FURTHER OBSERVATIONS ON THE RELATION OF pH VALUE TO TOXICITY OF PRESERVATIVES TO MICROÖRGANISMS

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In a previous paper (1929) it was shown that much more sodium benzoate was required to prevent growth of yeasts, molds and bacteria at pH values near neutrality (pH 5 to 8) than in the distinctly acid range of pH 2.5 to 4.5. The rate of multiplication of yeast and the rate of yeast fermentation, used as criteria of the toxicity of sodium benzoate to yeast, were also markedly affected by the pH value. In that paper were cited several investigators who had found that disinfecting power (killing effect) of several reagents is a function of the pH value. In the present paper is given he results of our recent experiments conducted to determine the effect of pH value on the preservative action of several weak acids (or their salts) and of two other preservatives, sodium chloride and formaldehyde.

EXPERIMENTAL

In these studies, the approximate concentrations of the preservatives required at three different pH values to prevent growth of *Penicillium glaucum*, *Saccharomyces ellipsoideus* and a mixed culture of *B. aceti* and lactic acid bacteria were determined. In addition, the relation of pH value to the retarding effect of these preservatives on yeast fermentation was studied.

Apple juice was used as the culture medium and was adjusted to pH values of 2.5, 3.5 and 7.0 by acidification with citric acid or addition of sodium hydroxide. For determining the concentrations of preservatives required to prevent growth at the three pH

values, test tubes were used as containers for the medium. An inoculum of one loopful of an active culture per tube was used. Growth or absence of growth was determined by visual inspection, except in borderline and doubtful cases in which visual inspection

TABLE 1

Relation of pH value to the concentrations of several preservatives required to prevent growth

PRESERVATIVE	pH VALUE	CONCENTRATION TO PREVENT GROWTH			
		S. ellipsoideus	Penicillium mold	Mucor mold	Mixed bacteria
		grams per 100 cc.	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*
Sodium salicylate	2.5	0.02	0.02	0.04	0.02
	3.5–3.8	0.06	0.15	0.10	0.08
	7.0	3.0+	3.0+	3.0+	3.0+
SO_2	2.5	0.02	0.02	0.03	0.01
	3.5	0.08	0.06	0.06	0.03
	7.0	Above 0.50	Above 0.50	Above 0.50	0.100
Acetic acid	3.5	0.8–1.0	0.8–1.0	0.8–1.0	No data
	7.0	Above 4.0	Above 4.0	Above 4.0	No data
NaCl	2.5	14	16	18	No data
	3.5	20	20	20	No data
	7.0	20	20	20	No data
Formaldehyde	2.5	0.015	0.015	0.015	0.015
	3.5	0.030±	0.030±	0.030±	0.030±
	7.0	0.030±	0.030±	0.030±	0.030±

Note: While, in the table the term "SO_2" is used in order to conform to usual practice, sodium sulfite was the reagent added. Acetic acid as such was added at pH 2.5 and 3.5 whereas, potassium acetate was added at pH 7.0. Its equivalent as "acetic acid" is reported in the table; i.e., the concentration of acetic acid corresponding to the total concentration of acetate radicle appears in the table.

was supplemented by microscopical examination. Tubes were stored at room temperature for three months or longer.

Portions of 100 cc. of the juice of the three pH values were inoculated with an active culture of *S. ellipsoideus* and the rate of fermentation was followed by daily determinations of loss in weight caused by evolution of carbon dioxide.

The relation of pH value to the preservative action of the several reagents studied is shown in table 1.

The data show that the preservative action of the weak acids (or their salts) salicylic acid, sulfurous acid and acetic acid is markedly affected by the pH value of the medium. Thus, at pH 7.0 more than 150 times as much sodium salicylate was required to prevent growth as at pH 2.5. These preservatives resemble sodium benzoate in their action as reported in the paper cited

TABLE 2

Effect of pH value on the retarding action of sodium salicylate on the rate of fermentation by S. ellipsoideus

(Loss in weight, grams per 100 cc.)

TIME	pH 2.5		pH 3.5		pH 7.0		
	0 gram in 100 cc.	0.02 gram in 100 cc.	0 gram in 100 cc.	0.04 gram in 100 cc.	0 gram in 100 cc.	0.08 gram in 100 cc.	2.0 grams in 100 cc.
days							
1	0.13	0	0.38	0	0	0	0
2	1.40	0	1 70	0	0.75	0.82	1.20
3	2.58	0	2.96	0	2.05	2.14	2.98
4	3.58	0	4.06	0.735	3.12	3.29	4.11
6	5.40	0.43	5.80	2.20	5.21	5.40	5.64
10	7.01	0.56	7.30	3.66	6.85	6.71	7.04
13	7.92	0.85	7.34	4.11	7.22	7.02	7.56
16	8.25	1.34	7.95	4.84	7.86	7.53	8.26
21	8.78	1.69	8.36	5.65	8.19	7.82	8.57
24	9.06	2.08	8.42	6.45	8.50	8.11	8.88
32	9.59	2.97	9.40	7.85	9.25	8.83	9.60
34	9.69	3.15	9.60	8.14	9.40	8.98	9.77
36	9.98	3.81	10.08	8.90	9.85	9.41	10.19

earlier in this report. The preservative action of sodium chloride and formaldehyde was only slightly or very moderately affected by the pH value. Evidently, the undissociated weak acids and not their ions are the preservative agents. With salt, osmotic action is probably the factor involved and with formaldehyde, some other factor.

The retarding effect on fermentation of concentrations of the various preservatives insufficient to prevent growth completely varied in a manner similar to that of the concentration required

to prevent growth. In order to conserve space, the data for only one preservative, sodium salicylate will be presented. (See table 2.)

At pH 7 none of the concentrations of sodium salicylate used appreciably affected the rate of fermentation. At pH 2.5 and 3.5 beginning of fermentation was delayed by small concentrations of salicylate and the rate of fermentation was retarded.

The results with acetic acid and sulfurous acid were similar to those reported for the salicylate; while the retarding action of sodium chloride and formaldehyde was but little affected by the pH value. At very low concentrations it was observed that most of the preservatives exerted an appreciable stimulating effect on the initial rate of fermentation; this effect possibly being due to stimulation of multiplication of the cells.

SUMMARY

The concentrations of the weak acids, benzoic, salicylic, sulfurous and acetic acids required to prevent the growth of typical microörganisms from fruit juices were found to be much greater at or near neutrality than in the acid range, pH 2.5 to 3.5. Their retarding action on fermentation was similarly affected. The preservative action and retarding effect of sodium chloride and formaldehyde on fermentation were but little effected by pH value.

REFERENCE

CRUESS, W. V., AND RICHERT, P. H. 1929 Effect of hydrogen ion concentration on the toxicity of sodium benzoate to microörganisms. Jour. Bact., 17, 363-371.