. Fructose and dextrose were the only sugars having a germicidal action on all the yeasts. Sucrose was germicidal to only one yeast, _Saccharomyces cerevisiae_.

4. If the same method is used for evaluating the preserving and germicidal action of acids as was used for sugars, viz,, the number of grams of each acid in 10 mls. of broth, then the preserving value of the acids for the bacteria is lactic > acetic > citric. If, however, the preserving and germicidal values are based on pH, the order is acetic > citric > lactic. The streptococci were considerably more resistant to acids than were the thermophiles.

5. The pH values showed that each acid does not depend on the hydrogen-ion alone for its action on the bacteria but depends partly on the un-ionized molecule or the anion or both.

6. The yeasts were more tolerant to the acids than the bacteria since it required an acid strength of 0.5 to 5 normal to bring about a

preserving and germicidal action while the bacteria required a normality of 0.1 to 0.3. The order of effectiveness of the acids in the preserving and germicidal range was acetic lactic citric for the yeasts, irrespective of whether it was based on pH or the per cent acid added.

7. For bacteria, the order of effectiveness of the acids in combination with sugars was lactic> acetic> citric. Fructose and dextrose were more effective than either sucrose or lactose in combination with the acids.

8. The thermophiles were not able to withstand a combination of fructose or dextrose with any of the acids as it took less than 2.5 per cent of the sugars to exert a germicidal action. *Streptococcus lactis* and *Streptococcus liquefaciens* still retained their greater resistance since higher percentages of the sugars in combination with acids were needed to kill them than for the thermophiles.

9. It was found for the yeasts that dextrose and fructose in combination with the preserving quantity of the respective acids were germicidal at a lower percentage than either sucrose or lactose in combination with the same amount of acid. In general, it took less dextrose and fructose to bring about a germicidal action with acetic than with lactic and less with lactic than with citric acid.

10. With the preserving quantity of acid, sucrose was more effective with lactic than with acetic acid for yeasts. Sucrose in combination with the preserving quantity of citric acid exerted a germicidal action on only two of the yeasts, namely, *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus*. No germicidal action was exerted by

lactose in combination with any of the preserving quantities of the acids.

Table 16. Influence of the germicidal quantity of organic acids and sugars on the viability of a thermophile isolated from milk powder.

	Sucrose 47.5% by weight	Dextrose 27.5% by weight	Fructose 17.5% by weight	Acetic* 2.25 ml. 0.1N	Citric* 1.5 ml. 0.1N	Lactic* 1.4 ml. 0.1N
Inoculum in bacterial ml.	151,000	93,500	93,500	151,000	93,500	93,500
after 1 day	0	0	0	0	210	2,390
2	11				3	300
3	18				0	12
4	5					0
5	0					
6						
7						

* made up to 10 ml.

Table 17. Influence of the germicidal quantity of organic acids and sugars on the viability of a thermophile isolated from raw milk.

	Sucrose 47.5% by weight	Dextrose 27.5% by weight	Fructose 17.5% by weight	Acetic* 3.0 ml. 0.1N	Citric* 1.9 ml. 0.1N	Lactic* 1.4 ml. 0.1N
Inoculum in bacterial ml.	51,000	51,000	51,000	51,000	51,000	51,000
after 1 day	0	0	0	0	0	0
2	0	0	0	0	0	0
3	199	0	0	0	0	0
4	58	0	0	0	0	0
5	37					
6	2					
7	0					

* made up to 10 ml.

Table 18. Influence of the germicidal quantity of organic acids and sugars on the viability of Bacillus coagulans.

	Sucrose 47.5% by weight	Dextrose 27.5% by weight	Fructose 17.5% by weight	Acetic* 2.25 ml. 0.1N	Citric* 2.0 ml. 0.1N	Lactic* 1.25 ml. 0.1N
Inoculum in bacterial ml.	145,000	65,000	65,000	65,000	145,000	65,000
after 1 day	0	0	0	800	4,400	50
2	0	0	0	140	132	25
3	0	0	0	181	0	0
4				750	0	0
5				185		
6				0		
7						

* made up to 10 ml.

Table 19. Influence of the germicidal quantity of organic acids and sugars on the viability of *Bacillus calidolactis*.

	Sucrose 47.5% by weight	Dextrose 27.5% by weight	Fructose 17.5% by weight	Acetic* 2.0 ml. 0.1N	Citric* 1.5 ml. 0.1N	Lactic* 1.25 ml. 0.1N
Inoculum in bacterial ml.	138,000	138,000	138,000	138,000	138,000	138,000
after 1 day	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4						
5						
6						
7						

* made up to 10 ml.

Table 20. Influence of the germicidal quantity of organic acids and sugars on the viability of <u>Streptococcus lactis</u>.

	Dextrose 35.0% by weight	Fructose 27.5% by weight	Acetic* 2.5 ml. 0.3N	Citric* 2.5 ml. 0.3N	Lactic* 2.0 ml. 0.2N
Inoculum in bacterial ml.	98,000	98,000	85,000	85,000	85,000
after 1 day	88,000	25,300	390	2,057	395
2	52,500	8,000	75	345	19
3	11,700	6,950	44	75	2
4	3,500	495	4	4	0
5	115	115	0	0	
6	36	0			
7	0				

* made up to 10 ml.

Table 21. Influence of the germicidal quantity of organic acids and sugars on the viability of Streptococcus liquefaciens.

	Dextrose 35.0% by weight	Fructose 27.5% by weight	Acetic* 2.5 ml. 0.2N	Citric* 3.0 ml. 0.2N	Lactic* 1.75 ml. 0.2N
Inoculum in bacterial ml.	144,500	144,500	84,500	84,500	84,500
after 1 day	74,000	20,000	21,100	11,850	29,500
2	34,000	10,800	7,900	10	7,350
3	3,900	5,450	2,850	0	1,260
4	3,300	4,550	38		80
5	260	360	0		0
6	33	60			
7	0	0			

* made up to 10 ml.

Table 22. Influence of the germicidal quantity of organic acids and sugars on the viability of *Saccharomyces cerevisiae*.

	Sucrose 60.0% by weight	Dextrose 50.0% by weight	Fructose 47.5% by weight	Acetic* 1.75 ml. 0.5N	Citric* 3.5 ml.	Lactic* 4.0 ml. 1N
Inoculum in bacterial ml.	167,000	165,000	167,000	137,000	165,000	154,500
after 1 day	30	0	0	3,200	10	50
2	18	0	3	110	2	34
3	10	0	4	0	0	7
4	9		12	0	0	0
5	5		53			
6	0		18			
7	0		0			

* made up to 10 ml.

Table 23. Influence of the germicidal quantity of organic acids and sugars on the viability of *Saccharomyces ellipsoideus*.

	Dextrose 47.5% by weight	Fructose 47.5% by weight	Acetic* 2.0 ml. 0.5N	Citric" 1.5 ml. 5N	Lactic* 4.0 ml. 1N
Inoculum in bacterial ml.	158,000	158,000	132,000	181,500	132,000
after 1 day	50	5	570	30	170
2	22	2	20	0	47
3	13	0	0	0	16
4	0	0	0	0	2
5	0				0
6					0
7					

* made up to 10 ml.

Table 24. Influence of the germicidal quantity of organic acids and sugars on the viability of <u>Torula lactis-condensi</u>.

	Dextrose 60.0% by weight	Fructose 60.0% by weight	Acetic* 2.0 ml. 0.5N	Citric* 3.0 m. 5N	Lactic* 4.5 ml. 1N
Inoculum in bacterial ml.	111,000	111,000	104,000	176,500	120,000
after 1 day	5,450	2,800	850	1,000	5
2	1,790	830	20	220	0
3	1,490	208	0	35	
4	715	33		7	
5	590	5		3	
6	10	2		0	
7	0	0			

* made up to 10 ml.

Table 25. Influence of the germicidal quantity of organic acids and sugars on the viability of <u>Zygosaccharomyces mellis</u>.

	Dextrose 60.0% by weight	Fructose 60.0% by weight	Acetic* 2.0 ml. 0.5N	Citric* 3.5 ml. 5N	Lactic* 3.5 ml. 1N
Inoculum in bacterial ml.	143,000	143,000	168,000	120,000	132,000
after 1 day	3,088	2,800	1,270	65	170
2	1,530	340	185	18	47
3	445	122	0	3	16
4	355	27		0	2
5	93	3			0
6	51	0			
7	0				

* made up to 10 ml.

Table 26. Influence of the germicidal quantity of organic acids and sugars on the viability of a yeast isolated from pickles.

	Dextrose 60.0% by weight	Fructose 60.0% by weight	Acetic* 2.25 ml. 0.5N	Citric* 4.5 ml. 5N	Lactic* 5.0 ml. 1N
Inoculum in bacterial ml.	145,000	145,000	149,000	180,000	150,000
after 1 day	10,100	3,400	70	19,100	350
2	3,365	800	0	9,450	220
3	1,545	300	0	1,110	82
4	1,130	82		63	16
5	435	22		2	3
6	97	12		2	0
7	0	0		0	

* made up to 10 ml.

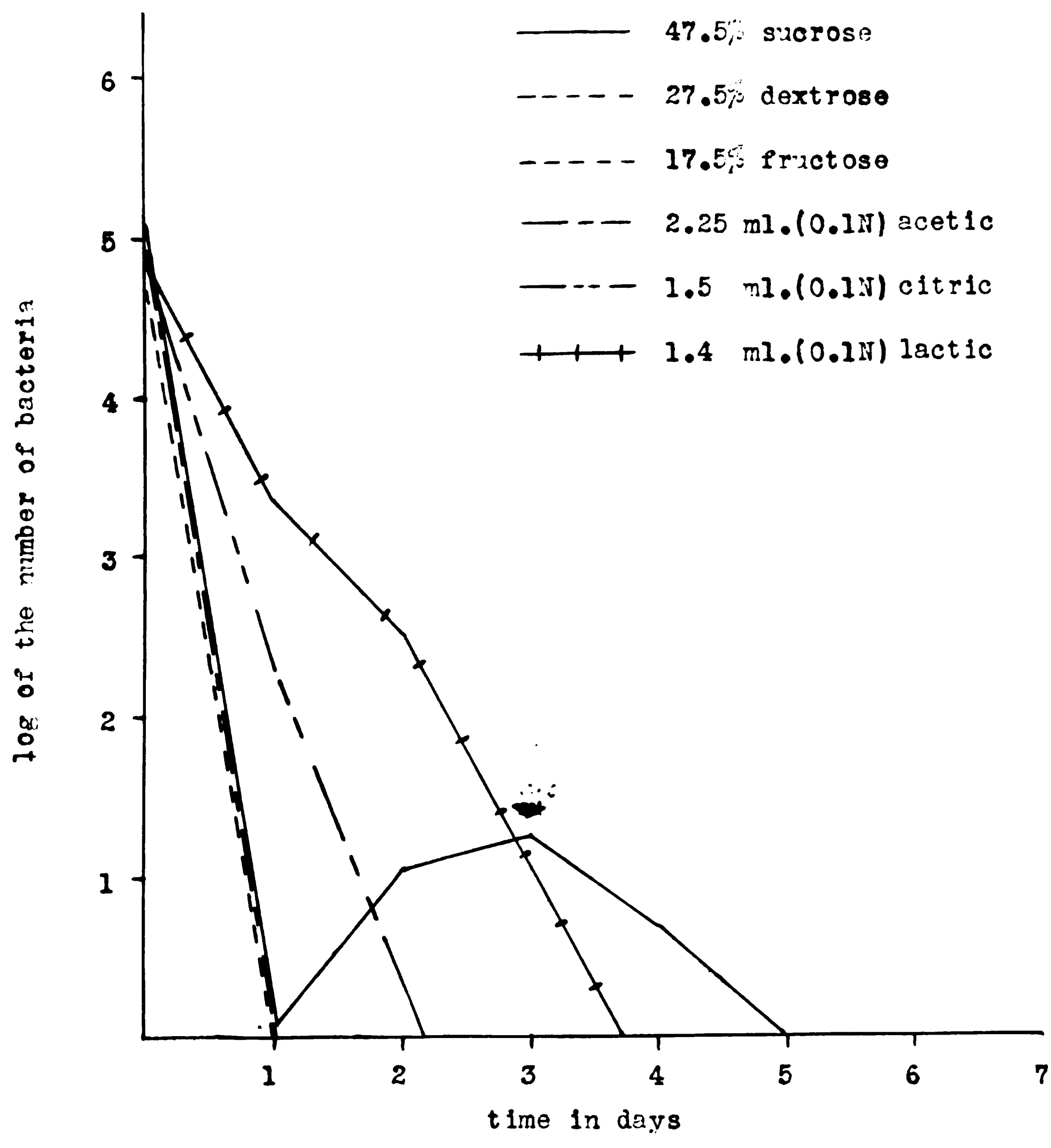

Figure 1. Influence of the germicidal quantity of organic acids and sugars on the viability of a thermophile isolated from milk powder.

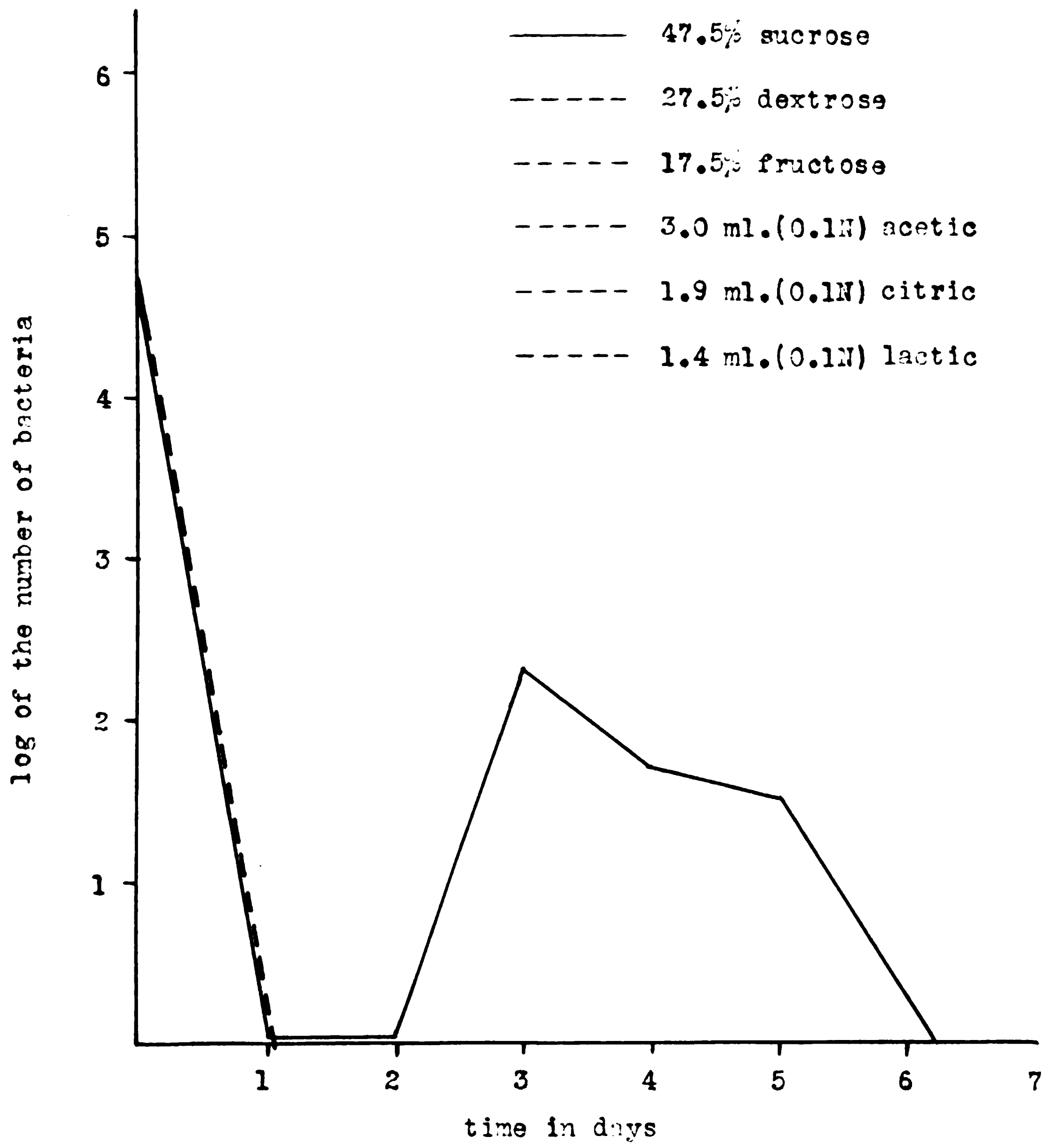

Figure 2. Influence of the germicidal quantity of organic acids and sugars on the viability of a thermophile isolated from raw milk.

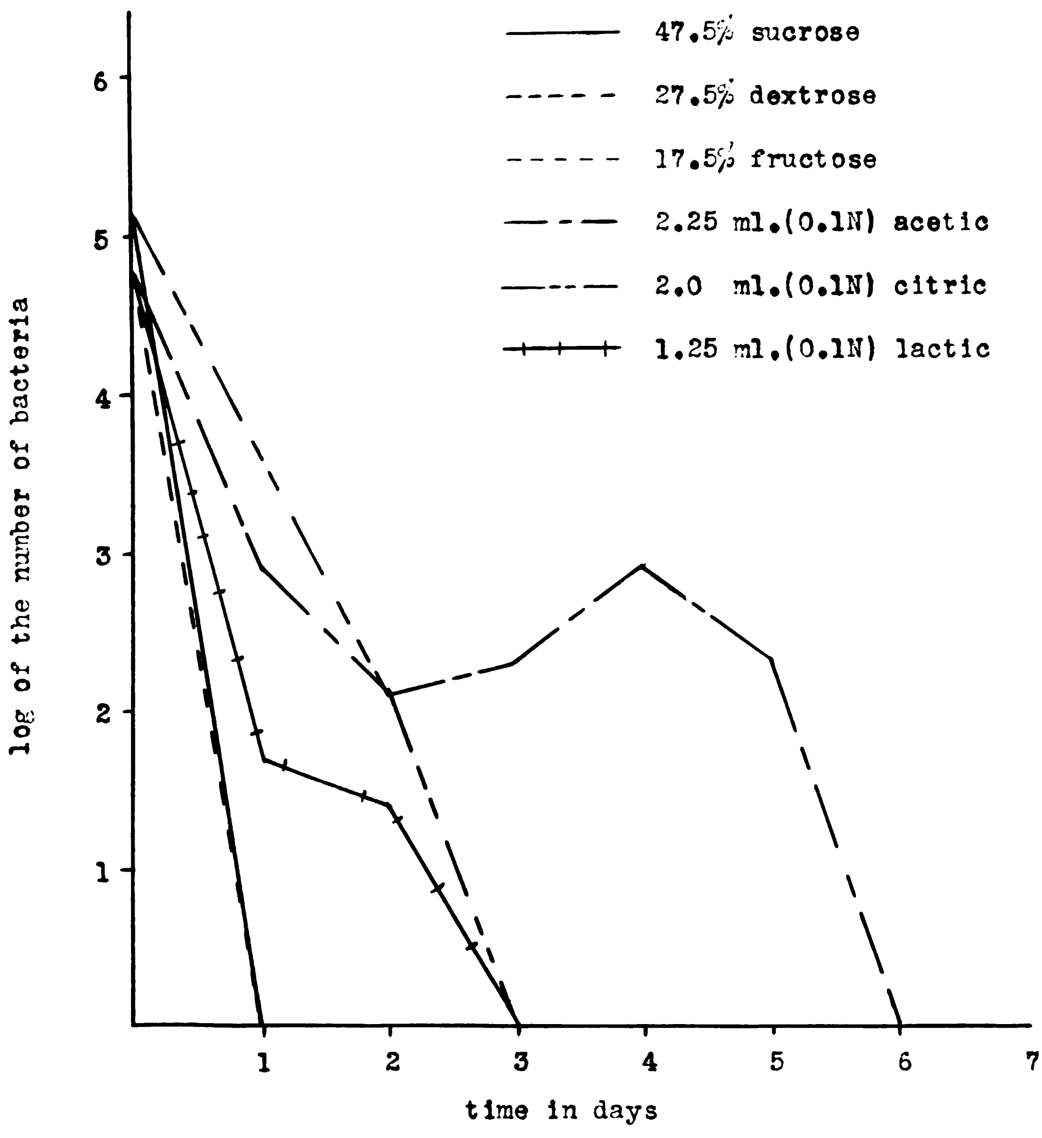

Figure 3. Influence of the germicidal quantity of organic acids and sugars on the viability of *Bacillus coagulans*.

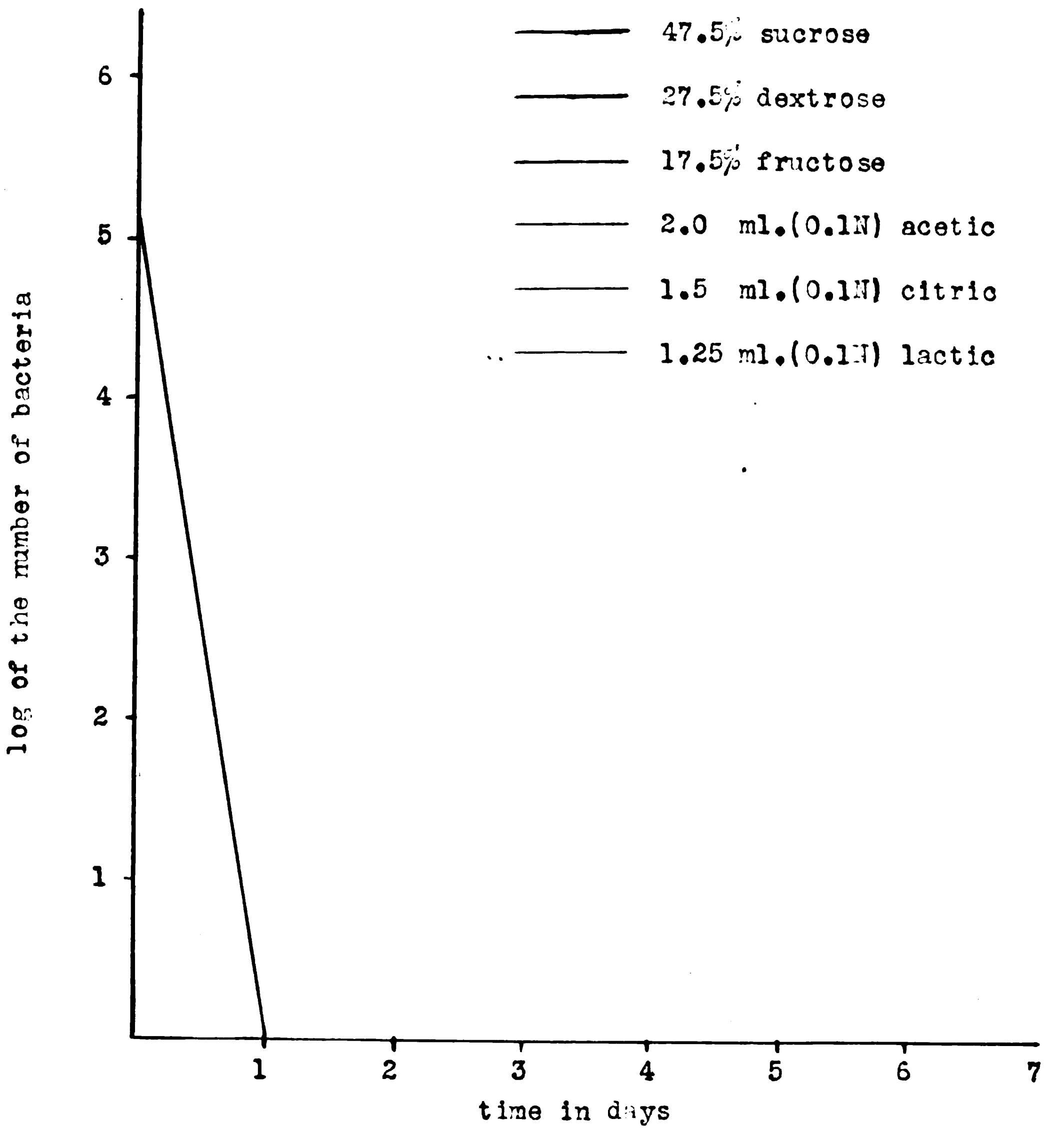

Figure 4. Influence of the germicidal quantity of organic acids and sugars on the viability of <u>Bacillus calidolactis</u>.

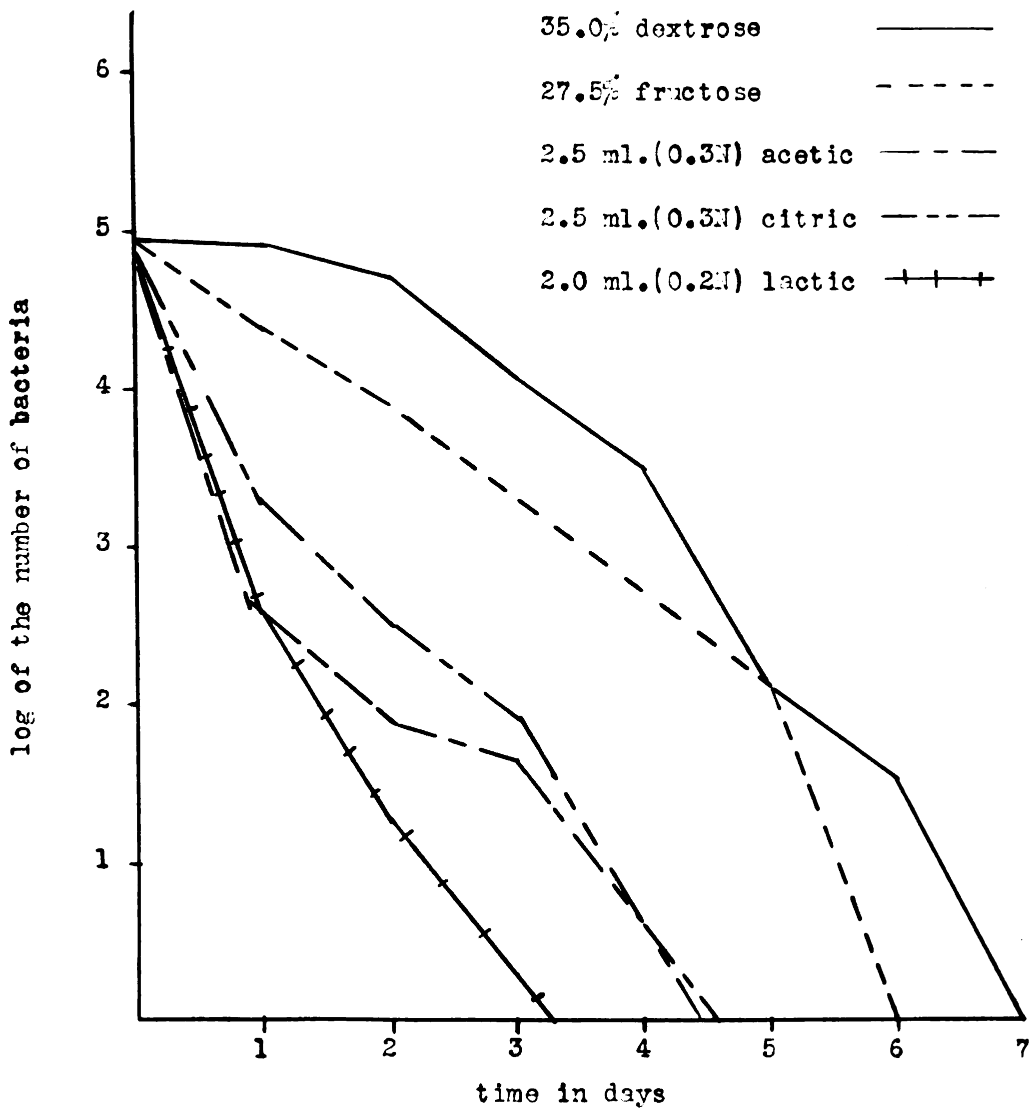

Figure 5. Influence of the germicidal quantity of organic acids and sugars on the viability of Streptococcus lactis.

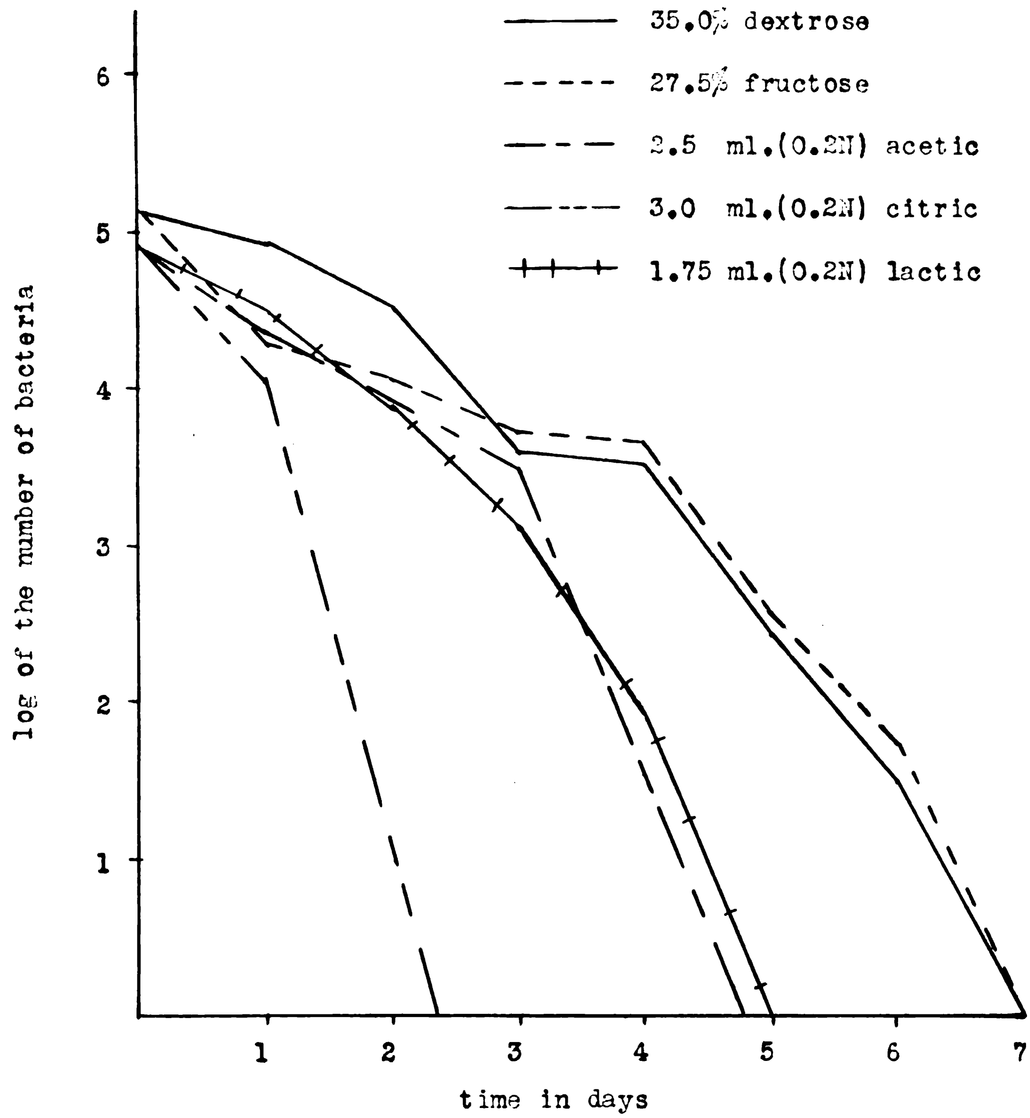

Figure 6. Influence of the germicidal quantity of organic acids and sugars on the viability of Streptococcus liquefaciens.

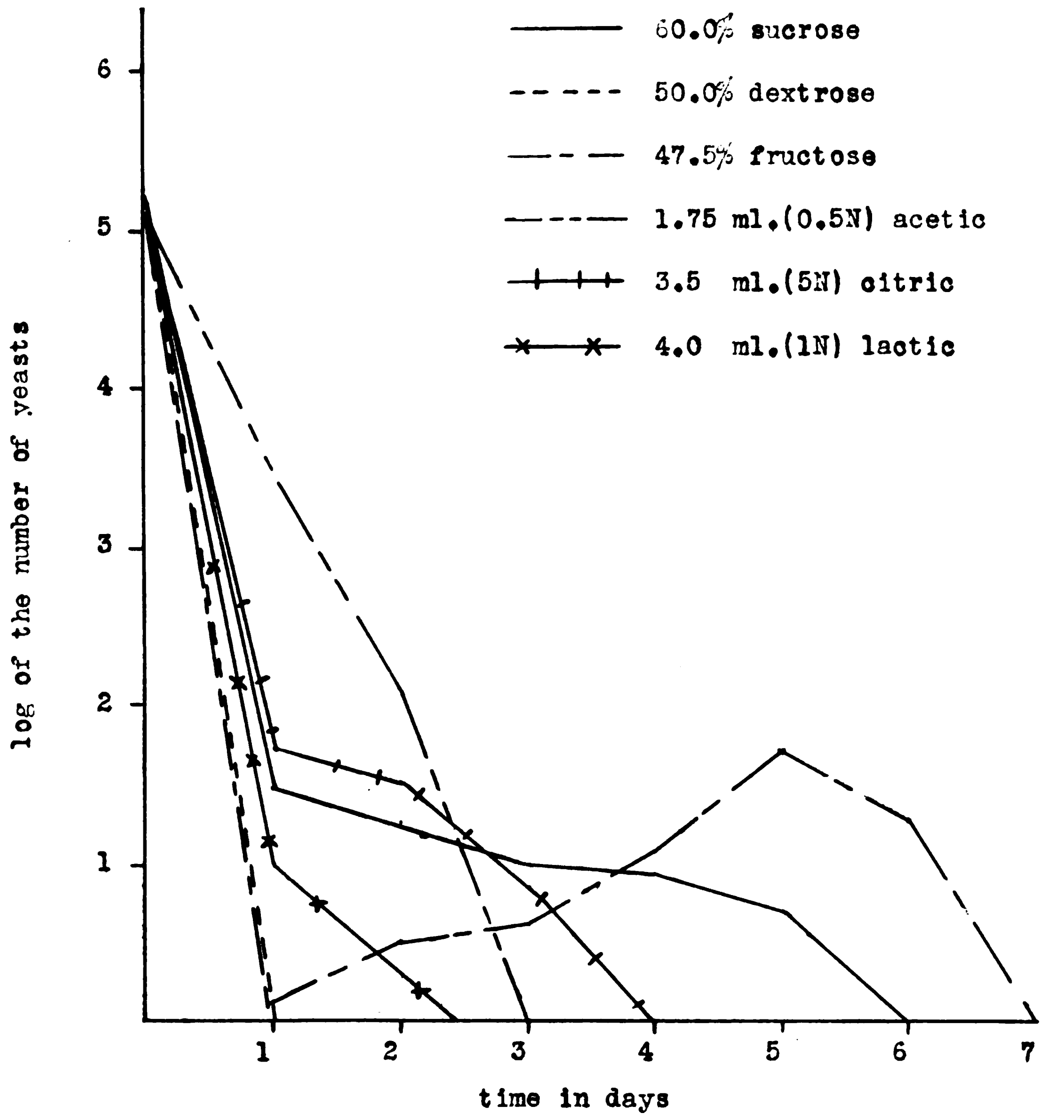

Figure 7. Influence of the germicidal quantity of organic acids and sugars on the viability of Saccharomyces cerevisiae.

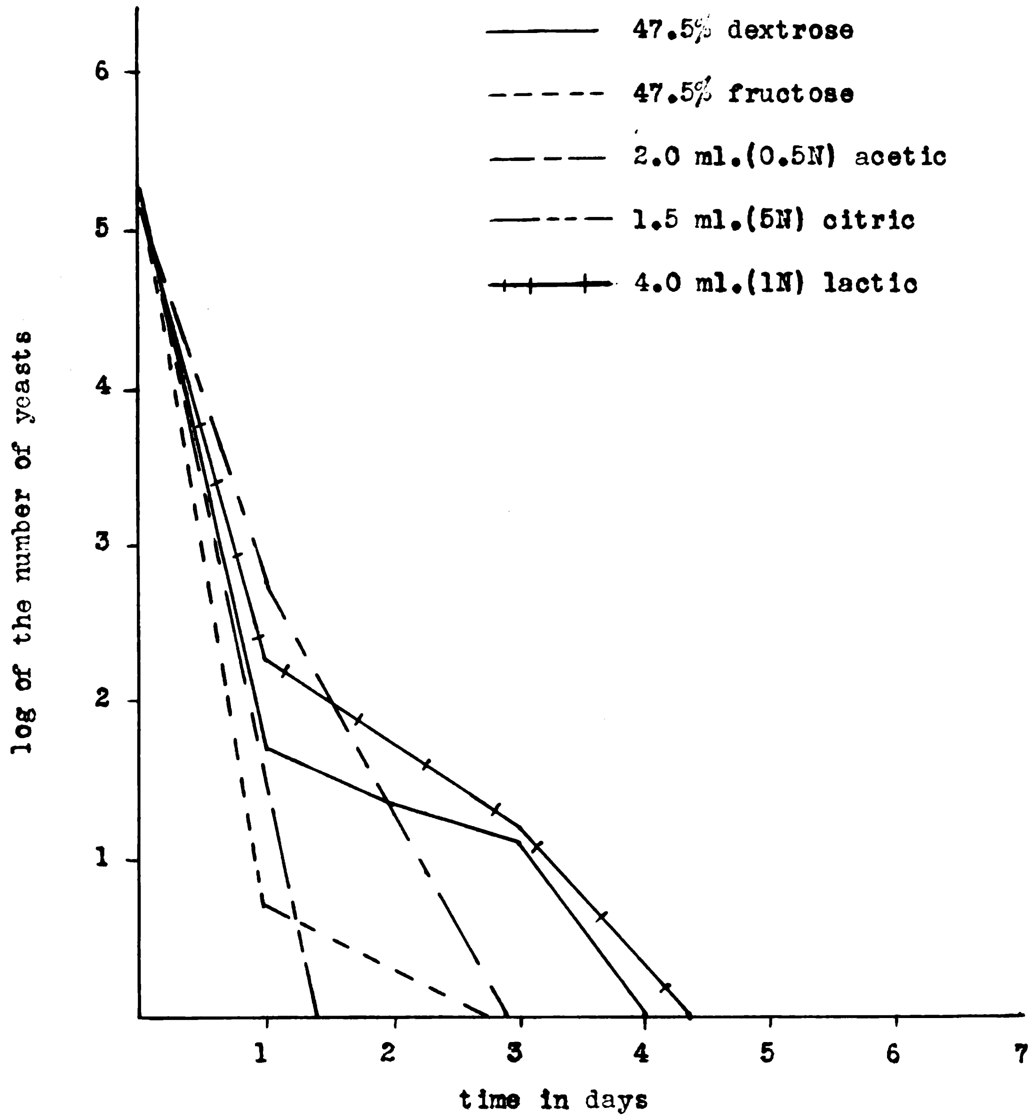

Figure 8. Influence of the germicidal quantity of organic acids and sugars on the viability of <u>Saccharomyces ellipsoideus</u>.

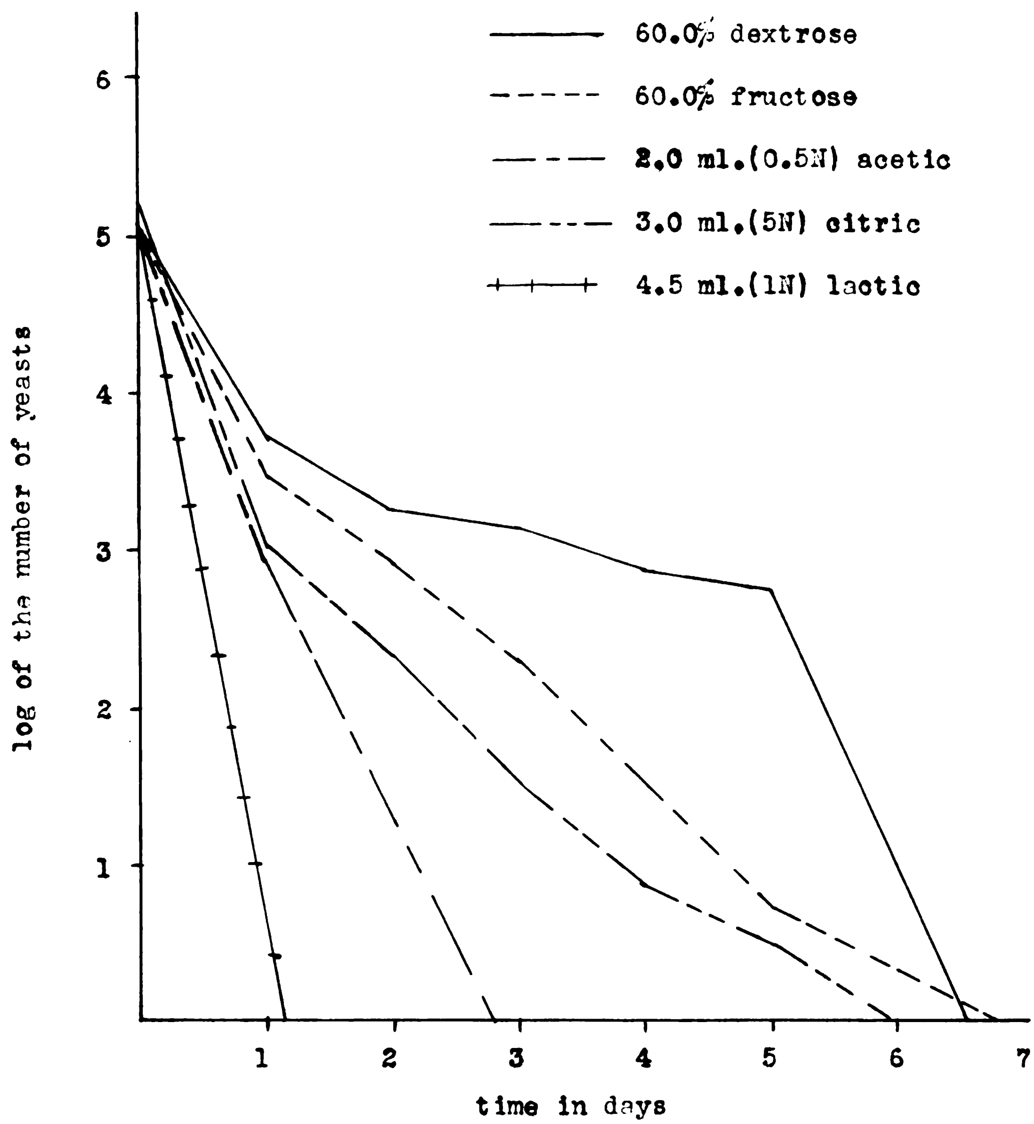

Figure 9. Influence of the germicidal quantity of organic acids and sugars on the viability of *Torula lactis-condensi*.

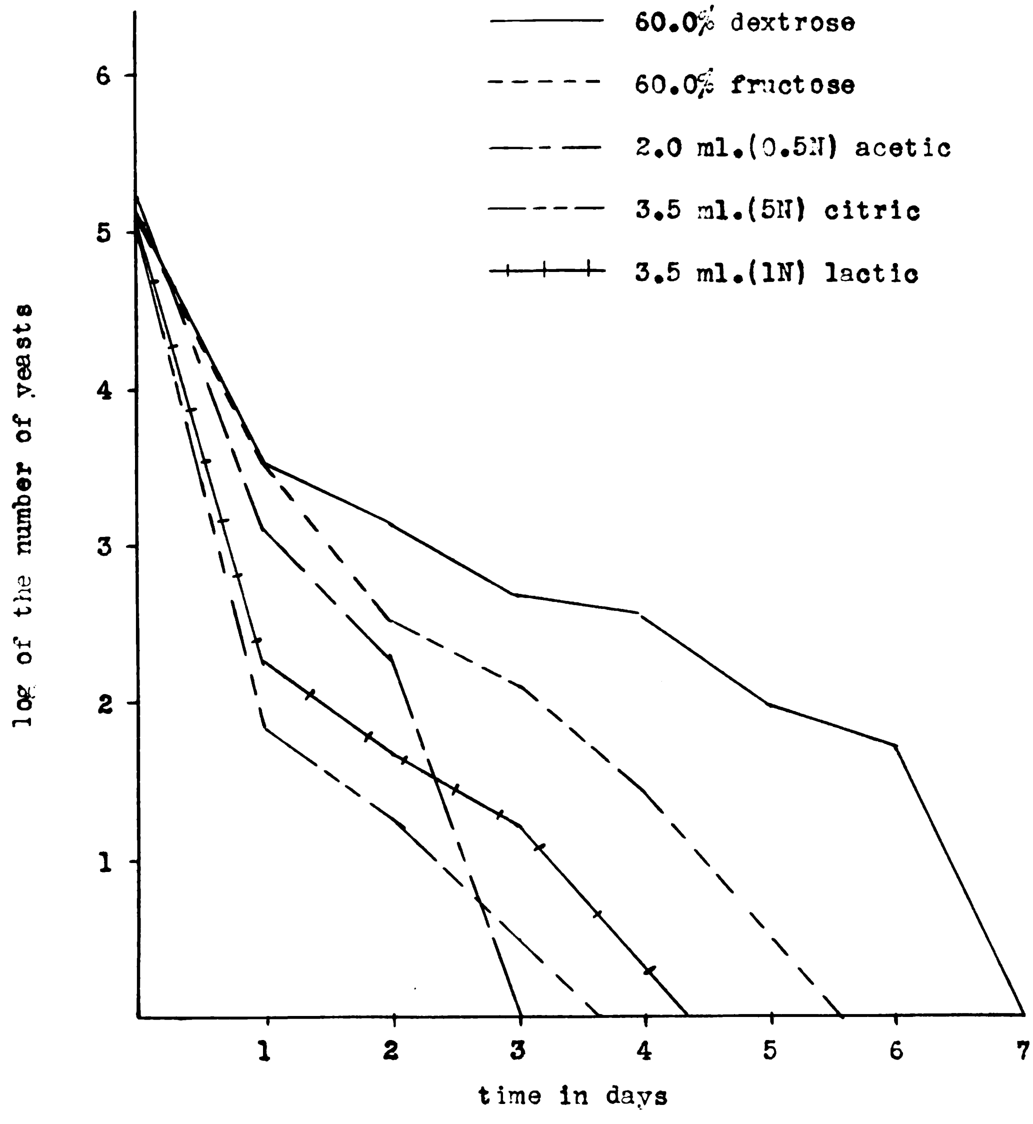

Figure 10. Influence of the germicidal quantity of organic acids and sugars on the viability of *Zygosaccharomyces mellis*.

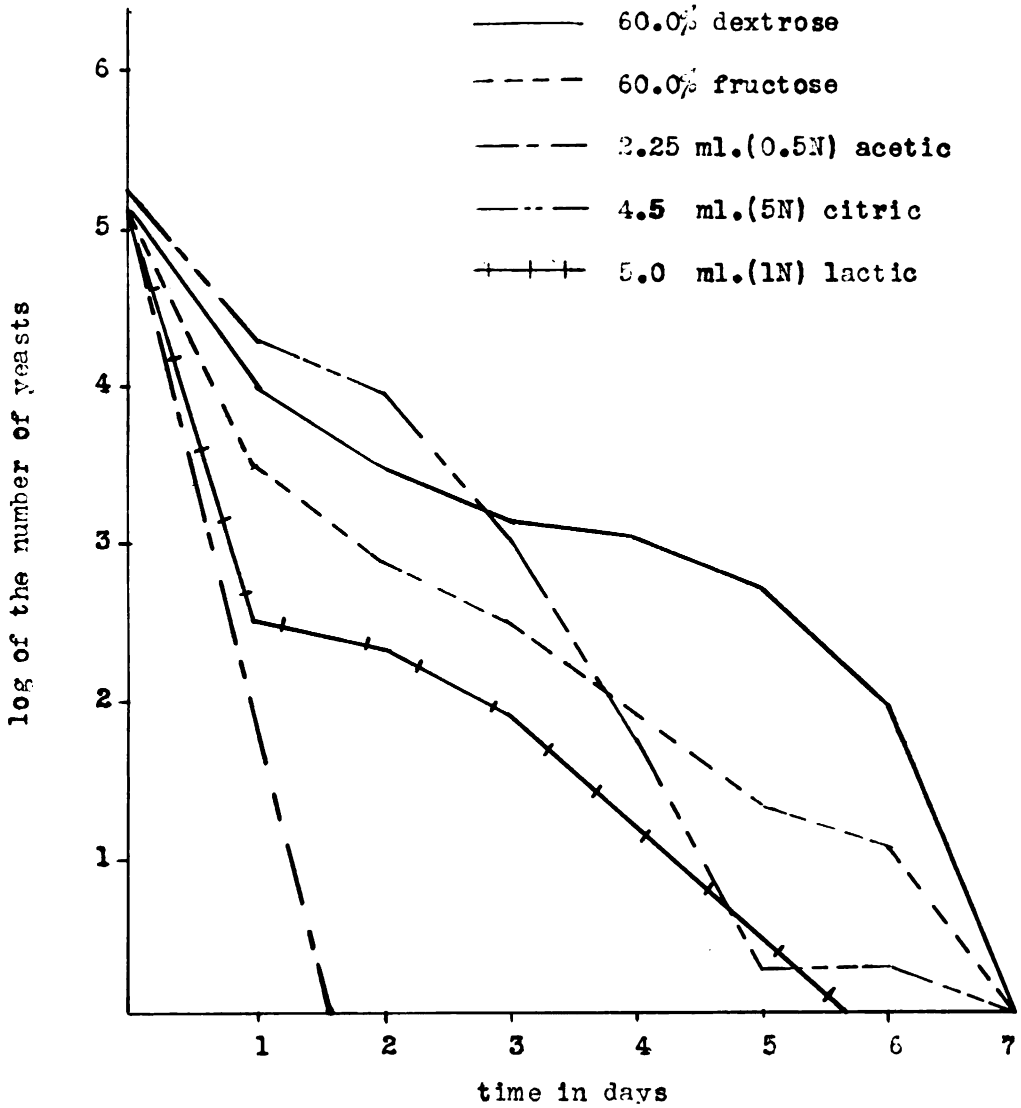

Figure 11. Influence of the germicidal quantity of organic acids and sugars on the viability of a yeast isolated from pickles.

Bibliography

1. Bial, M. Ueber die antiseptische Funktion des H-ions verdunnter Sauren. Ztschr. f. phys. Chemie. 40: 515 (1902) Cited from Nunheimer and Fabian Am. Jour. Public Health 30: 1040-1049 (1940).

2. Bitting, A.W. Experiments on the spoilage of tomato ketchup. U.S. Dept. Agr. Bull. 119 (1909).

3. Clark, J.F. On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. 28: 289-327. (1899).

4. Fabian, F.W. and Johnson, E.A. Experimental work on cucumber fermentation. Mich. Agr. Exp. Sta. Bull. 157 (1935).

5. Fabian, F.W. and Quinet, R.I. A study of the cause of honey fermentation. Mich. State Agr. Exp. Sta. Bull. 92 (1928).

6. Fabian, F.W. and Wadsworth, C.K. Preserving value of acetic and lactic acids in the presence of sucrose. Food Research 4: 511-519 (1939).

7. Johannessohn, E. Einfluss organischer Sauren auf die Hefegarung. Biochem. Zeitsch 47: 97-117 (1912) Cited from Chem Abstracts 7: 1934 (1913).

8. Kahlengerb, L. and True, R.H. On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. 22: 81-124 (1896).

9. Kronig, B. and Paul, T.H. Die Chemischen Grundlagen der Lehre von der Giftwirkung und Disinfection. Ztschr. fur Hyg. 25: 1 (1897) Cited from Nunheimer and Fabian. Am. Jour. Public Health 30: 1040-1049 (1940).

10. Levine, A.S. and Fellers, C.R. Action of acetic acid on food spoilage microorganisms. Jour. of Bact. 39: 499-514 (1939).

11. Levine, A.S. and Fellers, C.R. Inhibiting effect of acetic acid upon microorganisms in the presence of sodium chloride and sucrose. Jour. of Bact. 40: 255-269 (1940).

12. Nunheimer, T.D. and Fabian, F.W. Influence of organic acids, sugars, and sodium chloride upon strains of food poisoning staphylococci. Am. Jour. Pub. Health 30: 1040-1049 (1940).

13. Norton, J.F. and Hsu, P.H. The physical chemistry of disinfection. Jour. Inf. Dis. 18: 180-194 (1916).

14. Paus, N.N. Ueber des Wachstum der Typhus-und Colibacillon auf Nahrboden, denon verschiedene organische Sauren zugestetst sind. Centralb. f. Bakt. 45 Orig. 81 (1908) Cited from Chem. Abstracts 2: 283 (1908).

15. Pederson, C.S. and Breed, R.S. The preserving action in catsup of salt, sugar, benzoate and acid. N.Y. State Agr. Expt. Sta. Bull. 538 (1926).

16. Reid, J.D. The disinfectant action of certain organic acids. Am. Jour. Hyg. 16: 540-556 (1932).

17. Sackett, W. G. Honey as a carrier of intestional diseases. Col. Agr. Exp. Sta. Bull. 252 (1919).

18. Winslow, C.E.A. and Lochridge, E.E. The toxic effect of certain acids upon typhoid and colon bacilli in relation to the degree of dissociation. Jour. Inf. Dis. 3: 547-571 (1906).

19. Wolf, C.G.L. and Harris, J.E.C. Contributions to the biochemistry of pathogenic anaerobes. Jour. Path. and Bact. 21: 386-452 (1917).