STUDIES ON THE GERMICIDAL ACTIVITY OF SORBIC ACID

by

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INTRODUCTION

The problem of how to preserve foods without heating has never been completely solved. Brining and fermentation have their uses but are applicable only to a very few products. Sterilization by radiation shows some promise but still appears to be far from practical utilization. A more direct approach is the addition to food of specific chemical compounds which effectively control spoilage but which are not injurious to human beings consuming the food. Such compounds must be cheap, colorless, tasteless, soluble, and otherwise compatible with products to be consumed as human food.

The search for such ideal compounds has been remarkably fruitless. Until recently the only substance acceptable as a chemical food preservative was sodium benzoate. Unfortunately sodium benzoate in the maximum concentration allowed is not very effective in preventing microbial spoilage, particularly in non-acid foods. This picture was changed considerably by Gooding's discovery that sorbic acid might be a practical fungistatic agent. Later work showed that this six carbon unsaturated fatty acid is metabolized readily in the human body and is non-toxic.

Thus sorbic acid has come in to use as a practical preservative in food products such as cheese, fruit juice, and cakes. It has been used experimentally in products such as brined cucumbers, fish and candy. Manufacturers of sorbic acid have recommended its use, but with little understanding of the manner in which it acts or the effect of environment on its ability to destroy or inhibit growth of microbes.

The work done in this area has emphasized the fungistatic and

bacteriostatic activity of sorbic acid. These studies have not yielded the quantitative data which would allow one to evaluate the effect of environment on this compound. Inasmuch as sorbic acid is becoming an important food additive it seems that fundamental information on the effect of environment would be both desirable and necessary.

The work reported here deals mainly with the effect of pH and temperature on the activity of sorbic acid. Germicidal activity was used as a convenient measure since plate counts could be made and quantitative data accumulated. A set of preliminary experiments on development of resistance to sorbic acid in a yeast are also recorded.

REVIEW OF LITERATURE

Gooding (1945) was the first to discover that certain alpha-beta unsaturated fatty acids have the ability to inhibit growth of microorganisms. He called attention particularly to crotonic acid ($CH_3CH{=}CH\ COOH$) and its six carbon homologue, sorbic acid ($CH_3CH{=}CH{-}CH{=}CH\ COOH$). This latter compound seemed to show some promise as a food preservative and extensive animal tests were made to determine whether or not it might prove toxic to higher organisms (Deuel et al., 1954 A, B). These workers found that sorbic acid is non-toxic to animals, is metabolized in the same manner as caproic acid, and is actually utilized for growth. These reports encouraged industry to use sorbic acid as a practical food preservative.

The interest of food processors in sorbic acid as a preservative is indicated by the following reports. Smith and Rollin (1954) reported that 0.05 per cent sorbic acid mixed with processed American cheese was sufficient to inhibit growth of mold. Melnick et al. (1954) reported that in

heavily contaminated cheese the sorbic acid disappeared from the cheese surface. These workers suggested that this was due to degradation of sorbic acid by the mold. Boyd and Tarr (1955) showed that the growth of molds in yeast and smoked fish were strongly delayed when about 0.05 to 0.1 per cent sorbic acid was incorporated into the flesh during the brining process. In 1957 Ferguson and Powrie published evidence that sorbic acid in a very low concentration (.035 per cent) effectively prevented fermentation in fresh unpasteurized apple juice. Some other products in which sorbic acid has been used include cakes (Melnick et al., 1956), tangerine sherbet base (Patrick and Atkins, 1954), and pharmaceuticals (Puls, et al., 1955).

One property of sorbic acid has had special interest to microbiologists. This is its selective action for certain microorganisms. Phillips and Mundt (1950) reported that 0.1 per cent of sorbic acid acted effectively against the growth of film yeasts in cucumber brines in the laboratory, but did not effect the lactic fermentation. Costilow et al. (1955) using larger scale fermentations obtained essentially the same results. However, Borg et al. (1955) agreed only partially with these findings. They demonstrated that in commercial cucumber fermentations 1/10 per cent sorbic acid inhibited development of the lactic flora as well as that of the common yeasts.

In laboratory studies Vaughn and Emard (1951) found that sorbic acid selectively favored the growth of Lactobacillus and Leuconostoc strains and inhibited all test cultures of yeasts, molds and catalase negative bacteria used. In a more detailed report, Emard and Vaughn (1951) reported that 0.12 per cent sorbic acid contained in liver broth agar permitted the growth

of lactic acid bacteria and clostridia, but inhibited the catalase positive actinomyces, bacteria, molds and yeasts. Its effectiveness was found to depend upon concentration, the type of basal medium, and the pH of the medium. Because of the failure of sorbic acid to inhibit growth of catalase negative bacteria Hanson and Appleman (1955) checked further into the activity of this compound against clostridia. They found that concentrations of 0.12 per cent and one per cent sorbic acid are neither inhibitory nor stimulatory to Clostridium sporogenes and Clostridium botulinum types A and B in unbuffered and buffered complex media at pH 6.7.

Those workers concerning themselves with selective activity of sorbic acid were not long in recognizing that pH has a marked effect upon the activity of this compound. Emard and Vaughn (1952) showed that reduction of pH values from neutrality to the range of 5.0 to 5.5 improves both the selectivity of the 0.12 per cent sorbic acid medium for growth of lactic acid bacteria and the suppression of catalase positive microorganisms. Sheneman and Costilow (1955) found that the inhibitory action of sorbic acid toward yeast was greatly dependent upon pH. Also Costilow et al. (1955) reported that 0.1 per cent sorbic acid had very little effect upon most yeasts at pH 6.0. However, when the pH was lowered to 5.0 the yeasts were completely inhibited by this concentration of sorbic acid. They showed further that yeasts were completely inhibited by 0.1 per cent sorbic acid in 8 per cent salt solution when the medium had a pH of 4.6. As the pH was increased and/or salt concentration decreased, more sorbic acid was required for a complete inhibition of the yeasts tested. Bell et al. (1959) made more detailed studies on the inhibitory properties of sorbic acid against 66 species of filamentous fungi, 32 species of yeasts, and 6

species of lactic acid bacteria. The pH of the culture medium was found to be the principal factor controlling the effectiveness of the sorbic acid as an inhibitor for microbial growth. All of the organisms studied grew in media containing 0.1 per cent sorbic acid at pH 7.0. The yeasts and filamentous fungi were inhibited in media containing 0.1 per cent sorbic acid at pH 4.5. The lactic acid bacteria were inhibited at this concentration of the chemical at pH 3.5. From data obtained in detailed studies with certain species of yeasts and lactic acid bacteria made to determine the effectiveness of 0.1 per cent of sorbic acid over the pH range from 3.5 to 6.8, they concluded that reduction in growth of these organisms paralleled the dissociation of sorbic acid over the pH range studied. From this the investigators inferred that the toxic action of sorbic acid is probably directly related to the concentration of undissociated acid.

It has been known for many years that the bactericidal activity of organic acids is usually due to the undissociated molecule. Kahlenberg and Rodney (1896) found that the toxic action of highly dissociated acids, such as hydrochloric acid, for the plant Lupinus albus was due to the hydrogen ions. They suggested that in weakly dissociated acids the undissociated molecule and ions may play a role. Clark (1899) observed that acetic acid at a dilution where it was only 2 per cent ionized inhibited the germination of spores of filamentous fungi while highly dissociated mineral acids failed to show so marked a retarding effect. Clark also sugested that the activity of the weakly dissociated acids was due to the undissociated molecule. Winslow and Lochridge (1906) found that inorganic acids such as hydrochloric acid and sulfuric acid were more toxic for Bacillus coli and Bacillus typhosa in concentrations where the degree of

dissociation was greatest. However, organic acids exerted a marked toxic effect in dilutions at which they were only slightly dissociated. Thus the authors concluded that the active germicidal portion of organic acids was the undissociated molecule. Reid (1932) found that some monobasic organic acids exerted a germicidal action upon Bacillus pyocyaneus and that this action was inversely proportional to the hydrogen-ion concentration. However, he attributed at least part of the activity to a corresponding decrease in surface tension. The work of Osterhout (1935) indicates that only the undissociated molecule can penetrate the cell membrane. Later Rahn and Conn (1944) also found that the pH has a great effect on the dissociation of benzoic and salicylic acids and they were able to show a parallel between the degree of undissociation and toxic activity.

Such a conclusion would be in line with earlier work showing that weak organic acids such as phenol (McCulloch, 1936), halogenated phenols (Ordal and Denomedi, 1943), and benzoic and salicylic acids (Rahn and Conn, 1944) owe their germicidal activity to the undissociated form of the acid. See also the report of Cowles (1941) on the action of acetic, propionic, butyric, valeric, caproic, and caprylic acids on Staph. aureus and E. coli showing that the bactericidal action was due to the undissociated acid fraction.

Another environmental factor having a marked effect on activity of germicidal compounds is temperature. The magnitude of this effect can be expressed in terms of a temperature coefficient calculated from velocity of lethal actions at various temperatures (Rahn, 1945). In general as the temperature increases in arithmetic progression, the velocity of the reaction

increases in geometric progression. Thus

$$k'/k = \phi\ (T'-T)$$

where k' and k are the velocity constants of the reaction at temperatures T' and T respectively and ϕ is the temperature coefficient.

Jordan and Jacobs (1946) who made observations on the disinfection rate of Bacterium coli by different concentrations of phenol at different temperatures, found that at all concentrations the value of ϕ increased toward the upper end of the temperature range. Jordan, Jacobs and Davis (1947) observed a variation in the value of ϕ when cells of B. coli were heated at temperatures ranging from 47°C to 55°C. It was of some interest to compare the temperature coefficients calculated for sorbic acid with those given for phenol by the above author.

MATERIALS AND METHODS

The cultures used were obtained from the following sources: Escherichia coli NRRL B-281--originally from the collection of the Northern Utilization Research and Development Division, U.S.D.A., Peoria, Illinois in 1952, and carried since that time by periodic transfers on nutrient agar slants; Staphylococcus albus--Department of Bacteriology at Kansas State University; Saccharomyces cerevisiae strain L-508--isolated originally by A. F. Borg and J. L. Etchells from an experimental cucumber brine in 1956 at Ayden, North Carolina and maintained in the lyophilized state.

A specially purified sorbic acid was obtained through the courtesy of Mr. Winston Bradshaw of Carbide and Carbon Chemical Corporation, New York. For some purposes it was desirable to prepare solutions of different pH but containing the same concentration of undissociated sorbic acid. The

amounts to be used were calculated from the ionization constant of sorbic acid as follows:

$$K = \frac{[H+]\,[Sorbate^-]}{[H\ Sorbate]} = 1.75 \times 10^{-5} \text{ (at 25°C)}$$

Suppose that the concentration of undissociated acid at pH = 4 equals C. At pH = 5 how much sorbic acid must be placed in solution to give a concentration of undissociated acid equal to C? If one gram of sorbic acid per liter has been used, the molarity of the solution must be:

$$\frac{1}{112} = 8.93 \times 10^{-3} \text{ mole/liter}$$

where the molecular weight of sorbic acid is 112. In a 0.1 per cent solution at pH 4 the total acid present = 8.93×10^{-3} moles/liter.

If concentrations of sorbate ion = X,

then $\frac{(10^{-4})(X)}{(8.93 \times 10^{-3} - X)} = 1.73 \times 10^{-5}$

$10^{-4}X = 1.5 \times 10^{-7} - 1.73 \times 10^{-5}X$

$11.73 \times 10^{-5}X = 1.5 \times 10^{-7}$

$X = \frac{1.5 \times 10^{-7}}{1.2 \times 10^{-4}} = 1.25 \times 10^{-3}$ moles/liter = concentration of sorbate ion

$8.93 \times 10^{-3} - 1.25 \times 10^{-3} = 7.68 \times 10^{-3}$ moles/liter = concentration of undissociated acid in a 0.1 per cent solution at pH 4.0

The total amount of sorbic acid which must be dissolved in a liter of solution to obtain this concentration of undissociated sorbic acid when the pH is 5.0 can be calculated as follows:

Let SA = total sorbic acid added

Let HS = concentration of undissociated acid = 7.68×10^{-3} moles/liter

Let y = concentration of sorbate ions at pH 5.0

Then SA = HS + y

Thus $\frac{(10^{-5})(y)}{7.68 \times 10^{-3}} = 1.73 \times 10^{-5}$

$y\ 10^{-5} = (1.73 \times 10^{-5})(7.68 \times 10^{-3})$

$y = 13.2864 \times 10^{-3}$

$= 13.29 \times 10^{-3}$

$SA = HS + y$

$= (7.68 + 13.29)\ 10^{-3}$

$= 20.97 \times 10^{-3}$ mole/liter

The weight in grams $= 2.097 \times 10^{-2} \times 12$

$= 234.864 \times 10^{-2}$

235×10^{-2}

= 2.35 gms/liter

= .235 gms per cent

Similar calculations yielded values for total sorbic acid to be used in producing identical concentration of undissociated acid at different pH levels. Thus:

pH	Grams of sorbic acid/100 ml
4.0	0.110
4.5	0.134
5.0	0.235
6.0	1.57

Each of these amounts of sorbic acid was dissolved in 95 ml of buffer of appropriate pH. The bottles were heated in a steamer for a few minutes to dissolve the crystals and cooled to room temperature before the pH of the solutions was readjusted with HCl or NaOH (1N) to the values desired. The final volumes of the solutions were then adjusted to 100 ml.

The following ingredients were used in the preparation of nutrient broth employed for cultivating E. coli and Staph. albus.

Peptone	10 g
Beef extract	3 g
Sodium chloride	5.0 g
Distilled water	1000 ml

The pH was adjusted to neutrality using brom-thymol-blue as indicator. Nutrient agar was prepared by adding 1.5 per cent agar to the nutrient broth before sterilizing.

Potassium acid phthalate buffer was used in most of the experiments. This buffer is effective in the pH range 4 to 6. The stock of potassium phthalate was prepared by dissolving one fifth of a mole (40.83 g) in a liter of distilled water. Sodium hydroxide used in neutralizing to the desired pH was prepared by dissolving one fifth of mole (8 g) of dry NaOH in a liter of distilled water. By combining these two stock solutions in varying proportions and diluting with distilled water, buffers with different pH values could be obtained. Potassium phosphate buffers were prepared in the same manner.

To avoid bacterial losses, 0.1 per cent peptone water was used for diluting cultures for plate counts (Strak, et al. 1957).

The broth medium was used for growth of the inoculum. The inoculum itself was prepared by transferring from the stock culture to 10 ml nutrient broth and incubating at 37°C for 24 hours.

The experimental procedure consisted of making plate counts at specified intervals on cultures exposed to sorbic acid solutions at different pH and at different temperatures. Tubes containing 9 ml buffer or 9 ml of buffer plus sorbic acid received 1 ml of inoculum after 5-10 minutes temperature equilibration in a water bath.

The tube of broth culture inoculum was shaken vigorously just prior to use and the inoculum was thoroughly mixed with the test solutions. From

these tubes (A and B) 1 ml aliquots were removed at given times to determine the number of viable cells. Each 1 ml aliquot was serially diluted to 10^{-2}, 10^{-4}, and 10^{-6} with the peptone water diluent. From a suitable dilution samples in triplicate were transferred to sterile Petri dishes, liquefied nutrient agar (held at 45°C) was added and the sample mixed. All plates were incubated at 37°C. After a suitable period of incubation, usually 48 hours, the colonies were counted with the aid of a Quebec colony counter.

In the studies on the effect of sorbic acid on yeast, S. cerevisiae was cultivated on malt-yeast-extract broth (MY broth). The composition of this medium was as follows:

Malt extract	3 g
Yeast extract	3 g
Peptone	5 g
Glucose	10 g
Distilled water	1000 ml

The broth was autoclaved at 121°C for 15 minutes. The MY agar was prepared by adding 2 per cent shredded agar to MY broth before sterilization.

The culture was prepared by inoculating MY broth from an MY agar slant and incubating at 30°C for 48 hours.

In studies on the resistance of Sacch. cerevisiae to sorbic acid, different concentrations of sorbic acid (0.02%, 0.04%, 0.07%, 0.1% and 0.2%) were dissolved in MY broth. The pH of the broth was adjusted to 4 with 1N HCl. Five tubes were prepared from each concentration. To each tube was added 4.5 ml of broth and 0.5 ml of 48 hour broth culture as inoculum.

A control was included for each concentration; these tubes were left uninoculated. Because the medium was somewhat turbid, the colorimeter used (Bausch and Lomb Spectronic 20) could not be adjusted to give 100 per cent

transmittance for the control tubes. Thus the values given in Appendix Table 17 must be referred to the corresponding control reading. Transmission readings for all tubes were made at 0, 48, 72, and 168 hours.

Ultra violet irradiation was used in an attempt to increase the mutation rate of Sacch. cerevisiae. About 8 ml of a 48 hour culture in MY broth was poured into a sterile Petri dish and exposed to the UV rays at a distance of 28.12 cm. for ten minutes with the lid removed. Aliquots (0.5 ml) of the culture were then transferred to each of five replicate tubes of each of the five different sorbic acid concentrations. Control tubes containing no sorbic acid were inoculated and treated as above.

In interpreting plate counts it was necessary to apply a statistical test in order to judge whether certain observed differences were significant. The method of Least Significant Difference was chosen and the LSD was calculated in those cases where small differences in populations had to be evaluated. The calculation was made according to the method outlined by Snedecor (1957).

RESULTS

Effect of pH on Germicidal Activity of Sorbic Acid

Because of the absence of specific data on the bactericidal activity of sorbic acid it was necessary to determine the approximate concentrations of this substance suitable for use in studies on rate of killing bacteria. To expedite experimental work it was convenient to use concentrations of bactericide which would kill all or the major portion of the bacteria in a test culture in 20 to 90 minutes. If the test culture is destroyed in less than 20 minutes, it is difficult to make the number of platings

necessary for accurate determination of the population change. Experiments running longer than 90 minutes often become excessively time consuming. In some instances it was necessary to extend the time of exposure of the test culture to 180 minutes.

In the initial experiments sorbic acid was used in a concentration of 0.1 per cent. This corresponds to the concentration recommended when this chemical is used as a fungistat. The data from two separate experiments shown in Table 1 show clearly that a 0.1 per cent solution of sorbic acid is germicidal for Escherichia coli in phthalate buffer at pH 4.0. The phthalate buffer used was toxic to the bacteria and reduced the viable population to a considerable degree. However, when sorbic acid was present, killing took place much more rapidly and its effect is unmistakable.

Table 1. Effect of 0.1 per cent sorbic acid at pH 4.0 on population of Escherichia coli at 30°C.

Experiment	Time of exposure in minutes	No sorbic acid	0.1 per cent sorbic acid
A	0	42.3M[L1]	42.3M
	5	32.0M	4.6M
	10	15.9M	1.2M
	20	9.7M	143T
	30	5.7M	<30
B	0	45.3M	39.3M
	5	22.5M	3.4M
	10	14.9M	73T
	20	6.5T	<1H[L1]
	30	690T[L1]	<30

L1 M = Million; T = Thousand; H = Hundred

Bell, Etchells, and Borg (1959) have shown that pH has a marked effect on the fungistatic and bacteriostatic action of sorbic acid.

Therefore it was of interest to determine whether the pH of the environment had a similar effect on bactericidal activity of this compound. Accordingly experiments similar to the ones described above, were set up at pH 5.0 and pH 6.0. Concentrations of sorbic acid used were those which would yield the same concentration of undissociated acid as exist in a 0.1 per cent solution at pH 4.0. At pH 5.0 this equivalent concentration is 0.23 per cent; at pH 6.0 it is 1.6 per cent. For details of the calculation see section on Materials and Methods.

The data recorded in Table 2 show that sorbic acid has some lethal effect on E. coli at pH 5.0 but that it causes no appreciable drop in viable count of the test culture at pH 6.0. It should also be noted that the buffer controls, unlike those at pH 4.0 do not show any significant drop in population even though the time of exposure was triple that at pH 4.0.

The results of Experiments A and B are in good agreement. Lethal effect can be evaluated by comparing the difference between control counts and test counts with the L.S.D. When the difference exceeds the L.S.D., the decrease in population must be due to the activity of the test solution. Such a comparison shows that a significant drop in population occurred at pH 5.0 between 60 and 90 minutes in Experiment A and between 30 and 60 minutes in Experiment B.

The inability of 1.6 per cent sorbic acid at pH 6.0 to kill a significant portion of the E. coli population even on 90 minutes exposure was unexpected. This raised the question of what, if any, concentration of sorbic acid is lethal to E. coli at pH 6.0 and pH 7.0. Concentrations of potassium sorbate as high as 3.6 per cent at pH 6.0 and 14.4 per cent at

pH 7.0 were used. The data obtained in typical experiments are recorded in Table 3.

Table 2. Effect of sorbic acid at pH 5.0 and pH 6.0 on populations of Escherichia coli at 30°C.

Experiment	Time of exposure in minutes	L.S.D.[L1]	No sorbic acid	0.23% sorbic acid at pH 5.0
A	0		44.8M[L2]	44.8M[L2]
	10			38.3M
	30			33.3M
	60	12.91		41.3M
	90		44.3M	31.3M
B	0		65.8M	65.8M
	10	17.5		62.0M
	30			60.7M
	60			45.3M
	90		66.7M	31.8M
				1.6% sorbic acid at pH 6.0
A	0		44.8M[L2]	44.8M[L2]
	10	18.77		40.3M
	30			47.7M
	60			43.7M
	90		57.3M	44.0M
B	0		65.8M	65.8M
	10			61.0M
	30			56.7M
	60	51.44		50.3M
	90		71.3M	50.0M

L1 L.S.D. = Least significant difference
L2 Average of four separate determinations. M = Million

Table 3. Effect of various concentrations of potassium sorbate on populations of Escherichia coli at pH 6.0 and pH 7.0 (30°c).

Time of exposure in minutes	pH	L.S.D.[L1]	Concentrations of potassium sorbate				
			0	1.8%	3.6%	7.2%	14.4%
0	6[L2]		36.3M[L4]	42.7M	43.0M	-	-
10	6	9.23	39.3M	38.7M	48.7M	-	-
0	7[L3]	9.0736	45.0M	45.0M	40.0M	40.0M	23.6M
10	7		52.0M	46.7M	49.7M	32.1M	<300T[L4]

L1 L.S.D. = Least significant difference
L2 phthalate buffer
L3 phosphate buffer
L4 M = Million; T = Thousand

It is clear from these data that even in high concentrations sorbic acid lacks germicidal activity at pH 6.0 and pH 7.0. Only when the concentration reached 14.4 per cent at pH 7.0 was E. coli adversely affected in ten minutes time.

The striking effect of pH on the activity of sorbic acid can be seen by examining the curves plotted in Figure 1. These show a comparison of rates of destruction of E. coli at pH 4.0, 5.0, and 6.0 when the concentration of sorbic acid is adjusted to give equal concentrations of the undissociated compound in each case. The curves for pH 5.0 and 6.0 are plotted from data included in Table 2 (B) while that for pH 4.0 is plotted from data in Table 1 (A). These curves are included to emphasize the marked effect of pH in this situation.

For purposes of comparison, experiments similar to those described above were performed using the gram positive coccus Staphylococcus albus as the test organism. It soon became clear that it would not be possible to compare results obtained with the two different bacteria at pH 4.0. Staph. albus was so sensitive to the buffer that meaningful experiments on

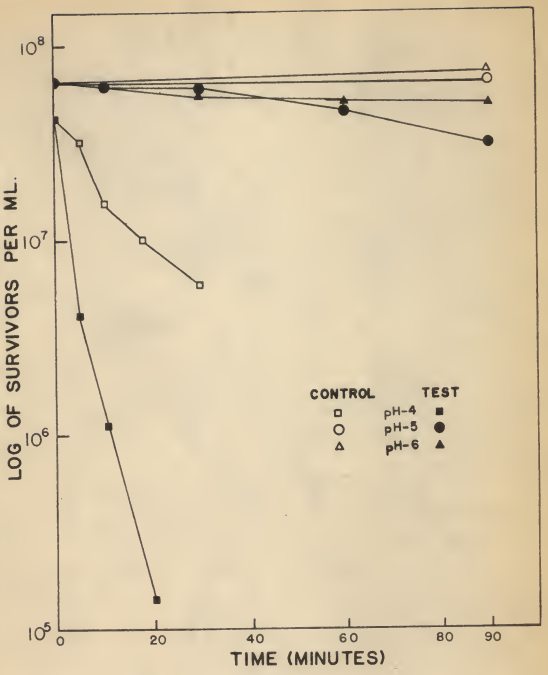

Figure 1. Rate of destruction of Escherichia coli exposed at 30°C to identical concentrations of undissociated sorbic acid at pH 4.0, 5.0, and 6.0.

death rate were impractical at this pH. For example, an initial population of 6.9 million per ml (M/ml) was reduced to less than 300,000 per ml in 5 minutes and there were no detectable survivors after 10 minutes exposure. Similar results were obtained when sorbic acid was present. For this reason experiments with Staph. albus were carried out at pH 5.0.

The data of three replicate experiments are presented in Table 4. In general these indicate that moderate lethal action can be observed, particularly if the time of exposure is increased. In two cases (B and C) the viable population was reduced by about one half on exposures of 30 and 60 minutes respectively.

Table 4. Effect of 0.23 per cent sorbic acid at pH 5.0 on populations of Staphylococcus albus at 30°C.

Experiment	Time exposure in minutes	No sorbic acid	0.23% sorbic acid	L.S.D.[L1]
A	0	9.6M[L2]	8.3M	
	10		8.5M	
	20		6.7M	
	30		6.9M	2.7
	40		6.2M	
	50		6.0M	
	60	9.2M	5.1M	
B	0	12.1M	14.1M	
	5	12.2M	13.8M	
	10	13.5M	11.4M	2.6
	20	10.7M	11.6M	
	30	12.0M	5.1M	
C	0	11.7M	11.7M	
	5		9.5M	
	10		7.2M	0.41
	20		8.0M	
	30	8.0M	8.9M	

L1 L.S.D. = Least significant difference
L2 M = Million

Effect of Temperature on the Germicidal Activity of Sorbic Acid

Temperature may have a marked effect on the activity of a given germicide. In view of the fact that sorbic acid is used as a food preservative and many foods are normally refrigerated, it seemed desirable to determine the effect of temperature on its germicidal activity. Temperatures from 0° to 45°C were used, the tests being performed at pH 4.5 or 5.0 with E. coli and at pH 5.0 with Staph. albus.

Data obtained in preliminary experiments showed that populations of E. coli were not reduced appreciably after 30 minutes exposure to 0.13 per cent sorbic acid at pH 4.5 when temperatures of 0°, 10°, or 20°C were used. At 37°C, however, the viable count dropped rapidly. The evidence presented in Table 5 makes it clear that E. coli shows essentially 100 per cent survival under these circumstances when the temperature is 20°C or below, but is rapidly destroyed at 37°C.

Table 5. Effect of 0.13 per cent sorbic acid at pH 4.5 on population of Escherichia coli at various temperatures.

Experiment	Temperature in °C	Time of exposure in minutes	No sorbic acid	0.13% sorbic acid
A	0	0	42.7M[L1]	-
		60	51.7M	52.0M
	10	30	-	48.0M
	20	30	-	44.7M
	37	0	33.7M	-
		30	2.3M	<300T[L2]
B	0	0	-	29.7M
		60	-	30.6M
		120	-	29.0M
	10	60	-	26.6M
		120	-	30.3M

Table 5 (Concl.)

Experiment	Temperature in °C	Time of exposure in minutes	No sorbic acid	0.13% sorbic acid
	20	0	40.0M	-
		60	45.7M	33.7M
		120	31.5M	25.7M
	37	0	29.1M	-
		30	24.9M	2.8M
		60	3.7T	3.7T

L1 M = Million
L2 T = Thousand

Because the culture was killed so rapidly at 37°C at pH 4.5 it seemed more practical to perform experiments at 30°, 37°, and 45° at a pH where killing was known to take place more slowly. It seemed likely that more meaningful data could be obtained if pH 5.0 were used.

The data from two separate experiments recorded in Table 6 follow the same pattern indicated in Table 5. At temperatures of 0° and 10°C populations remain virtually unchanged after 180 minutes exposure to 0.23 per cent sorbic acid at pH 5.0. At 20°C, 150 minutes exposure did not reduce the population markedly as compared to the control. At 30°C, however, an initial population of about 64M bacteria per ml was reduced to about 34 M/ml in 60 minutes (See Experiment B). This effect is more marked at 37°C and at 45°C. The drop in viable count after only 10 minutes exposure to the sorbic acid solution was very noticeable. Experiment A does not allow a similar comparison between effects at 20° and 37°C because experimental error was relatively large and differences between populations fall within the L.S.D.

Table 6. Effect of 0.23 per cent sorbic acid at pH 5.0 on population of *Escherichia coli* exposed at various temperatures.

Experiment	Temperature in °C	Time of exposure in minutes	No sorbic acid	0.23% sorbic acid	L.S.D.[L1]
A	0	0	47.5M[L2]	-	
		90	-	42.7M	
		180	50.7M[L3]	43.9M	
A	10	0	47.5M[L2]	-	
		90	-	43.0M	
		150	-	50.3M	
		180	51.3M	40.1M	
A	20	0	47.5M[L2]	-	
		60	-	46.7M	
		120	-	48.3M	27.9
		150	52.0M	45.3M	
B	30	0	63.7M	-	
		10	-	60.7M	
		20	-	54.3M	12.6
		40	-	44.0M	
		60	65.3M	34.4M	
A	37	0	47.5M[L2]	-	
		30	-	40.0M	
		60	-	29.0M	28.7
		90	38.3M	19.7M	
B	37	0	63.7M	-	
		10	-	55.3M	
		20	-	57.3M	14.7
		40	-	11.7M	
		60	40.4M	5.6M	
B	45	0	63.7M	-	
		10	-	7.3M	
		20	-	225T[L3]	
		40	-	<30T	
		60	25.8M	Appx. 29	

L1 L.S.D. = Least significant difference
L2 Average of five separate determinations
L3 M = Million; T = Thousand

Similar experiments were performed with Staph. albus at 20°, 30°, 37°, and 40°C. Typical data obtained are recorded in Table 7.

Table 7. Effect of 0.23 per cent sorbic acid at pH 5.0 on populations of Staphylococcus albus exposed at various temperatures.

Experiment	Temperature in °C	Time of exposure in minutes	No sorbic acid	0.23% sorbic acid
A	20	0	6.4M[L1]	-
		10	6.1M[L2]	6.2M
		20	6.0M	6.0M
		40	6.1M	5.3M
		60	6.1M	3.9M
A	30	0	6.4M[L1]	-
		10	5.5M	6.2M
		20	6.0M	5.1M
		40	4.9M	<3M
		60	5.0M	<300T
B	37	0	15.2M	-
		10	-	620T
		20	-	<30T
		40	-	<3T
		60	1.7M	<30
A	40	0	6.4M[L1]	-
		10	Appx. 1.5M	<300T
		20	<3M	<3T
		40	<30T	<300
		60	<3T	<30

L1 Represents an average of six separate determinations
L2 M = Million; T = Thousand

The values reported for survivors at 30°C do not correspond particularly well with those obtained in experiments under similar conditions performed in the investigations on the effect of the pH. See Table 4 for comparison. No obvious explanation suggests itself. It seems possible that 30°C is at or near a "critical temperature" where germicidal activity changes

markedly. If this were true, then slight variations in experimental conditions could give rise to inconsistent results. Indeed, Table 4 shows some of this inconsistency.

Velocity Constant of the Reaction

The rate at which destruction of microbes proceeds can be expressed in terms of the velocity constant of the reaction. Comparison of the velocity constants at different temperatures gives a measure of the effect of temperature on the rate of the particular reaction being considered.

The velocity constant may be calculated if initial and final bacterial populations and duration of the experiment are known. The relationship of these factors is expressed by the following equation:

$k = 1/t \log B/b$ where t = time interval in minutes
B = initial population (bact/ml)
b = final population (bact/ml)

Where k is determined at two or more temperatures a temperature coefficient (θ) expressing the relative change in reaction rate with temperatures can be expressed as follows:

$$\theta^{(T_2-T_1)} = \frac{k_2}{k_1}$$

Where k_1 and k_2 are velocity constants at temperatures T_1 and T_2 respectively.

Suitable data for making such calculations are available in Experiment B, Table 6. The velocity constants derived from these data are presented in Table 8 below.

Table 8. Velocity constants for destruction of Escherichia coli.*

Temperature	Time in volume	Velocity constant	Average
30°C	0 - 10	2.1×10^{-3}	
	10 - 20	4.8×10^{-3}	
	20 - 40	4.6×10^{-3}	4.2×10^{-3}
	40 - 60	5.4×10^{-3}	
37°C	0 - 10	2.8×10^{-3}	
	10 - 20	-3.6×10^{-3}	
	20 - 40	30.1×10^{-3}	10.6×10^{-3}
	40 - 60	13.1×10^{-3}	
45°C	0 - 10	88.4×10^{-3}	
	10 - 20	144.4×10^{-3}	116.4×10^{-3}

* Calculated from data of Experiment B, Table 6.

The following temperature coefficients are calculated from average values of the velocity constants.

$$\theta^{(37-30)} = \frac{10.6 \times 10^{-3}}{4.2 \times 10^{-3}} = 2.52$$

$$\theta^{7} = 2.52$$

$$\log \theta = 1/7 \log 2.52 = \frac{0.40140}{7} = 0.05734$$

$$\theta_{(30-37)} = 1.14$$

$$\theta^{(45-37)} = \frac{116.4 \times 10^{-3}}{10.6 \times 10^{-3}} = 11.0$$

$$\theta^{8} = 11.0$$

$$\log \theta = 1/8 \log 11.0 = \frac{(1.04139)}{8} = 0.13017$$

$$\theta_{(37-45)} = 1.35$$

For comparison with coefficients calculated for phenol see Table 9 of Discussion.

Attempts to Detect Strains of *Saccharomyces cerevisiae* Resistant to Sorbic Acid

It was of interest to know whether a common yeast such as *Saccharomyces cerevisiae* would develop resistance to sorbic acid readily. To test this possibility cultures containing approximately 60 million viable cells per ml were set up with and without various amounts of sorbic acid. Duplicate experiments were carried out with yeast cells which had been irradiated with ultra-violet light to increase their rate of mutation.

In general, growth, as measured by turbidity, was related inversely to the concentration of sorbic acid in both normal and irradiated cultures. The relationship between growth and concentration of sorbic acid is shown by the curves plotted in Figure 2 and Figure 3. No tube containing sorbic acid showed turbidity approaching that of the control tubes. The transmittance readings recorded are to be found in Appendix Table 17.

DISCUSSION

Previous work with sorbic acid has emphasized its inhibitory properties, that is, its ability to prevent growth of yeasts and higher fungi. Relatively little work has been done with bacteria and most of the information available concerns the failure of sorbic acid to inhibit catalase negative bacteria. The work undertaken here deals mostly with the bactericidal effect of sorbic acid. This approach was used deliberately since it is possible to follow the lethal effect in a quantitative manner and to determine the effect of environment upon this bactericidal action. Because the two approaches are quite different it is not possible to make direct comparisons between the findings on inhibitory properties and the bactericidal properties of sorbic acid. However, certain similarities exist

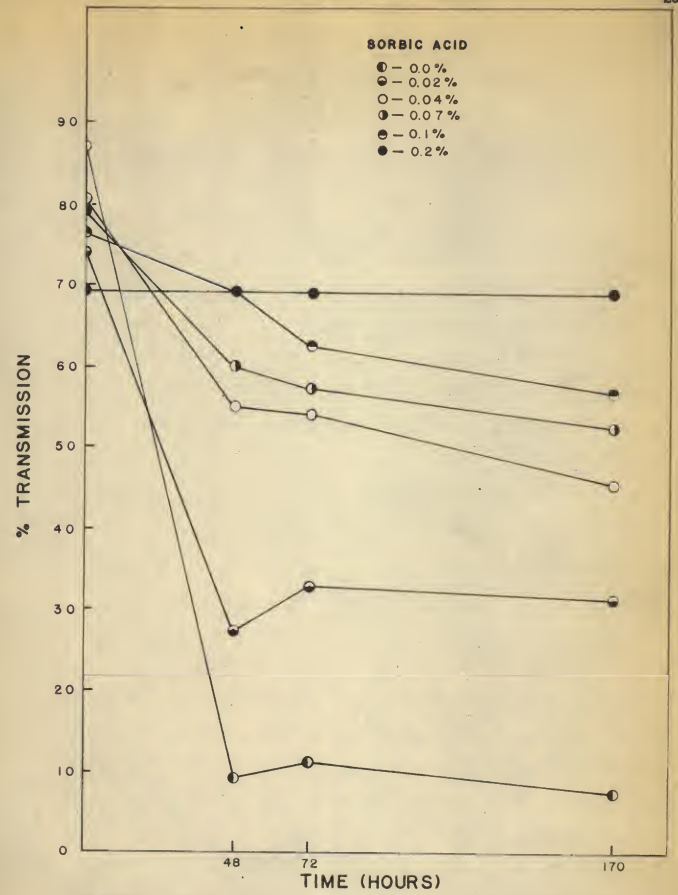

Figure 2. Transmittance of unirradiated cultures of Saccharomyces cerevisiae growing in the presence of various concentrations of sorbic acid.

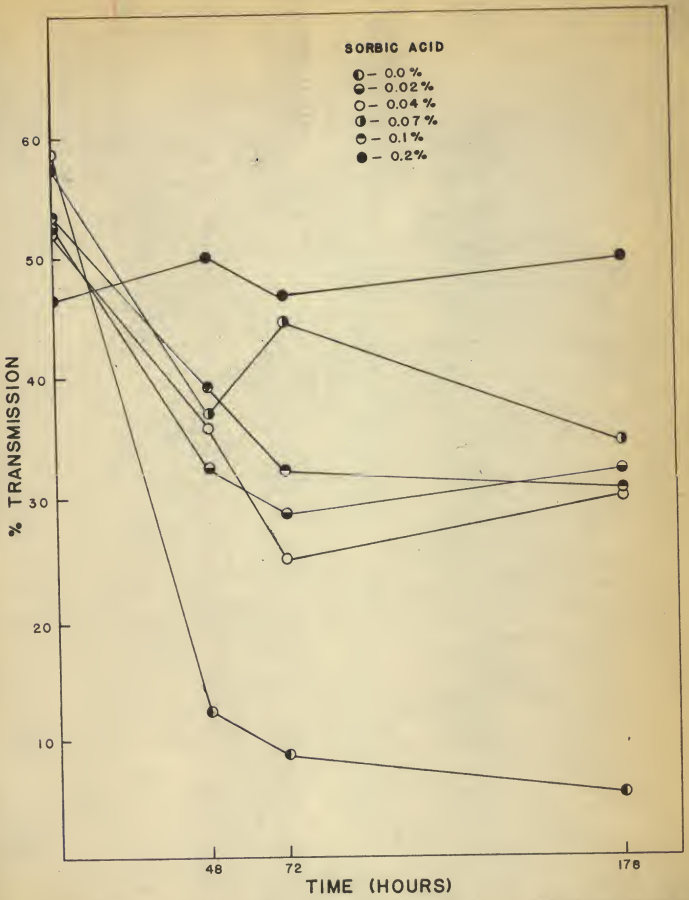

Figure 3. Transmittance of UV irradiated cultures of Saccharomyces cerevisiae growing in the presence of various concentrations of sorbic acid.

between inhibition and lethal action and the data reported suggest some interesting experiments that might be done relative to the effect of environment on the inhibitory properties of sorbic acid.

It was pointed out in the previous section that pH has a marked effect on the activity of sorbic acid. At 30°C Escherichia coli is killed rapidly by sorbic acid at pH 4.0. In similar solutions at pH 5.0 the organisms were killed at a much slower rate. At pH 6.0 no appreciable germicidal effect was observed. Interpretation of the results obtained is complicated somewhat by the fact that at pH 4.0 the buffer controls themselves, brought about a considerable reduction in population of the test organism. However, comparison of the curves in Figure 1 shows clearly that the death rate was much higher in the sorbic acid solution than in the buffer control.

Staphylococcus albus is more sensitive to the reagents used and no data could be obtained at pH 4.0. Death in the buffer control was so rapid as to preclude any meaningful comparison with a test solution containing sorbic acid. At pH 5.0 the lethal effect of 0.23 per cent sorbic acid was moderate. Thus at this pH S. albus appears to show approximately the same degree of sensitivity to sorbic acid as does the Gram negative rod, E. coli.

It was postulated that the bactericidal action of sorbic acid was due mainly to the undissociated molecule. This follows the interpretation given by Bell, Etchells and Borg (1959) that undissociated sorbic acid is responsible for the fungistatic and bacteriostatic action of sorbic acid.

Sorbic acid is a weak organic acid. The ionization constant of 1.75×10^{-5} is almost identical to the dissociation constant of acetic acid which is given as 1.8×10^{-5}. It is a white crystalline organic acid

slightly soluble in water. It is an alpha-beta unsaturated fatty acid having the formula CH_3-CH=CH-CH=CH-COOH. The corresponding saturated fatty acid, caproic acid, can be found in butter fat in concentrations of 1.4 - 2.5 per cent. Sorbic acid, which is a shorter chain compound in the series of unsaturated fatty acids, contains double bonds in conjugated position. It has not been found to occur in nature. Sorbic acid has the following physical properties:

Molecular weight	112.2
Melting point	134.5°C
Ionization constant at 25°C	1.75×10^{-5}

From this information it is possible to calculate values which can be used in plotting a dissociation curve (see section on Materials and Methods). An examination of the dissociation curve of sorbic acid shows that between pH 4.0 and pH 6.0 the dissociation increases markedly (see Figure 4). Thus it would appear that the germicidal action might decrease as the pH is increased from 4.0 to 6.0. If it is true that the germicidal action is due to the undissociated molecule, it should be possible to duplicate at pH 5.0 the rate of destruction of bacteria observed at pH 4.0. Theoretically this can be done by increasing the total concentration of the sorbic acid at pH 5.0 to the point where the concentration of undissociated acid is equal to that obtained in a 0.1 per cent solution at pH 4.0. This approach can be extended to higher pH values and was actually used at pH 6.0 as well as at pH 5.0. Contrary to what was expected, the rate of decrease in the population at pH 5.0 did not approach that in a similar solution at pH 4.0. At pH 6.0 the germicidal effect of the sorbic acid was still less even though the concentration of the undissociated acid was maintained by appropriate increases in the total concentration added. Thus it appears that while the

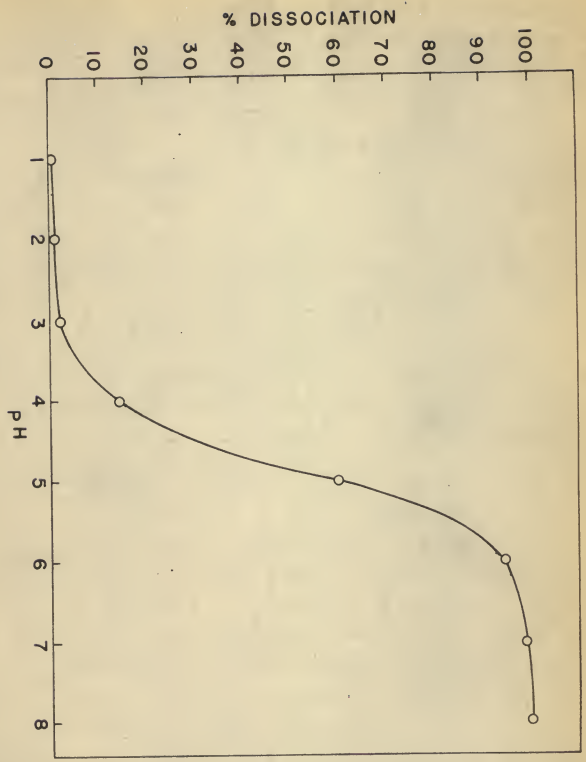

Figure 4. Dissociation curve of sorbic acid.

undissociated molecule may have considerable effect at low pH values, it alone is not responsible for germicidal action.

It was outside the scope of these experiments to investigate the mode of action of sorbic acid and to try to separate the pH effect from the direct effect of sorbic acid itself. However, some speculation on these points seems justified. One might suspect that the dramatic increase in germicidal activity with decrease in pH could be due to altered permeability of the cell membrane thus allowing sorbic acid access to certain vulnerable sites of metabolic activity. Experiments by Costilow et al. (1959) have shown that this peculiar pH effect is much less marked when one tests cell-free preparations of yeast cells rather than whole yeast cells. Thus permeability could be a factor in these organisms. The experiments of Gale and Epps (1942) are interesting in this connection. They concluded that in certain cases pH brings about an effective starvation of cells by changing enzymatic content. This would not seem to be a factor in the particular case considered here since cells left for three hours and longer were viable and the total population did not show any detectable decrease.

Data presented in the previous section show that populations of Escherichia coli were not reduced significantly after 30 minutes exposure at pH 4.5 when temperatures were at 0°, 10°, and 20°C. At 37°C, however, the viable count dropped rapidly (refer to Table 5). Similarly at pH 5.0 Escherichia coli showed essentially 100 per cent survival when temperatures were at 0° and 10°, even when the exposure was extended to 180 minutes. Likewise, at 20°, 150 minutes exposure did not result in significant drop in population. However, decrease in the viable count was noticeable when

the temperature was raised to 30° and was greatly accelerated at temperatures of 37°, 40°, and 45°C (refer to Table 6).

Similar experiments were performed with Staphylococcus albus at 20°, 30°, 37°, and 40°C at pH 5.0. The results obtained were essentially the same as those reported for Escherichia coli. At the lower temperature, 20°C, the population was reduced but not markedly so after 60 minutes of exposure. As the temperature was increased, the rate of killing increased and reached maximum at temperature of 40°C.

It is tempting to speculate that sorbic acid exerts its effect by interfering with some metabolic system when this system is in a relatively active state. When the temperature is reduced, it might be conjectured that the organism would be able to survive a partial blocking of this system when the total activity of the cell is reduced by decreasing the temperature, but that it could not survive the block at a higher temperature where the normal products of the block system were required at a rapid rate. It should be possible to test this hypothesis by using psychrophilic bacteria. These bacteria continue to metabolize at an appreciable rate at low temperatures and thus they should continue to show sensitivity to sorbic acid even though temperatures were reduced to the neighborhood of 0°C. On the other hand, the temperature may exert its effect by altering the permeability of the cell to sorbic acid.

The experiments were continued up to limiting temperatures. At 45°C killing was quite rapid in the controls and here again interpretation of the result in terms of activity of sorbic acid was not possible.

The increase of bactericidal activity of various agents with temperature is well known (see van Eseltine and Rahahn, 1949). It is sometimes

useful to express temperature effects in terms of a coefficient θ which reflects the relative increase in reaction rate per 1°C rise in temperature. A corresponding coefficient Q_{10} expresses a relative increase in rate for 10°C rise in temperature. These values can be calculated from plate count data obtained in experiments where test organisms are exposed to a lethal agent at a series of temperatures. The figures obtained are not highly accurate and reproducible but general trends can be indicated. For purposes of comparison calculations have been made of the data obtained in the experiments reported here and are placed side by side in a table prepared by using data from Jordan and Jacobs (1946). The values of data they obtained using phenol are quite comparable to those determined here for sorbic acid.

Table 9. Comparison of temperature coefficients for phenol and sorbic acid.

Phenol conc.	Temperature range °C	θ
4.62 g/L	30.0 - 32.5	1.29
	32.5 - 35.0	1.40
	35.0 - 38.0	1.19
	38.0 - 39.5	1.04
	39.5 - 42.0	1.41
Sorbic acid conc.		
2.35 g/L	30 - 37	1.14
	37 - 45	1.35

The sorbic acid calculations are based upon only a very few temperatures and for this reasons cannot be considered highly reliable. However, they do indicate that sorbic acid is probably not greatly different from a compound such as phenol in its change of activity with temperature.

Since sorbic acid is relatively ineffective as a lethal agent for bacteria at low temperatures, it would be most useful to know whether its ability to inhibit growth of spoilage microorganisms is subject to a similar effect. If so, sorbic acid might be relatively useless as a preservative for foods kept at low temperatures. Data on this point have not been found in the literature.

An attempt was made to obtain a mutant of Saccharomyces cerevisiae resistant to sorbic acid. Large numbers of cells were placed in nutrient medium containing various concentrations of sorbic acid (0.02, 0.04, 0.07, 0.1 and 0.2 per cent). In some experiments the cells were treated with ultra violet light to increase mutation rate before they were added to the sorbic acid medium. The mutagen was used after it was found that unirradiated cells of Sacch. cerevisiae failed to grow well in any concentration of sorbic acid used.

Theoretically if a mutant resistant to sorbic acid appears, it should grow in the presence of sorbic acid, at least at low concentration of the inhibitor. Such a mutant should grow well enough to produce detectable turbidity in the medium, and if it is sufficiently resistant, it should show light transmittance essentially equal to that in control tubes containing no sorbic acid. Experiments of this kind were carried out. The data obtained showed that the irradiated cells as well as the unirradiated ones were effectively inhibited by all concentrations of sorbic acid used. In no case did the optical density of test cultures reach that in control tubes where no sorbic acid was present. Thus no mutation to sorbic acid resistance was detected in the limited number of simple experiments performed.

In considering the possibility of mutation to resistance it is

interesting to review evidence concerning the site of action of sorbic acid. To be successful, a hypothetical mutant would have to bypass any metabolic block caused by the action of sorbic acid. Just what such a block or blocks may consist of, has not been determined definitely. It is known that sorbic acid can prevent germination of wheat (1957). Microbiologists have searched for vulnerable metabolic sites in the known aerobic pathways since anaerobic bacteria are generally quite resistant to sorbic acid. Mukherjee (1952) presented data to show that in the presence of inhibitory amounts of cyanide, butyric acid ($CH_3-CH_2-CH_2-COOH$) was oxidized to crotonic acid ($CH_3-CH=CH-COOH$) up to a certain point but after this point was reached the oxidation could not continue further because of inhibition of the enzyme by the accumulated crotonic acid. He found that cyanide was a selective inhibitor of the oxidation of butyric acid to ketones by its action on the enzyme system responsible for converting crotonic acid to β hydroxy butyric acid. On the basis of this work, Melnick, et al. (1954), postulated that accumulation of $\alpha\ \beta$ unsaturated fatty acid in a test medium can inhibit the dehydrogenase system in molds. Thus sorbic acid may block the dehydrogenase system in microorganisms. Whitaker (1959) demonstrated that sorbic acid is a sulfhydryl enzyme inhibitor and postulated that this blockage inhibits the growth of microorganisms. Azukas, et al., (1959) disagreed with Whitaker's hypothesis. They pointed out the structural analogy of sorbic acid and phosphoenolpyruvate and suggested that sorbic acid might compete with phosphoenolpyruvate for enolase.

While the mode of action of sorbic acid is not definitely known, present evidence indicates that it affects the basic respiratory mechanisms.

Thus it appears that resistant mutants would have to have alternate respiratory pathways which would allow them to bypass the block in their normal scheme of oxidative metabolism.

Information on the relative frequency of mutations to sorbic acid resistance can be of great practical value. The usefulness of the compound as a general food preservative would be greatly decreased if resistant mutants occurred at a high rate. If the metabolic block produced by sorbic acid occurs at some critical point, then mutation to resistance might be a very rare event. We still do not know for certain the precise reaction or reactions affected by this agent, and the data presented here are too meager to provide a basis for generalization. However, no mutants resistant to sorbic acid have been reported in the literature, despite the fact that this chemical has now been in widespread use for several years.

SUMMARY AND CONCLUSIONS

The work reported here was undertaken in an attempt to obtain basic information about the effect of certain environmental factors on the activity of the important new food preservative, sorbic acid. Specifically, the influence of pH and temperature were considered. Germicidal activity was used as a convenient measure since quantitative data in the form of plate counts could be accumulated. A set of preliminary experiments on development of resistance to sorbic acid in a yeast are also recorded.

Germicidal activity of sorbic acid against Escherichia coli and Staphylococcus albus was determined at 30°C at pH 4.0 (except S. albus), 5.0, 6.0 and 7.0 (except S. albus). Total concentrations of sorbic acid were varied so as to yield equal concentrations of undissociated acid at

pH 4.0, 5.0, and 6.0. E. coli was destroyed rapidly at pH 4.0, slowly at pH 5.0 and was not affected at pH 6.0. S. albus followed the same pattern, but was killed more rapidly than E. coli exposed to the same solution. E. coli was destroyed rapidly at pH 7.0 when the sorbic acid concentration reached 14.4 per cent but was unaffected by 7.2 per cent of this substance at pH 7.0.

The acidity of the medium appears to have a direct effect on the organism, altering its permeability or some other factor; equal concentrations of undissociated sorbic acid show unequal germicidal activity at different pH values.

Similar tests of germicidal activity were performed at 0°, 10°, 20°, 30°, 37°, 40°, and 45°C. Resistance of E. coli was determined at pH 4.5 and 5.0, that of S. albus at pH 5.0. There was a striking absence of lethal effect at 20°C and below even after 2½ to 3 hours exposure. At 30°C the decrease in viable population was noticeable, but not marked. However, at the higher temperatures the rate of destruction of the test organisms was much more rapid, being greatest at the highest temperature used. Temperature coefficients calculated from experimental data yielded values of 1.14 and 1.35 for E. coli. These are comparable to similar values given for phenol in the literature.

In a limited series of experiments neither unirradiated nor ultra violet irradiated cultures of Saccharomyces cerevisiae showed evidence of resistance to graded concentrations of sorbic acid.

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LITERATURE CITED

Asukas, J. J., R. N. Costilow, H. L. Sadoff, and Wen Tah Hsu.
Inhibition of Glycosis by Sorbic Acid. Personal communication. 1959.

Boyd, J. W., and H. L. A. Tarr.
Inhibition of Mold and Yeast Development in Fish Products. J. of Food Technology. 9:411-412. 1955.

Borg, A. F., J. L. Etchells, and T. A. Bell.
The Influence of Sorbic Acid on Microbial Activity in Commercial Cucumber Fermentation. Bact. Proc., Abstract. 19: 1955.

Bell, T. A., J. L. Etchells, and A. F. Borg.
Influence of Sorbic Acid on the Growth of Certain Species of Bacteria, Yeasts, and Filamentous Fungi. J. of Bact. 77:5, 573-579. 1959.

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.

Cowles, P. B.
The Germicidal Action of the Hydrogen Ion and the Lower Fatty Acids. Yale J. Biol. Med. 13:571-578. 1941.

Costilow, R. N., W. E. Ferguson, and S. Ray.
Sorbic Acid as a Selective Agent in Cucumber Fermentation. I. Effect of Sorbic Acid on Microorganisms Associated with Cucumber Fermentation. Appl. Microbiol. 3:341-345. 1955.

Costilow, R. N., F. M. Coughlin, E. K. Robbins, and Wen Tah Hsu.
Sorbic Acid as Selective Agent in Cucumber Fermentation. II. Effect of Sorbic Acid on the Yeast and Lactic Acid Fermentation in Brined Cucumbers. Appl. Microbiol. 5:6, 373-379. 1957.

Deuel, H. J., and R. Alfin-Slater.
Sorbic Acid as a Fungistatic Agent for Foods. I. Harmlessness of Sorbic Acid as a Dietary Component. Food Research. 19:1, 1-11. 1954.

Deuel, H. J., C. E. Calbert, L. Anisfeld, H. McKeehan, and H. D. Blunden.
Sorbic Acid as a Fungistatic Agent in Foods. II. Metabolism of γ,δ Unsaturated Fatty Acids with Emphasis on Sorbic Acid. Food Research. 19:1, 13-19. 1954.

Emard, L. O., and R. H. Vaughn.
Selectivity of Sorbic Acid Media for the Catalase Negative Lactic Acid and Clostridia. J. Bact. 63:487-494. 1952.

Ferguson, W. E., and W. B. Powrie.
Studies on the Preservation of Fresh Apple Juice with Sorbic Acid. Appl. Microbiol. 5:1, 41-43. 1957.

Gale, E. F., and H. M. R. Epps.
The Effect of the pH of the Medium During Growth on the Enzymic Activities of Bacteria (Escherichia coli and Micrococcus lysodeibticus) and the Biological Significance of the Changes Produced. Biochem. J. 36:600. 1942.

Gooding, C. M. Q.
Process of Inhibiting Growth of Molds. U.S. Patent 2,379,244. 1945.

Hansen, J. D., and M. D. Appleman.
Effect of Sorbic, Propionic, and Caproic Acids on the Growth of Certain Clostridia. Food Research. 20:92-96. 1955.

Jordan, R. C., and S. E. Jacobs.
Studies in the Dynamics of Disinfection. V. The Temperature Coefficient of the Reaction Between Phenol and Bact. coli, Derived from Data Obtained by an Improved Technique. J. Hyg. Comb. 44:243. 1946.

Jordan, R. C., S. E. Jacobs, and H. E. F. Davis.
Studies in the Dynamics of Disinfection. J. Hyg. Comb. 46:2, 33. 1947.

Kahlenberg, L., and H. T. Rodney.
On the Toxic Action of Dissolved Salts and Their Electrolytic Dissociation. Bot. Gaz. 22:81-124. 1896.

McCulloch, E. C.
Disinfection and Sterilization. Philadelphia, Lea and Febiger. 525 pp. 1936.

Mukherjee, S.
Studies on Degradation of Fats by Microorganisms. III. The Metabolism of Butyric Acid by A. niger. Arch. Biochem. 35:34. 1952.

Melnick, D., F. H. Luckmann, and M. C. Gooding.
Sorbic Acid as Fungistatic Agent for Foods. VI. Metabolic Degradation of Sorbic Acid in Cheese by Molds and the Mechanism of Mold Inhibition. Food Research. 19:1, 44-57. 1954.

Melnick, D., F. H. Luckmann, and M. C. Gooding.
Sorbic Acid as Fungistatic Agent for Foods. XI. Effectiveness of Sorbic Acid in Protecting Cakes. Food Research. 21:1, 133. 1956.

Osterhout, W. J. V.
The Absorption of Electrolytes in Large Plant Cells. Bot. Rev. 2:6, 283. 1935.

Ordal, E. J., and F. Deromedi.
Studies on the Action of Wetting Agents on Microorganisms. II. The Synergistic Effect of Synthetic Wetting Agents on the Germicidal Action of Halogenated Phenols. J. of Bact. 45:3. 1943.

Phillips, G. F., and J. O. Mundt.
Sorbic Acid as an Inhibitor of Scum Yeast in Cucumber Fermentation. J. Food Technol. 4:291-293. 1950.

Patrick, R., and C. D. Atkins.
Effectiveness of Sorbic Acid as a Preservative for Tangerine Sherbet Base. Proceedings of the Florida State Horticultural Society. 67:194. 1954.

Puls, D. D., L. F. Lindgren, and F. P. Cosgrove.
Sorbic Acid as a Fungistatic Agent for Certain Pharmaceuticals. J. Amer. Pharm. Assoc. 44:85. 1955.

Pomeranz, Y.
Effectiveness of Sorbic Acid in Preservation of Damp Wheat. Food Research. 22:6, 553-561. 1957.

Reid, I. D.
Disinfection Action of Certain Organic Acids. Am. J. Hyg. 16:540-556. 1932.

Rahn, O., and J. E. Conn.
Effect of Increase in Acidity on Antiseptic Efficiency. Ind. Eng. Chem. 36:185-187. 1944.

Rahn, Otto.
Physical Method of Sterilization of Microorganisms. Bact. Review. 9:1. 1945.

Sheneman, J. M., and R. N. Costilow.
Sorbic Acid as Preservative for Sweet Cucumber Pickles. Appl. Micro. 3:3, 186. 1955.

Smith, D. P., and N. J. Rollin.
Sorbic Acid as a Fungistatic Agent for Foods. VIII. Effectiveness of Sorbic Acid in Protecting Cheese. Food Research. 19:1, 59. 1954.

Straka, R. P., and J. L. Stokes.
Rapid Destruction of Bacteria in Commonly Used Diluents and Its Elimination. Appl. Micro. 5:1. 1957.

Vaughn, R. H., and L. O. Emard.
Selectivity of Sorbic Acid Media for Catalase Negative Lactic Acid and Obligate Sporulating Anaerobes. Bact. Proc., Abstract. 38: 1951.

Union Carbide Chemicals Co.
Technical Information, BULLETIN F-8526A, Dec., 1956.

Whitaker, J. R.
Inhibition of Sulphydryl Enzymes with Sorbic Acid. Food Research. 24:37-43. 1959.

Winslow, C. E. A., and E. E. Lochridge.
The Toxic Effect of Certain Acids Upon Typhoid and Colon Bacilli in Relation to the Degree of Their Dissociation. J. Inf. Dis. 3:547-570. 1906.

APPENDIX

APPENDIX

Table 10. Plate counts: *Escherichia coli* treated with 0.1 per cent sorbic acid at pH 4.0 and 30°C.

Section A - Control

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	45	45M	
	b		44	44M	42.3M
	c		38	38M	
	3 a,b,c	10^{-7}	<30		
5	4 a,b,c	10^{-4}	>300		
	5 a	10^{-5}	245		
	b		236		
	c		308		
	6 a	10^{-6}	29	29M	
	b		35	35M	32.0M
	c		32	32M	
10	8 a,b,c	10^{-4}	>300		
	9 a	10^{-5}			
	b		173	17.3M	
	c		153	15.3M	15.9M
20	11 a,b,c	10^{-4}	>300		
	12 a	10^{-5}	95	9.5M	
	b		106	10.6M	9.7M
	c		89	8.9M	
30	14 a,b,c	10^{-4}	>300		
	15 a	10^{-5}	59	5.9M	
	b		65	6.5M	5.7M
	c		47	4.7M	

Table 10. (Cont.)

Section A - Test

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
5	17 a,b,c	10^{-4}	>300		
	18 a	10^{-5}	49	4.9M	
	b		46	4.6M	4.6M
	c		42	4.2M	
10	20 a,b,c	10^{-3}	>300		
	21 a	10^{-4}	148	1.5M	
	b		119	1.2M	1.2M
	c		104	1.0M	
20	23 a,b,c	10^{-2}	>300		
	24 a	10^{-3}	108	108T	
	b		126	126T	143T
	c		196	196T	
30	25 a,b,c	10^{0}	0	0	0

Section B - Control

0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	39	39M	
	b		49	49M	45.3M
	c		48	48M	
	3 a,b,c	10^{-7}	<30		
5	4 a	10^{-5}	220	22.0M	
	b		200	20.0M	22.5M
	c		256	25.6M	
	5 a	10^{-6}	30		
	b		30		
	c		23		

Table 10 (Concl.)

Section B - Control (Cont.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
10	6 a	10^{-5}	116	11.6M	
	b		196	19.6M	14.9M
	c		136	13.6M	
	7 a,b,c	10^{-6}	<30		
20	8 a,b,c	10^{-4}	>300		
	9 a	10^{-5}	79	7.9M	
	b		59	5.9M	6.5M
	c		56	5.6M	
30	10 a	10^{-4}	59	59T	
	b		87	87T	69T
	c		61	61T	

Section B - Test

1/2	11 a,b,c	10^{-5}	>300		
	12 a	10^{-6}	35	35M	
	b		41	41M	39.3M
	c		42	42M	
5	13 a,b,c	10^{-4}	>300		
	14 a	10^{-5}	?	?	
	b		37	3.7M	
	c		30	3.0M	3.4M
10	15 a	10^{-3}	74	74T	
	b		72	72T	73T
	c		73	73T	
	16 a,b,c	10^{-4}	<30		
20	17 a,b,c	10^{-2}	0	0	0
30	18 a,b,c	10^{0}	0	0	0

Table 11. Plate counts: Escherichia coli treated with 0.23 per cent sorbic acid at pH 5.0 and pH 6.0 at 30°C.

Section A - Control at pH 5.0

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	32	32M	38.7M
	b		46	46M	
	c		38	38M	
90	4 a,b,c	10^{-5}	>300		
	5 a	10^{-6}	46	46M	
	b		36	36M	44.3M
	c		51	51M	

Section B - Control at pH 5.0

0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	64	64M	
	b		71	71M	73.7M
	c		86	86M	
90	4 a,b,c	10^{-5}	>300		
	5 a	10^{-6}	67	67M	
	b		64	64M	66.7M
	c		69	69M	

Section A - Test at pH 5.0

0	11 a,b,c	10^{-5}	>300		
	12 a	10^{-6}	39	39M	
	b		37	37M	41.0M
	c		47	47M	

Table 11 (Cont.)

Section A - Test at pH 5.0 (Cont.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
10	13 a,b,c	10^{-5}	>300		
	14 a	10^{-6}	37	37M	38.3M
	b		35	35M	
	c		43	43M	
30	16 a,b,c	10^{-5}	>300		
	17 a	10^{-6}	35	35M	
	b		33	33M	33.3M
	c		32	32M	
60	19 a,b,c	10^{-5}	>300		
	20 a	10^{-6}	40	40M	
	b		37	37M	41.3M
	c		47	47M	
90	22 a,b,c	10^{-5}	>300		
	23 a	10^{-6}	28	28M	
	b		33	33M	31.3M
	c		33	33M	

Section B - Test at pH 5.0

0	11 a,b,c	10^{-5}	>300		
	12 a	10^{-6}	64	64M	
	b		71	71M	63.0M
	c		54	54M	
10	13 a,b,c	10^{-5}	>300		
	14 a	10^{-6}	52	52M	
	b		74	74M	62.0M
	c		60	60M	

Table 11 (Cont.)

Section B - Test at pH 5.0 (Cont.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
30	15 a,b,c	10^{-5}	>300		
	16 a	10^{-6}	52	52M	
	b		68	68M	60.7M
	c		62	62M	
60	18 a,b,c	10^{-5}	>300		
	19 a	10^{-6}	53	53M	
	b		37	37M	45.3M
	c		46	46M	
90	20 a,b,c	10^{-4}	>300		
	21 a	10^{-5}	183	18.3M	
	b		223	15.3M	
	c		150	15.0M	31.8M
	22 a	10^{-6}	42	42M	
	b		55	55M	
	c		38	38M	
Section A - Control at pH 6.0					
0	6 a,b,c	10^{-5}	>300		
	7 a	10^{-6}	57	57M	
	b		45	45M	52.7M
	c		56	56M	
90	9 a,b,c	10^{-5}	>300		
	10 a	10^{-6}	58	58M	
	b		59	59M	57.3M
	c		55	55M	

Table 11 (Cont.)

Section B - Control at pH 6.0

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	6 a,b,c	10^{-5}	>300		
	7 a	10^{-6}	56	56M	
	b		59	59M	59.3M
	c		63	63M	
90	9 a,b,c	10^{-5}	>300		
	10 a	10^{-6}	70	70M	
	b		80	80M	71.3M
	c		64	64M	

Section A - Test at pH 6.0

0	24 a,b,c	10^{-5}	>300		
	25 a	10^{-6}	43	43M	
	b		38	38M	47.0M
	c		60	60M	
10	26 a,b,c	10^{-5}	>300		
	27 a	10^{-6}	34	34M	
	b		43	43M	40.3M
	c		44	44M	
30	29 a,b,c	10^{-5}	>300		
	30 a	10^{-6}	41	41M	
	b		46	46M	47.7M
	c		56	56M	
60	32 a,b,c	10^{-5}	>300		
	33 a	10^{-6}	34	34M	
	b		44	44M	43.7M
	c		53	53M	

Table 11 (Concl.)

Section A - Test at pH 6.0 (Concl.)

Time, minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
90	35 a,b,c	10^{-5}	>300		
	36 a	10^{-6}	42	42M	44.0M
	b		40	40M	
	c		50	50M	

Section B - Test at pH 6.0

0	23 a,b,c	10^{-5}	>300		
	24 a	10^{-6}	79	79M	
	b		60	60M	67.3M
	c		63	63M	
10	25 a,b,c	10^{-5}	>300		
	24 a	10^{-6}	69	69M	
	b		56	56M	61.0M
	c		58	58M	
30	27 a,b,c	10^{-5}	>300		
	28 a	10^{-6}	65	65M	
	b		60	60M	56.7M
	c		55	55M	
60	30 a,b,c	10^{-5}	>300		
	31 a	10^{-6}	47	47M	
	b		48	48M	50.3M
	c		56	56M	
90	33 a,b,c	10^{-5}	>300		
	34 a	10^{-6}	49	49M	
	b		59	59M	50.0M
	c		47	47M	

Table 12. Plate counts: Escherichia coli treated with potassium sorbate at pH 6.0 and pH 7.0 at 30°C.

Section A - Control at pH 6.0

Time/ minutes	Plate numbers	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	31	31M	36.3M
	b		38	38M	
	c		40	40M	
	3 a,b,c	10^{-7}	<30		
10	4 a,b,c	10^{-5}	>300		
	5 a	10^{-6}	36	36M	
	b		42	42M	39.3M
	c		40	40M	
	6 a,b,c	10^{-7}	<30		

Section A - Test at pH 6.0, potassium sorbate 1.8 per cent

0	7 a,b,c	10^{-5}	>300		
	8 a	10^{-6}	49	49M	
	b		34	34M	42.7M
	c		45	45M	
	9 a,b,c	10^{-7}	<30		
10	11 a,b,c	10^{-5}	>300		
	12 a	10^{-6}	40	40M	
	b		41	41M	38.7M
	c		35	35M	

Table 12 (Cont.)

Section A - Test at pH 6.0, potassium sorbate 3.6 per cent

Time/ minutes	Plate numbers	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	13 a,b,c	10^{-5}	>300		
	14 a	10^{-6}	40	40M	
	b		38	38M	43.0M
	c		51	51M	
	15 a,b,c	10^{-7}	<30		
10	18 a,b,c	10^{-5}	>300		
	19 a	10^{-6}	52	52M	
	b		46	46M	48.7M
	c		48	48M	
Section B - Control at pH 7.0					
0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	47	47M	
	b		41	41M	45.0M
	c		47	47M	
10	3 a,b,c	10^{-5}	>300		
	4 a	10^{-6}	52	52M	
	b		45	45M	52.0M
	c		59	59M	
Section B - Test at pH 7.0, potassium sorbate 1.8 per cent					
0	5 a,b,c	10^{-5}	>300		
	6 a	10^{-6}	49	49M	
	b		45	45M	45.0M
	c		41	41M	

Table 12 (Cont.)

Section B - Test at pH 7.0, potassium sorbate 1.8 per cent (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
10	8 a,b,c	10^{-5}	>300		
	9 a	10^{-6}	39	39M	
	b		48	48M	46.7M
	c		53	53M	
Section B - Test at pH 7.0, potassium sorbate 3.6 per cent					
0	10 a,b,c	10^{-5}	>300		
	11 a	10^{-6}	37	37M	
	b		50	50M	40.0M
	c		33	33M	
10	13 a,b,c	10^{-5}	>300		
	14 a	10^{-6}	38	38M	
	b		56	56M	49.7M
	c		55	55M	
Section B - Test at pH 7.0, potassium sorbate 7.2 per cent					
0	15 a,b,c	10^{-5}	>300		
	16 a	10^{-6}	41	41M	
	b		39	39M	40.0M
	c		40	40M	
10	18 a	10^{-5}	284	28.4M	
	b		324	32.4M	27.6M
	c		220	22.0M	
	19 a	10^{-6}	35		
	b		28		
	c		47		

Table 12 (Concl.)

Section B - Test at pH 7.0, potassium sorbate 14.4 per cent

Time, minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	20 a	10^{-5}	115	11.5M	
	b		112	11.2M	
	c		119	11.9M	
	21 a	10^{-6}	18	18M	
	b		37	37M	23.6M
	c		52	52M	
10	22 a,b,c	10^{-4}	<30		<300T

Table 13. Plate counts: Staphylococcus albus treated with 0.23 per cent sorbic acid at pH 5.0 and 30°C.

Section A - Control

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a	10^{-5}	108	10.8M	
	b		94	9.4M	9.6M
	c		87	8.7M	
	2 a,b,c	10^{-6}	<30		
60	4 a,b,c	10^{-4}	>300		
	5 a	10^{-5}	110	11.0M	
	b		81	8.1M	9.2M
	c		84	8.4M	

Section A - Test

0	6 a	10^{-5}	100	10.0M	
	b		73	7.3M	8.3M
	c		77	7.7M	
	7 a,b,c	10^{-6}	<30		
10	8 a	10^{-5}	96	9.6M	
	b		85	8.5M	8.5M
	c		74	7.4M	
	9 a,b,c	10^{-6}	<30		
20	10 a	10^{-5}	66	6.6M	
	b		62	6.2M	6.7M
	c		74	7.4M	
	11 a,b,c	10^{-6}	<30		

Table 13 (Cont.)

Section A - Test (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
30	12 a,b,c	10^{-4}	>300		
	13 a	10^{-5}	78	7.8M	
	b		66	6.6M	6.9M
	c		64	6.4M	
	14 a,b,c	10^{-6}	<30		
40	16 a,b,c	10^{-4}	>300		
	17 a	10^{-5}	60	6.0M	
	b		61	6.1M	6.2M
	c		64	6.4M	
50	19 a,b,c	10^{-4}	>300		
	20 a	10^{-5}	70	7.0M	
	b		60	6.0M	6.0M
	c		50	5.0M	
60	23 a,b,c	10^{-4}	>300		
	24 a	10^{-5}	70	7.0M	
	b		43	4.3M	5.1M
	c		41	4.1M	

Section B - Control

0	1 a,b,c	10^{-4}	>300		
	2 a	10^{-5}	130	13.0M	
	b		110	11.0M	12.1M
	c		123	12.3M	
	3 a,b,c	10^{-6}	< 30		

Table 13 (Cont.)

Section B - Control (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
5	4 a,b,c	10^{-4}	>300		
	5 a	10^{-5}	141	14.1M	
	b		120	12.0M	12.2M
	c		104	10.4M	
	6 a,b,c	10^{-6}	< 30		
10	7 a,b,c	10^{-4}	>300		
	8 a	10^{-5}	124	12.4M	
	b		130	13.0M	13.5M
	c		150	15.0M	
	9 a,b,c	10^{-6}	< 30		
20	10 a,b,c	10^{-4}	>300		
	11 a	10^{-5}	96	9.6M	
	b		95	9.5M	10.7M
	c		130	13.0M	
	12 a,b,c	10^{-6}	< 30		
30	13 a,b,c	10^{-4}	>300		
	14 a	10^{-5}	123	12.3M	
	b		110	11.0M	12.0M
	c		127	12.7M	
	15 a,b,c	10^{-6}	< 30		

Table 13 (Cont.)

Section B - Test

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	16 a,b,c	10^{-4}	>300		
	17 a	10^{-5}	134	13.4M	
	b		146	14.6M	14.1M
	c		143	14.3M	
	18 a,b,c	10^{-6}	<30		
5	19 a,b,c	10^{-4}	>300		
	20 a	10^{-5}	144	14.4M	
	b		124	12.4M	13.8M
	c		145	14.5M	
	21 a,b,c	10^{-6}	< 30		
10	22 a,b,c	10^{-4}	>300		
	23 a	10^{-5}	95	9.5M	
	b		114	11.4M	11.4M
	c		132	13.2M	
	24 a,b,c	10^{-6}	< 30		
20	26 a,b,c	10^{-4}	>300		
	27 a	10^{-5}	115	11.5M	
	b		106	10.6M	11.6M
	c		128	12.8M	
	28 a,b,c	10^{-6}	<30		
30	30 a,b,c	10^{-4}	>300		
	31 a	10^{-5}	42	4.2M	
	b		46	4.6M	5.1M
	c		65	6.5M	
	32 a,b,c	10^{-6}	<30		

Table 13 (Cont.)

Section C - Control

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a,b,c	10^{-4}	>300		
	2 a	10^{-5}	107	10.7M	
	b		101	10.1M	11.7M
	c		142	14.2M	
	3 a,b,c	10^{-6}	<30		
30	4 a,b,c	10^{-4}	>300		
	5 a	10^{-5}	84	8.4M	
	b		80	8.0M	8.0M
	c		76	7.6M	

Section C - Test

0	7 a,b,c	10^{-4}	>300		
	8 a	10^{-5}	124	12.4M	
	b		102	10.2M	11.7M
	c		124	12.4M	
	9 a,b,c	10^{-6}	<30		
5	10 a,b,c	10^{-4}	>300		
	11 a	10^{-5}	97	9.7M	
	b		92	9.2M	9.5M
	c		11?	?	
	12 a,b,c	10^{-6}	<30		
10	13 a,b,c	10^{-4}	>300		
	14 a	10^{-5}	25?	?	
	b		68	6.8M	
	c		76	7.6M	7.2M
	15 a,b,c	10^{-6}	<30		

Table 13 (Concl.)

Section C - Test (Concl.)

Time/ minutes	Plate number	Dilution	Number of Bacteria per plate	Number of bacteria per ml	Average
20	16 a,b,c	10^{-4}	>300		
	17 a	10^{-5}	85	8.5M	
	b		87	8.7M	8.0M
	c		69	6.9M	
	18 a,b,c	10^{-6}	<30		
30	19 a,b,c	10^{-4}	>300		
	20 a	10^{-5}	100	10.0M	
	b		81	8.1M	8.9M
	c		85	8.5M	
	21 a,b,c	10^{-6}	<30		

Table 14. Plate counts: Escherichia coli treated with 0.13 per cent sorbic acid at pH 4.5 and various temperatures.

Section A - Control at 0°C

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	44	44M	
	b		46	46M	42.7M
	c		38	38M	
60	3 a,b,c	10^{-5}	> 300		
	4 a	10^{-6}	47	47M	
	b		52	52M	51.7M
	c		56	56M	

Section A - Control at 37°C

0	5 a,b,c	10^{-5}	> 300		
	6 a	10^{-6}	35	35M	
	b		34	34M	33.7M
	c		32	32M	
30	7 a	10^{-4}	244	2.4M	
	b		208	2.1M	2.3M
	c		228	2.3M	

Section A - Test at 0°C

60	10 a,b,c	10^{-5}	> 300		
	11 a	10^{-6}	47	47M	
	b		52	52M	52.0M
	c		57	57M	

Table 14. (Cont.)

Section A - Test at 10°C

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
30	13 a,b,c	10^{-5}	>300		
	14 a	10^{-6}	54	54M	
	b		41	41M	48.0M
	c		49	49M	
Section A - Test at 20°C					
30	16 a,b,c	10^{-5}	>300		
	17 a	10^{-6}	42	42M	
	b		43	43M	44.7M
	c		49	49M	
Section A - Test at 37°C					
30	18 a,b,c	10^{-4}	<30		<300T
Section B - Control at 20°C					
0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	42	42M	
	b		44	44M	40.0M
	c		34	34M	
60	3 a,b,c	10^{-5}	>300		
	4 a	10^{-6}	48	48M	
	b		50	50M	45.7M
	c		39	39M	

Table 14 (Cont.)

Section B - Control at 20°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
120	5 a	10^{-5}	336	33.6M	
	b		296	29.6M	31.5M
	c		304	30.4M	
	6 a	10^{-6}	19		
	b		39		
	c		38		
Section B - Control at 37°C					
0	7 a	10^{-5}	252	25.2M	
	b		380	38.0M	29.1M
	c		268	26.8M	
	8 a	10^{-6}	31	31M	
	b		30	30M	
	c		24	24M	
30	10 a,b,c	10^{-4}	>300		
	11 a	10^{-5}	292	29.2M	
	b		208	20.8M	24.9M
	c		248	24.8M	
60	12 a	10^{-2}	52	5.2T	
	b		26	2.6T	3.7T
	c		33	3.3T	
Section B - Test at 0°C					
0	15 a	10^{-5}	220	22.0M	
	b		268	26.8M	
	c		304	30.4M	
	16 a	10^{-6}	39	39M	
	b		28	28M	29.7M
	c		32	32M	

Table 14 (Cont.)

Section B - Test at 0°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
60	17 a	10^{-5}	256	25.6M	
	b		300	30.0M	
	c		312	31.2M	30.6M
	18 a	10^{-6}	39	39M	
	b		28	28M	
	c		30	30M	
120	19 a	10^{-5}	248	24.8	
	b		304	30.4	
	c		300	30.0	29.0M
	20 a	10^{-6}	28	28M	
	b		37	37M	
	c		24	24M	

Section B - Test at 10°C

60	21 a	10^{-5}	316	31.6M	
	b		260	26.0M	
	c		232	23.2M	
	22 a	10^{-6}	23	23M	26.6M
	b		25	25M	
	c		31	31M	
120	23 a,b,c	10^{-5}	>300		
	24 a	10^{-6}	31	31M	
	b		28	28M	30.3M
	c		32	32M	

Section B - Test at 20°C

60	25 a,b,c	10^{-5}	>300		
	26 a	10^{-6}	33	33M	
	b		30	30M	33.7M
	c		38	38M	

Table 14 (Concl.)

Section B - Test at 20°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
120	27 a,b,c	10^{-4}	>300		
	28 a	10^{-5}	216	21.6M	
	b		248	24.8M	25.7M
	c		320	32.0M	
	29 a	10^{-6}	24	24M	
	b		32	32M	
	c		20	20M	
Section B - Test at 37°C					
30	31 a	10^{-4}	324	3.24M	
	b		320	3.20M	2.8M
	c		300	3.0 M	
	32 a	10^{-5}	29	2.9 M	
	b		18	1.8 M	
	c		24	2.4 M	
60	33 a	10^{-2}	52	5.2 T	
	b		26	2.6 T	3.7T
	c		33	3.3 T	
	34 a,b,c	10^{-3}	<30		

Table 15. Plate counts: Escherichia coli treated with 0.23 per cent sorbic acid at pH 5.0 and various temperatures.

Section A - Control at 0°C

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a,b,c	10^{-5}	>300		
	2 a	10^{-6}	37	37M	
	b		30	30M	37.3M*
	c		45	45M	
180	4 a,b,c	10^{-5}	>300		
	5 a	10^{-6}	43	43M	
	b		52	52M	50.7M
	c		57	57M	

Section A - Control at 10°C

0	6 a,b,c	10^{-5}	>300		
	7 a	10^{-6}	45	45M	
	b		47	47M	46.0M*
	c		46	46M	
180	9 a,b,c	10^{-5}	>300		
	10 a	10^{-6}	44	44M	
	b		55	55M	51.3M
	c		55	55M	

Section A - Control at 20°C

0	11 a,b,c	10^{-5}	>300		
	12 a	10^{-6}	48	48M	
	b		52	52M	49.7 *
	c		49	49M	

* Average of these values used as a control count at zero time.

Table 15 (Cont.)

Section A - Control at 20°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
150	14 a,b,c	10^{-5}	>300		
	15 a	10^{-6}	52	52M	
	b		54	54M	52.0M*
	c		50	50M	
Section A - Control at 37°C					
0	16 a,b,c	10^{-5}	>300		
	17 a	10^{-6}	57	57M	
	b		58	58M	53.0M*
	c		44	44M	
90	19 a,b,c	10^{-5}	>300		
	20 a	10^{-6}	31	31M	
	b		41	41M	38.3M
	c		43	43M	
Section A - Test at 0°C					
0	21 a,b,c	10^{-5}	>300		
	22 a	10^{-6}	56	56M	
	b		60	60M	52.0M
	c		40	40M	
90	23 a,b,c	10^{-5}	>300		
	24 a	10^{-6}	42	42M	
	b		40	40M	42.7M
	c		46	46M	

* Average of these values used as control count at zero time.

Table 15 (Cont.)

Section A - Test at 0°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
180	26 a	10^{-5}	360	36.0M	
	b		408	40.8M	43.9M
	c		548	54.8M	
	27 a,b,c	10^{-6}	?	?	?
Section A - Test at 10°C					
90	28 a,b,c	10^{-5}	>300		
	29 a	10^{-6}	46	46M	43.0M
	b		42	42M	
	c		41	41M	
150	30 a,b,c	10^{-5}	>300		
	31 a	10^{-6}	52	52M	
	b		50	50M	50.0M
	c		39	39M	
180	33 a	10^{-5}	376	37.6M	
	b		412	41.2M	40.1M
	c		416	41.6M	
	34 a,b,c	10^{-6}	0?	?	?
Section A - Test at 20°C					
60	35 a,b,c	10^{-5}	>300		
	36 a	10^{-6}	46	46M	46.7M
	b		49	49M	
	c		45	45M	

Table 15 (Cont.)

Section A - Test at 20°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
120	37 a,b,c	10^{-5}	>300		
	38 a	10^{-6}	48	48M	
	b		49	49M	48.3M
	c		48	48M	
150	40 a,b,c	10^{-5}	>300		
	41 a	10^{-6}	42	42M	
	b		47	47M	45.3M
	c		47	47M	
Section A - Test at 37°C					
30	43 a,b,c	10^{-5}	>300		
	44 a	10^{-6}	32	32M	
	b		47	47M	40.0M
	c		41	41M	
60	46 a,b,c	10^{-5}	>300		
	47 a	10^{-6}	33	33M	
	b		29	29M	29.0M
	c		25	25M	
90	48 a,b,c	10^{-4}	>300		
	49 a	10^{-5}	260	26.0M	
	b		200	20.0M	19.7M
	c		132	13.2M	
	50 a	10^{-6}	21		
	b		29		
	c		25		

Table 15 (Cont.)

Section B - Control at 30°C

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	2 a,b,c	10^{-5}	>300		
	3 a	10^{-6}	61	61M	
	b		67	67M	63.7M
	c		63	63M	
60	5 a,b,c		>300		
	6 a	10^{-6}	64	64M	
	b		62	62M	65.3M
	c		70	70M	
Section B - Control at 37°C					
60	9 a	10^{-5}	428	42.8M	
	b		352	35.2M	40.4M
	c		432	43.2M	
Section B - Control at 45°C					
60	12 a,b,c	10^{-4}	>300		
	13 a	10^{-5}	271	27.1M	
	b		270	27.0M	25.8M
	c		232	23.2 M	
Section B - Test at 30°C					
0	15 a,b,c	10^{-5}	>300		
	16 a	10^{-6}	50	50M	
	b		71	71M	60.7M
	c		61	61M	

Table 15 (Cont.)

Section B - Test at 30°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
20	19 a,b,c	10^{-5}	>300		
	20 a	10^{-6}	46	46M	
	b		65	65M	60.7M
	c		52	52M	
40	23 a,b,c	10^{-5}	>300		
	24 a	10^{-6}	46	46M	
	b		41	41M	44.0M
	c		45	45M	
60	26 a	10^{-5}	316	31.6M	
	b		252	25.2M	34.4M
	c		464	46.4M	

Section B - Test at 37°C

10	28 a,b,c	10^{-5}	>300		
	29 a	10^{-6}	51	51M	
	b		58	58M	55.3M
	c		57	57M	
20	32 a,b,c	10^{-5}	>300		
	33 a	10^{-6}	57	57M	
	b		64	64M	57.3M
	c		51	51M	
40	34 a,b,c	10^{-4}	>300		
	35 a	10^{-5}	160	16.0M	
	b		91	9.1M	11.7M
	c		100	10.0M	
	36 a	10^{-6}	22		
	b		31		
	c		30		

Table 15 (Concl.)

Section B - Test at 37°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
60	38 a,b,c	10^{-4}	>300		
	39 a	10^{-5}	54	5.4M	
	b		48	4.8M	5.6M
	c		66	6.6M	

Section B - Test at 45°C

10	40 a,b,c	10^{-4}	>300		
	41 a	10^{-5}	63	6.3M	
	b		91	9.1M	7.2M
	c		64	6.4M	
20	42 a	10^{-3}	193	193T	
	b		262	260T	225T
	c		220	220T	
	43 a,b,c	10^{-4}	<30		
40	46 a,b,c	10^{-3}	<30		
60	49 a	10^{0}	22	22	
	b		27	27	<30
	c		37	37	

Table 16. Plate counts: Staphylococcus albus treated with 0.23 per cent sorbic acid at pH 5.0 and various temperatures.

Section A - Control at 20°C

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a	10^{-5}	59	5.9M	
	b		59	5.9M	6.1M*
	c		65	6.5M	
	2 a,b,c	10^{-6}	<30		
10	3 a	10^{-5}	57	5.7M	
	b		60	6.0M	6.1M
	c		65	6.5M	
	4 a,b,c	10^{-6}	<30		
20	5 a	10^{-5}	74	7.4M	
	b		66	6.6M	6.0M
	c		41	4.1M	
	6 a,b,c	10^{-6}	<30		
40	7 a	10^{-5}	55	5.5M	
	b		65	6.5M	6.1M
	c		62	6.2M	
	8 a,b,c	10^{-6}	<30		
60	9 a	10^{-5}	61	6.1M	
	b		63	6.3M	6.1M
	c		70	7.0M	
	10 a,b,c	10^{-6}	<30		

Section A - Control at 30°C

0	11 a	10^{-5}	69	6.9M	
	b		58	5.8M	6.4M*
	c		64	6.4M	
	12 a,b,c	10^{-6}	<30		

* 6.4 represents an average of six separate determinations.

Table 16 (Cont.)

Section A - Control at 30°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
10	13 a	10^{-5}	51	5.1M	
	b		65	6.5M	5.5M
	c		49	4.9M	
	14 a,b,c	10^{-6}	<30		
20	15 a	10^{-5}	59	5.9M	
	b		52	5.2M	6.0M
	c		69	6.9M	
	16 a,b,c	10^{-6}	<30		
40	17 a	10^{-5}	49	4.9M	
	b		61	6.1M	4.9M
	c		38	3.8M	
	18 a,b,c	10^{-6}	<30		
60	19 a	10^{-5}	50	5.0M	
	b		50	5.0M	5.0M
	c		51	5.1M	
	20 a,b,c	10^{-6}	<30		

Section A - Control at 40°C

0	21 a	10^{-5}	69	6.9M	
	b		69	6.9M	6.8M*
	c		65	6.5M	
	22 a,b,c	10^{-6}	<30		
10	23 a	10^{-5}	13	1.3M	
	b		17	1.7M	≃ 1.5M
	c		14	1.4M	
	24 a,b,c	10^{-6}	<30		

Table 16 (Cont.)

Section A - Control at 40°C (Concl.)

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
20	25 a,b,c	10^{-5}	< 30		< 3M
40	27 a,b,c	10^{-3}	< 30		< 30T
60	29 a,b,c	10^{-2}	< 30		< 3T

Section A - Test at 20°C

0	31 a	10^{-5}	51	5.1M	
	b		61	6.1M	5.6 *
	c		57	5.7M	
	32 a,b,c	10^{-6}	< 30		
10	33 a	10^{-5}	63	6.3M	
	b		62	6.2M	6.2M
	c		60	6.0M	
	34 a,b,c	10^{-6}	< 30		
20	35 a	10^{-5}	68	6.8M	
	b		44	4.4M	6.0M
	c		67	6.7M	
	36 a,b,c	10^{-6}	< 30		
40	37 a	10^{-5}	36	3.6M	
	b		67	6.7M	5.3M
	c		55	5.5M	
	38 a,b,c	10^{-6}	< 30		
60	39 a	10^{-5}	37	3.7M	
	b		40	4.0M	3.9M
	c		40	4.0M	
	40 a,b,c	10^{-6}	< 30		

Table 16 (Cont.)

Section A - Test at 30°C

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	41 a	10^{-5}	70	7.0M	
	b		73	7.3M	7.0 *
	c		68	6.8M	
	42 a,b,c	10^{-6}	<30		
10	43 a	10^{-5}	59	5.9M	
	b		74	7.4M	6.2M
	c		54	5.4M	
	44 a,b,c	10^{-6}	<30		
20	45 a	10^{-5}	52	5.2M	
	b		62	6.2M	5.1M
	c		40	4.0M	
	46 a,b,c	10^{-6}	<30		
40	47 a,b,c	10^{-5}	<30		< 3M
60	49 a,b,c	10^{-4}	<30		<300T

Section A - Test at 40°C

0	51 a	10^{-5}	66	6.6M	
	b		68	6.8M	6.8 *
	c		69	6.9M	
	52 a,b,c	10^{-6}	<30		
10	53 a,b,c	10^{-4}	<30		<300T
20	55 a,b,c	10^{-2}	<30		< 3T
40	56 a,b,c	10^{-1}	<30		<300
60	58 a,b,c	10^{0}	<30		<30

Table 16 (Concl.)

Section B - Control at 37°C

Time/ minutes	Plate number	Dilution	Number of bacteria per plate	Number of bacteria per ml	Average
0	1 a	10^{-4}	>300		
	b				
	c				
	2 a	10^{-5}	187	18.7M	
	b		134	13.4M	15.2M
	c		136	13.6M	
	3 a,b,c	10^{-6}	<30		
60	7 a,b,c	10^{-3}	>300		
	8 a	10^{-4}	235	2.3M	
	b		125	1.2M	1.7M
	c		148	1.5M	
	9 a,b,c	10^{-5}	<30		<30

Section B - Test at 37°C

10	22 a	10^{-4}	70	700T	
	b		56	560T	620T
	c		60	600T	
	23 a,b,c	10^{-5}	<30		
20	24 a,b,c	10^{-3}	<30		<30T
40	25 a,b,c	10^{-2}	<30		<3T
60	26 a,b,c	10^{0}	0		0

* 6.4 represents an average of six separate determinations.

Table 17. Transmittance of cultures of Sacch. cerevisiae in different concentrations of sorbic acid at pH 4.0.

Section A - Unirradiated

Sorbic Acid concentration	Tube number	Per cent Transmittance scale/hours 0	48	72	168
0.0%	1	77.5	9.0	11.0	8.5
	2	91.0	8.5	11.5	7.5
	3	88.0	8.5	13.5	7.0
	4	87.5	9.5	13.5	8.5
	5	90.0	9.0	12.0	8.0
Average		86.8	8.9	12.3	7.9
0.02%	1	75.5	20.0	23.0	27.0
	2	74.5	35.0	35.0	34.0
	3	70.0	23.0	26.0	26.0
	4	71.0	36.0	46.0	43.0
	5	79.5	22.0	24.0	26.5
Average		74.1	27.2	30.8	31.3
0.04%	1	77.5	47.0	51.5	44.5
	2	78.5	52.5	55.0	46.5
	3	84.0	55.0	58.0	42.0
	4	81.0	58.5	52.5	45.5
	5	81.5	61.5	52.5	47.5
Average		80.5	54.9	53.9	45.2
0.07%	1	79.5	63.0	61.0	55.0
	2	81.0	62.5	56.5	55.0
	3	79.5	58.5	51.5	52.0
	4	81.5	63.5	61.5	54.0
	5	79.5	56.0	58.5	48.0
Average		80.2	60.7	57.8	52.8
0.1%	1	78.5	71.5	59.0	60.0
	2	72.5	66.0	61.5	57.5
	3	77.5	74.5	67.0	60.0
	4	75.0	67.0	63.5	51.0
	5	77.0	66.0	61.0	55.0
Average		76.1	69.0	62.4	56.7
0.2%	1	83.0	68.5	68.0	68.0
	2	82.0	64.5	69.0	65.0
	3	81.0	70.0	70.5	67.0
	4	89.0	65.5	59.0	66.0
	5	92.0	77.0	80.0	78.5
Average		85.4	69.1	69.3	68.9

Table 17 (Concl.)

Section B - Irradiated

Sorbic Acid concentration	Tube number	Per cent Transmittance scale/hours 0	48	72	168
0.0%	1	62.0	12.0	8.0	4.5
	2	60.5	12.0	8.5	3.5
	3	58.5	12.5	9.0	4.0
	4	54.5	12.0	8.5	8.5
	5	58.5	12.0	9.0	6.5
Average		58.8	12.1	8.6	5.4
0.02%	1	54.0	30.5	28.0	30.0
	2	56.5	27.5	26.0	31.0
	3	50.0	34.5	30.5	33.0
	4	50.5	36.5	31.5	35.0
	5	51.5	32.5	26.5	31.0
Average		52.5	32.3	28.5	31.0
0.04%	1	51.0	39.0	25.5	33.0
	2	54.5	32.0	24.5	27.0
	3	57.0	40.5	27.0	31.0
	4	46.5	34.0	26.0	25.5
	5	51.0	34.0	26.5	32.0
Average		52.0	35.9	25.9	29.7
0.07%	1	55.0	38.0	46.5	31.0
	2	50.5	35.0	39.5	27.0
	3	59.5	35.5	47.5	35.5
	4	55.5	36.5	43.5	35.5
	5	66.5	39.0	47.5	42.5
Average		57.4	36.8	44.9	34.3
0.1%	1	51.0	38.0	24.5	21.0
	2	55.0	37.5	35.0	38.0
	3	56.5	44.0	38.5	38.0
	4	53.5	38.0	32.5	27.5
	5	51.5	38.0	29.5	26.0
Average		53.5	39.1	32.0	30.1
0.2%	1	49.5	54.0	46.0	54.5
	2	41.5	45.5	48.5	42.5
	3	55.0	52.5	53.0	54.5
	4	43.5	45.5	43.5	44.5
	5	45.5	53.5	45.0	51.5
Average		47.0	50.2	47.2	49.5

STUDIES ON THE GERMICIDAL ACTIVITY OF SORBIC ACID

by

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ABSTRACT

The discovery that sorbic acid is an effective fungistat and is non-toxic to human beings has lead to its use as a commercial preservative for certain foods. Practical use has far outdistanced basic knowledge about the effect of environment on the ability of this compound to inhibit or destroy microorganisms. The present study was initiated in an attempt to supply information on the effect of pH and temperature on the activity of sorbic acid. Special attention was given to the undissociated form of sorbic acid since the activity of weak organic acids as germicides or growth inhibitors usually resides in this form of the molecule.

The test organisms, Escherichia coli and Staphylococcus albus were exposed to sorbic acid at total concentrations designed to yield identical concentrations of undissociated acid at each of the pH values used. Activity of the compound was determined by plate counts of the viable population at various time intervals. Survival of E. coli was determined at pH 4.0, 5.0, 6.0, and 7.0; that of S. albus was determined at pH 5.0. This series of tests was made at 30°C.

E. coli was killed rapidly by 0.11 per cent sorbic acid at pH 4.0. At pH 5.0 the lethal effect was much less rapid even though the total concentration of sorbic acid was 0.235 per cent. At pH 6.0 and 1.57 per cent sorbic acid there was no detectable killing of the culture even after 90 minutes exposure. In similar experiments no lethal effect was found when 3.6 per cent sorbic acid was used at pH 6.0 or 7.2 per cent at pH 7.0. However, 14.4 per cent sorbic acid at pH 7.0 destroyed nearly all bacteria in the test culture in ten minutes. S. albus was generally more sensitive than E. coli. Thirty to sixty minutes exposure reduced the viable popula-

tion by about one half at pH 5.0. Lethality of sorbic acid for E. coli was related inversely to pH even when the concentration of undissociated sorbic acid was held constant. Thus it appears that the marked increase in germicidal activity of sorbic acid with decrease in pH is a function of factors other than the dissociation of the acid itself. It is suggested that the observed differences may be due to changes in permeability of the cell with pH.

Similar tests of germicidal activity were performed at 0°, 10°, 20°, 30°, 37°, 40°, and 45°C. Resistance of E. coli was determined at pH 4.5 and 5.0, that of S. albus at pH 5.0. There was a striking absence of lethal effect at 20°C and below even after 2½ to 3 hours exposure. At 30°C the decrease in viable population was noticeable, but not marked. However, at the higher temperatures the rate of destruction of the test organisms was much more rapid, being greatest at the highest temperature used. Temperature coefficients calculated from experimental data yielded values of 1.14 and 1.35 for E. coli. These are comparable to similar values given for phenol in the literature.

In a limited series of experiments neither unirradiated nor ultra violet irradiated cultures of Saccharomyces cerevisiae showed evidence of resistance to graded concentrations of sorbic acid placed in malt extract-yeast extract broth in the presence of 0.02, 0.04, 0.07, 0.1 and 0.2 sorbic acid at pH 4.0. Turbidity measurements were made periodically for one week. None of the cultures developed turbidity comparable to that of the control which contained no sorbic acid.