EFFECT OF HYDROGEN ION CONCENTRATION ON THE TOXICITY OF SODIUM BENZOATE TO MICROÖRGANISMS

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Observations made several years ago in this laboratory indicated that foods of low acidity were much more difficult to preserve with sodium benzoate than those of high acidity. Thus, the spoilage of apple and grape juice by yeast and mold was prevented by 0.1 per cent or less of sodium benzoate, whereas 0.2 per cent failed to prevent growth of bacteria and mold in avocado pulp and in non-acid vegetables stored in weak brine.

It was suspected that hydrogen ion concentration rather than total acidity might be the controlling factor.

A study of the literature showed that the attention of previous investigators had been given to the effect of hydrogen ion concentration on the disinfecting, that is killing action, of sodium benzoate and various other reagents rather than to their preservative action.

However, Herter in 1910 reported that 0.2 per cent sodium benzoate retarded the growth of and gas production by *B. coli* in plain glucose bouillon, but had no noticeable effect in the same medium in the presence of $CaCO_3$. He made no pH determinations and gave the factor of acidity only passing attention.

Barnard (1911) states that benzoic acid is a more effective preservative than sodium benzoate but cites no experimental evidence. Held (1915) found that benzoic acid is less effective as a disinfectant in a medium rich in protein than in one poor in this constituent and states that if the protein binding power of the benzoic acid is satisfied by some other acid, such as tartaric, the concentration of benzoic acid necessary for disinfection is

lessened. Perry and Beal (1920) found that growth of *S. cerevisiae* in glucose bouillon was prevented by 0.5 per cent sodium benzoate and all living cells killed by 3.0 per cent. They also state that benzoic acid is more effective than sodium benzoate in preventing growth of yeasts and molds. Bonacorsi (1923) states that the disinfecting power of several common disinfectants is affected by the pH value of the medium but makes no statement concerning the effect of pH value on their inhibitive action on growth, or activity of microörganisms. Fleischer and Amster (1922) found that the disinfecting power of acid dyes is increased by a decrease in pH value and that of basic dyes by increase in pH value. Kuroda (1926) found that the killing action of sodium benzoate and several other organic preservatives and disinfectants was markedly affected by the pH value of the medium. Disinfection was much less effective in the pH range 5.0 to 8.9 than in that of 1.4 to 3.5. He used *B. coli* and *B. prodigiosus*.

EXPERIMENTAL

Our experiments were conducted for the purpose of definitely determining the effect of hydrogen ion concentration on the inhibiting action of sodium benzoate on the more common food spoilage microörganisms. Those studied were *Saccharomyces ellipsoideus*, isolated from grapes; *S. cerevisiae*; several Mycoderma yeasts; penicillium mold, two species; a mucor mold; a lactic acid culture from E. B. Fred (his culture No. 124-2) vinegar bacteria; *B. coli*, *B. sporogenes* and *B. subtilis*. It will be impossible to present all of the data in detail in this brief report; instead only the more significant results will be given and for the most part in the form of curves.

For the budding fungi, that is yeasts and molds and for vinegar bacteria, two fruit juice media, grape and apple juices, were used. Different portions were brought to various pH values by the addition of powdered citric acid or N/1 sodium hydroxide or in some instances sodium bicarbonate. These portions of various pH values were subdivided and sodium benzoate added in amounts ranging from no benzoate to an amount at each pH value that it was believed from preliminary tests would prevent growth.

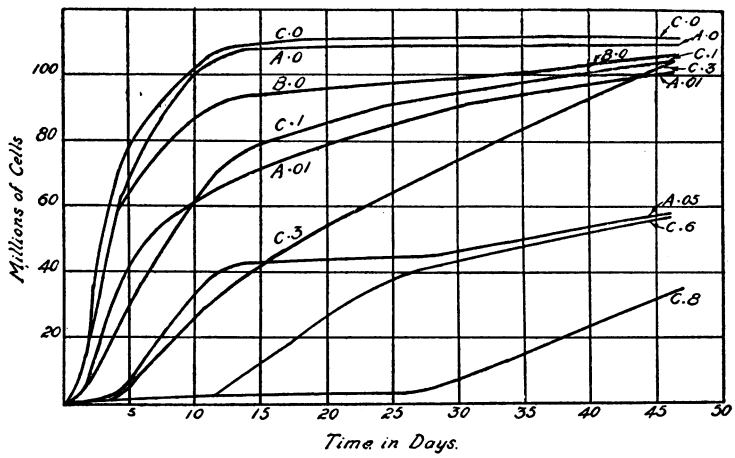

FIG. 1. EFFECT OF pH VALUE ON RETARDING ACTION OF SODIUM BENZOATE ON MULTIPLICATION OF S. ELLIPSOIDEUS

Curves labeled A 0, A 0.01 etc. represent juice of pH 3.8 and 0, 0.01 etc. gram benzoate per 100 cc. respectively; B, those of pH 3.0 and C those of pH 6.

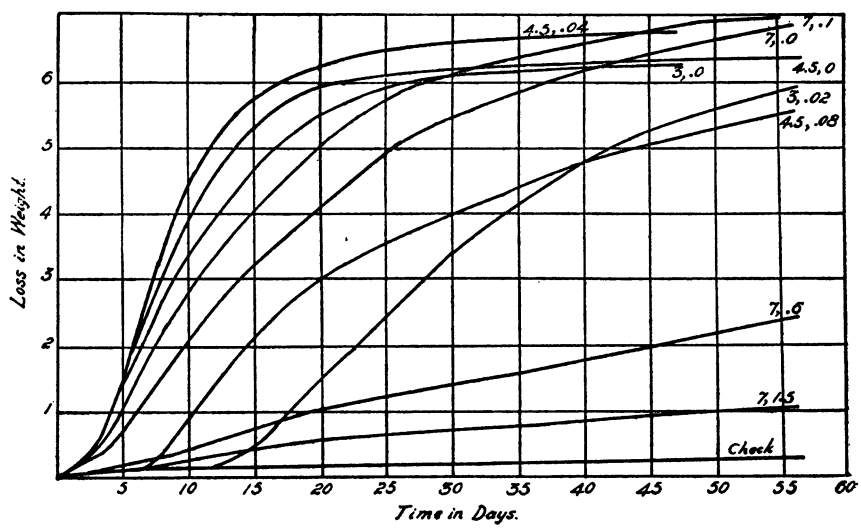

FIG. 2. EFFECT OF pH VALUE ON RETARDING ACTION OF SODIUM BENZOATE ON FERMENTATION BY S. ELLIPSOIDEUS

The tubed sterilized liquids were inoculated with pure cultures of the microörganisms previously listed. These were stored at room temperature, 18 to 25°C., for six months or longer and observations taken at intervals in order to determine at what

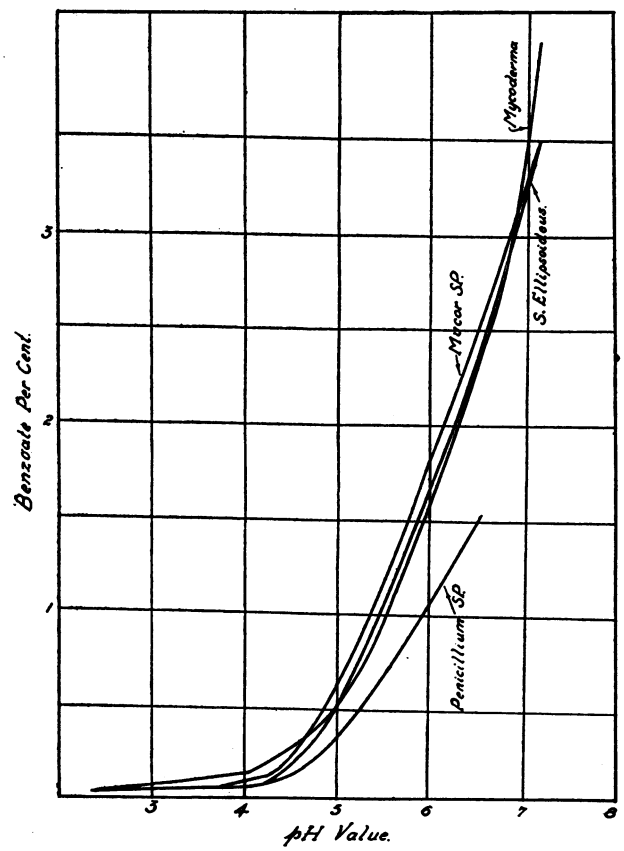

FIG. 3. EFFECT OF pH VALUE ON CONCENTRATION OF SODIUM BENZOATE REQUIRED TO PREVENT GROWTH OF CERTAIN YEASTS AND MOLDS

benzoate concentration in each of the various pH values growth was prevented.

With *S. ellipsoideus* additional observations were taken. In one series of tests the rate of multiplication of the cells at various

benzoate concentrations in juice of three pH values was determined. The results are shown in figure 1. In two other series of tests the rates of fermentation at three and at ten different pH values and various benzoate concentrations were determined. Typical results are given in figure 2.

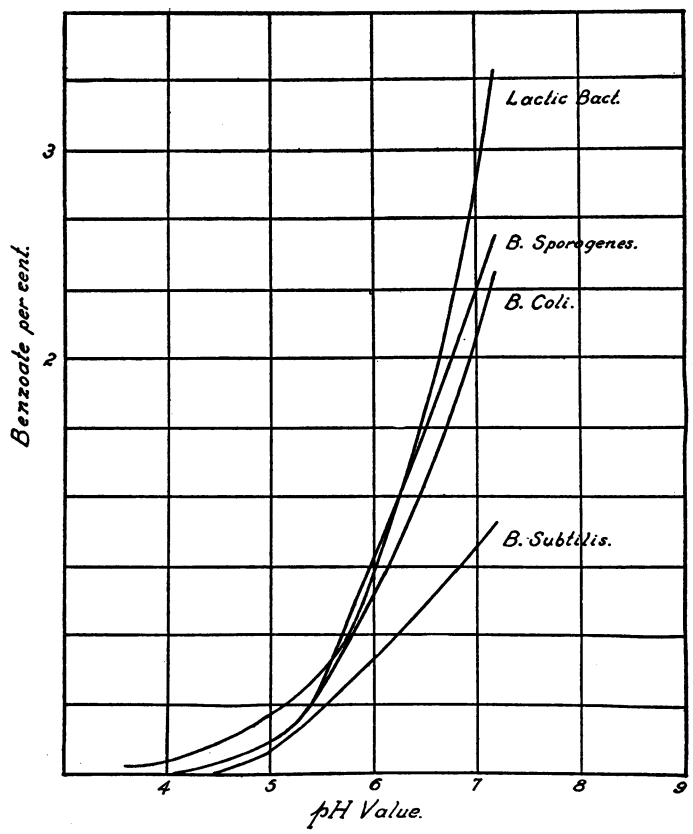

FIG. 4. EFFECT OF pH VALUE ON CONCENTRATION OF SODIUM BENZOATE REQUIRED TO PREVENT GROWTH OF CERTAIN BACTERIA

B. coli, *B. sporogenes*, *B. subtilis* and the lactic culture (E. B. Fred No. 124-2) were grown in a broth of the following composition; bacto pepton 10 grams, glucose 7.5 grams, Libby's extract of beef 10 grams, $MgSO_4$ 0.01 gram, KH_2PO_4 0.25 gram, $(NH_4)_2$

HPO_4 0.25 gram and water to make 1000 cc. The pH value of portions of the broth was adjusted by the addition of citric acid, N/1 NaOH or powdered $NaHCO_3$ to values ranging from 3.2 to 10.0 before sterilization. The range after sterilization was 3.6 to 8.7, changes occurring during sterilization being fairly pronounced at the lowest and highest pH values. Benzoate was added before sterilization to subdivisions of the various portions to give a wide range of benzoate concentrations. The tubed sterilized liquids were inoculated with a loopful of the respective cultures. The tubes were incubated a short time, about three days, at 37°C. and were then stored at room temperature 18° to 25°C. for more than six months. To the tubes of lactic and sporogenes cultures was added about $\frac{3}{4}$ inch of neutral mineral oil.

The benzoate concentrations required to prevent growth of the yeasts and molds at various pH values are given in figure 3; those for the bacteria in figure 4.

Experiments were conducted also with mixed cultures and with ripe olives in brine, avocado pulp, and certain non-acid vegetables. The results were similar to those obtained with pure cultures.

DISCUSSION

The effect of pH value on the inhibiting action of sodium benzoate on the multiplication of *S. ellipsoideus* was very pronounced. In figure 1 curves labeled A represent juice of pH 3.8, those labeled B, pH 3.0 and those labeled C, juice of pH 6.0. At pH 6 the highest concentration of benzoate 0.8 gram per 100 cc. used in this experiment failed to prevent growth, whereas at pH 3.0 growth was prevented by 0.05 gram of benzoate per 100 cc. At pH 6 and 0.8 gram benzoate per 100 cc. growth was delayed for twenty-five days, when slow multiplication ensued. At pH 6 and 0.3 gram benzoate per 100 cc., growth was slow but at 0.1 gram benzoate, growth was only moderately retarded. At the other pH values also, the retarding effect of the benzoate on growth varied with the concentration, although far less benzoate was required to produce a given effect at the lower pH values.

In figure 2 is given the effect of pH value on the retarding action of sodium benzoate on alcoholic fermentation by *S.*

ellipsoideus. As in its effect on the multiplication of this organism the retarding action of the benzoate was found to be dependent to a very marked degree on the pH value. The pH values used in this experiment were 3.0, 4.5 and 7.0. At pH 7.0 fermentation proceeded slowly even in the presence of 1.5 per cent sodium benzoate, the maximum concentration used. At pH 3.0 fermentation was completely prevented at 0.06 per cent of benzoate. At pH 4.5 more than 0.1 per cent of benzoate was required to prevent fermentation but 0.04 per cent appeared to stimulate fermentation. At benzoate concentrations between 0 per cent and the per cent required for prevention of fermentation the retarding action of the benzoate at each pH value in general varied with the concentration of benzoate present.

In figures 3 and 4 it will be seen that the benzoate concentration required to prevent growth varied greatly with the pH value. In the neighborhood of pH 4.5 for the yeasts and molds and at about 5.3 to 5.5 for *B. coli*, subtilis, sporogenes and lactic acid bacteria, there was great change in the slope of the curves. They became much steeper beyond these points; i.e. much more benzoate is required in the upper range of pH values than in that, say below pH 4.5.

Other data, not shown in the curves, indicate that the vinegar bacteria culture used behaved similarly to the lactic culture; and that at pH values 6.0 to about 9, growth of all organisms used was not prevented by 1.5 per cent of benzoate. At pH 10, less benzoate was required to prevent growth of one organism than at pH 7.3; in one test 0.7 per cent of benzoate prevented growth of one mycoderma culture at pH 10, but blue mold (a penicillium) grew even in the presence of 1.5 per cent benzoate at pH 10.0.

It is possible that the curves pass through a maximum somewhere between pH 7 and 10 and that the OH^- ion as well as the H^+ ion exerts an effect on the preservative action of sodium benzoate.

It is possible also that some of the inhibitive action of sodium benzoate at pH values in the range 5.0 to 7.0 is due to the Na^+ ion as Winslow (1928) and Doloff (see also the references given by

Winslow in article cited) show that sodium chloride at 0.5 M is slightly toxic in a pepton medium. At pH 7 in our tests concentrations of up to 4 per cent of sodium benzoate were used; giving at the higher percentages concentrations of Na^+ ions somewhat less than in a .5 M sodium chloride solution.

As would be expected, the pH growth range of the bacteria that are not tolerant to acid (*B. coli*, subtilis and sporogenes) was different from that for the acid tolerant organisms used in these tests and apparently also the turning point of the "pH versus benzoate to prevent growth" curves for these organisms was at a higher pH value than for the acid tolerant organisms. However, the pepton of the medium might have exerted some effect in the tests with these organisms.

It was found that ripe olives and non-acid vegetables in brine and avocado pulp inoculated with mixed cultures required many times more benzoate for preservation at pH values above 5 than at pH 3.5 to 4.0. One-tenth of 1 per cent benzoate had no apparent retarding effect on the spoilage of these products unless acid was added. It is therefore extremely dangerous to attempt to preserve such non-acid products with sodium benzoate unless acid is added. *B. subtilis* was considerably less tolerant to the acid and benzoate than *B. coli* and sporogenes.

SUMMARY

1. The retarding action of sodium benzoate on the rate of multiplication of *S. ellipsoideus* is much stronger at pH values of 2.5 to 4.5 than at 5.0 to 9.

2. A similar relationship holds for the retarding effect of sodium benzoate on alcoholic fermentation by this microörganism.

3. The concentrations of benzoate required to prevent the growth of *S. ellipsoideus*, *S. cerevisiae*, a mucor mold, two penicillium molds, three strains of mycoderma yeast, a lactic acid bacterium, a vinegar bacterium, *B. coli*, *B. subtilis* and *B. sporogenes* was greatly affected by the pH value of the medium. Much more benzoate was required at pH values near neutrality, e.g. pH 5 to 8 than at those in the moderately acid range 2.5 to 4.5.

4. While these observations are primarily of scientific interest

they also have an important bearing on the preservation of non-acid foods such as ripe olives, avocado pulp and non-acid vegetables by sodium benzoate.

5. Preliminary experiments have proved that similar relationships hold for certain other food preservatives.

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